Mediterranean cuisine and health: A multiapproach exploring the sofrito technique

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UNIVERSITAT DE BARCELONA

FACULTAT DE FARMÀCIA I CIÈNCIES DE L'ALIMENTACIÓ

MEDITERRANEAN CUISINE AND HEALTH: A MULTIAPPROACH EXPLORING THE SOFRITO TECHNIQUE

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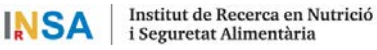

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ABSTRACT

1. ABSTRACT

Cooking made humans evolve. Cooking modified our gastrointestinal system, reducing the gut size and energy expenditure for digestion, which enabled the appearance of a more energy-dependent brain. Cooking process have emerged as a way to maximize the nutritional benefits from a limited amount of food. During the evolution, different diets appeared, in which culinary techniques and combination of ingredients led to what we know today as dietary patterns, such as Mediterranean Diet. This diet is characterized by a high intake of phytochemicals and recommended as a dietary standard in the prevention of cardiovascular and chronic diseases. However, the health outcomes of Mediterranean Diet are reportedly difficult to reproduce in non-Mediterranean populations, indicating the differences between cultures gastronomic techniques as one of the causes. Mediterranean cuisine was historically an excellent cooking model capable of exploring the healthy potential of different foods. Some traditional Mediterranean cooking process have a positive effect on nutritional quality of food, improving digestibility, retention of phytochemicals and caloric density. However, a scientific approach to how Mediterranean culinary process, the combination of their ingredients and their main dishes could influence bioactive compounds related to health has not yet been explored. The use of science in gastronomy is recent. In the 1980s, the term molecular gastronomy emerged as a discipline to approximate gastronomy and science in order to explain the mechanism and phenomena that occur during a dish preparation and consumption. This discipline is usually focus in physical and chemical process in relation to flavor but leaves aside nutritional aspects. Currently, some studies have characterized how different culinary techniques affect the content of phytochemicals, especially polyphenols and carotenoids, but with a simplistic approach, analyzing only one food and targeted compounds already known in raw food, forgetting the complexity of the dishes we eat and the formation of new chemical products. Therefore, an approximation between gastronomy and health needs to be more realistic, in which food consumption cannot be separated from culinary habits and cultural aspects. The aim of this thesis was studying the role of traditional Mediterranean cuisine, especially home-made preparations, could impact the content of bioactive compounds and promote health using the Mediterranean technique of *sofrito*. For this, different methodological approaches such as factorial designs and “omics” technique were used to describe a simple cooking technique that transforms dishes into complex systems.

HYPOTESIS AND AIMS

2. HYPOTESIS AND AIMS

HYPOTESIS

The main hypothesis of this thesis is: The Mediterranean cuisine have relevance on the health effects of the Mediterranean diet.

AIMS

The main aim of this thesis is to study the role of traditional Mediterranean cuisine, especially home-made preparations, in the content of bioactive compounds of tomato products with a special focus on the *sofrito* technique.

The general aims are:

- To study the effect of home-cooking tomato making on the bioactive compounds (Publication 1 and 2).
- To characterize the *sofrito* cooking technique and the influence of the ingredients on the bioactive compounds (publication 3, 4, 5, 6 and 7).
- To evaluate the tomato *sofrito* intake in health parameters (Publication 8).

The specific aims are:

- To study the role of olive oil as excipient on the extraction of bioactive compounds from food matrix during cooking process (Publication 1, 2 and 6).
- To characterize the *sofrito* sauces, a key component of Mediterranean diet (Publication 3).
- To evaluate the possible synergism between ingredients and cooking process on the content of bioactive compounds applying the *sofrito* technique (Publication 4, 5 and 6).
- To study the impact on cooking practices in the final composition of a sauce by non-targeted approach (Publication 7).
- To evalute the effects of *sofrito* intake on cardiovascular health in animal model (Publication 8).

INTRODUCTION

3. INTRODUCTION

3.1 Gastronomy, science and health

3.1.1 Cooking in evolution

During human evolution, the use of fire was a key element for its development[1]. Cooking influenced the gastrointestinal tract, decreasing the gut size and energy spent during digestion, which made possible the development of an energy demanding brain[2]. This happened thanks to food processing which decrease chewing demands and facilitated nutrients absorption[1–3]. Humans are fire dependent for food preparation[3]. The development of thermal food treatment contribute substantial on phenotypical, intellectual, societal and economic development of humanity[4]. However, there is still a gap on how cooked foods has influenced our diets and health.

All diets during evolution have one factor in common: cooked food[3]. Historically, human civilization develops different cooking techniques that was able to extract the maximum benefits from a limited food resource. For a long time, vegetables foods were sliced, mashed, fermented, baked, roasted or boiled, in which provide the calories, phytochemicals, macro and micro nutrients necessary for body maintaining[3]. However, the use of these culinary techniques and the preparation of meals at home diminish every day[5]. Since the middle of 20th century people have been cooking less from basic ingredients[6]. The modern life requires ready-to-eat and easy-to-prepare foods which move away from home cooking[7,8]. However, the consumption of these type of food leads to an increase in the caloric intake inducing diet-related diseases, such as diabetes and obesity[5,7].

In this way, governmental and non-governmental organizations have promoted home cooking as a key strategic to put down obesity and poor diets[9]. There are indications that home food preparations improve diet, health and social outcomes. Home cooking decrease the risk of obesity and chronic-degenerative diseases[5]. People that prepare their meals at home show higher consumption of vegetables and fruits[10], better diet[7] and improved adherence to models diets such as Mediterranean diet[11]. However, cooking practice have a multidisciplinary face, in which not only factors like know how to cook influence, but also including gender, available time and employment, personal relationships and culture background that hampers the incentive to cook[5].

To promote home cooking as a dietary strategy it is necessary to understand how cooking impacts the nutritional content of food. Studies have been carried out to see how the different cooking techniques influence the content of bioactive compounds and health related compounds, however solid results to elaborate recommendations have not yet been established[12–15]. Not only the retention of bioactive molecules but how these techniques affect food structure and bioaccessibility has been explored [14,15]. Since cooking is an essential part of our daily life, understand the mechanism of action with various cooking process, recipes and

cultural changes exert their effects on phytochemicals related with health effects merits further investigations[15,16].

3.1.2 When gastronomy meets science

Gastronomy (from greek: *gastèr*, stomach; *nomià*, law) is characterized as the set of techniques and culinary arts for the preparation of a good food, in which it relates culture and food[17]. Antonie-Laurent de Lavosier starts an approach between cooking and science publishing in 1783 an article about his studies of meat stock in order to determinate how much meat was needed for Paris hospital[18]. In 19th century the scientists such as Justus Liegbig and Benjamin Thompson closely this approach in order to help to feed the poor[19]. Other scientist like Parmentier (nutritional chemist), Pasteur (microbiologist), Chevreul (lipid chemist) and Maillard (food chemist) contribute to approximate food, cooking and science[20]. But only in 1835 the definition of the term gastronomy was accepted by the *Academie Francaise* as a synonymous of cuisine or ways that food are traditionally cooked and consumed in a region or country[21]. At the end of 20th century emerged the concept of molecular gastronomy using scientific evidence to reveal secrets of the culinary arts. Today, some chefs and scientist come closer to apply the principles of molecular gastronomy in their restaurant and later were recognized as a top in the world[19,21]. Top-ranked chefs such as Adrià, Blumenthal and the Roca brothers have or have had laboratories in their on local, however they do not consider themselves "molecular chefs"[22].

Molecular Gastronomy emerged as a discipline to approach science and gastronomy, in which it was defined as "*the scientific activity consisting in looking for the mechanism or phenomena occurring during dish preparation and consumption*"[20]. This discipline is considered part of food science and technology that aims to apply knowledge to improve dishes[17]. However, before 1980, food science neglected culinary process, in which textbooks of food chemistry containing almost nothing about culinary transformation[18]. This lack of information was probably by the complexity of culinary process and because science was focus on produce enough food for feed population, directing research to ingredients and solve technological problems[18]. Hervé This and Nicholas Kurti, in 1988[18], decided that this new disciple must be created and 1992 the discipline was recognized when the first International Conference of Science and Gastronomy was celebrated[14]. In a recent publication, Hervé This define the term "molecular gastronomy" as a science evidence approach to understand physical and chemical mechanism of a phenomenon in a culinary process, but cannot be confused with technology or engineering, once it is not focus on improving technique[23]. Currently there a still a debate whether molecular gastronomy is a really a discipline or would it be just food science[17].

Reporting molecular gastronomy in France, This describe dishes as system with bioactivity and culinary transformations could change that bioactivity[24]. Because most culinary transformations are thermal process of plant and animal tissues, a lot of work have been done in how different cooking techniques affect and preserve health-promote phytochemicals in order to approach cooking, nutrition and health[12,13,15,24]. However, literature only seek to verify how one

technique can influence one food, focus only the cooking technique and forgetting about the complexity of the dishes we eat. Besides, most of the articles are focus on targeted compounds found in raw foods, not exploiting other chemical classes or the formation of new compounds[25].

The approximation between gastronomy and health needs to be more realistic. Food consumption, as part of a diet, cannot be separated from cuisine habits and styles[16]. The complexity of homemade preparations that involve many factors such as utensils, ingredients, recipes and techniques affect the final product. Looking from this side, an approximation between food consumption, taking into account the traditional cuisine of a population, and nutrition has not yet been performed.

3.2 Mediterranean cuisine as a model for approach gastronomy and health

3.2.1 Could Mediterranean cuisine contribute to its diet beneficial effects?

The Mediterranean diet (MD), characterized by a high intake of phytochemicals from vegetables, has been correlated with improvements in metabolic and cardiovascular health[26,27]. This association is supported by evidence from PREDIMED study, in which a MD supplemented with extra virgin olive oil (EVOO) or dried fruits reduce the risk of cardiovascular events among high-risk population[27]. These findings has been reaffirmed by other MD clinical trials[28]. However, the health outcomes of a MD are reportedly difficult to reproduce in non-Mediterranean populations[26]. This difficulty indicates that MD practices cannot be separated from food production, or social and cultural habits which have been built historically around food and nutrition. Thus, the study of the Mediterranean cuisine allows us to visualize the multidimensionality of food consumption by its population, because it is in the kitchen where different patrimonial aspects of the MD converge and could influence its health effect[16].

Mediterranean cuisine was historically an excellent cooking model capable of exploring the healthy potential of different foods. This cuisine improved cooking techniques through an empirical process of trials in which sensory quality and well-being determined the food preparation[3]. Some traditional Mediterranean cooking process have a positive effect on nutritional quality of food, improving digestibility, retention of phytochemicals and caloric density. Literature pointed the marinating process[29], long and gently sauces preparations[30], soups[31] and use of extra virgin olive oil to cook[30,32] as an example of these health techniques.

In order to understand the Mediterranean cuisine, we should first begin to analyze the culinary system, which are combinations of ingredients, condiments and procedures that are within a common historical and territorial context. After that, we should examine the techniques, and finally, consider its elaboration, which is, in fact, what the population eats[16]. *Sofrito* is a culinary procedure used as a base to prepare Mediterranean dishes and sauces. This basic cuisine technique consists of lightly frying onion and garlic in olive oil and then add other

ingredients, such as tomato, to prepare tomato *sofrito* sauce as an example (Figure 1). The elaboration of tomato *sofrito* can be modified according to the region that prepares it by cultural diversity, varying the proportion of ingredients that impact its final chemical profile. This combinations of ingredients, called food pairing, has been used to understand the choice of ingredients by flavor in a culture by molecular gastronomy[33], but this approach could be explored to verify beneficial health effects in the traditional cuisine.

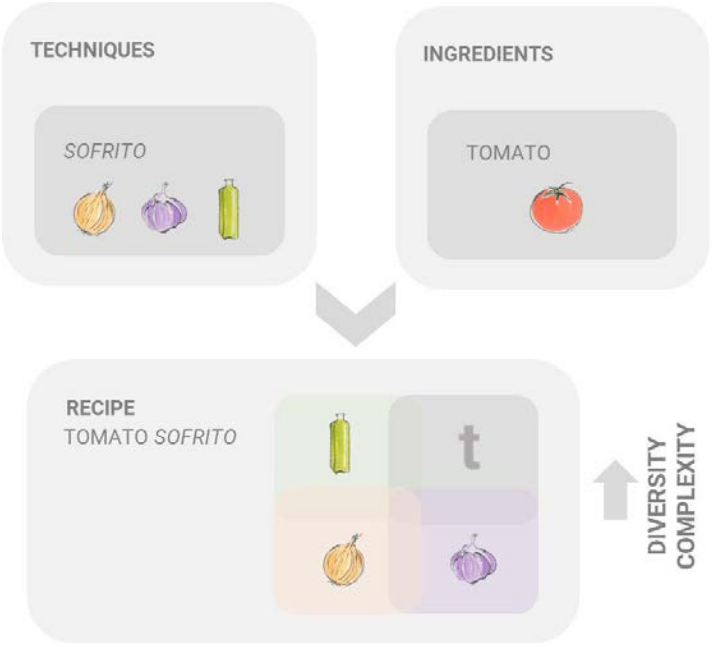

Figure 1. Contextualization of *sofrito* on the Mediterranean culinary system.

Tomatoes, the base of *sofrito* sauce, are one of the most consumed vegetable in the world[34]. In 2017, fresh tomato was the most consumed vegetable by Spanish houses, representing 16.8% of the total fresh vegetable volume and 18.0% in economic measures. It was estimated a consumption of 12.84 kg/year *per capita*[35]. Moreover tomato sauces represented a 40.8% of all sauce consumption in Spain[36]. Epidemiological studies have shown that tomatoes intake reduce prostate cancer risk and cardiovascular risk factors and has a greater correlation with a low incidence of chronic-degenerative diseases, especially cooked tomatoes products[26,37,38]. The tomato *sofrito* sauce show a high phenolic and carotenoids content[39] and its consumption is one of 14-item validated questionnaire used to evaluate the adherence of traditional MD[40]. Recently, it is associated with improved cardiovascular risk parameters and insulin sensitivity, activates thermogenesis by browning fat tissue and manifestation of less aggressive prostate cancer[36,41,42]. Therefore, these characteristics make *sofrito* an excellent candidate to corroborate cuisine and health.

3.2.2 A multiapproach to understand Mediterranean cuisine

As previously stated, research on cooking and nutrition has been focused on how a cooking technique influences the bioactive compounds of a food. This approach, considering nutrients in a targeted way, leaves behind the complexity of the culinary process. Therefore, a new step should be taken towards a more realistic approach to food preparation, looking for the culinary system, which considers a typical cooking process, the proportion of ingredients used and the variability of a recipe. For this, new strategies can be borrowed from other areas of science as experimental designs and foodomics to understand the cuisine in its entirety.

Experimental designs tools have been frequently applied to optimized analytical methods[43] and industrial process[44]. These techniques aim to reduce consumption of products, number of experiments and time for minimizing cost. For this, it studies different strategic factors together with the development of a mathematical models, which allow to reveal the statistical significance of each factor studied. Also evaluates a possible interaction between the factors. In multivariate design exist two types of variables: (I) factors that are the term that we want to verify their influence on the (II) response that is what we want to determine. In this case, factors must have a minimum and maximum values established in order to define the experimental domain to be investigated. First-order models are commonly used in exploratory studies when a large number of factors need to be screened. This type of method detects the factors that have an effect on the response in which it helps to discard those that do not contribute, being perfect for preliminary studies and first screening, and the most popular first-order designs are Full Factorial Design (FFD) and Fractional Factorial Design (FaFD)[43,45,46]. In our case, this approach could be useful to determinate how factors that influence the content of bioactive compounds in a dish preparation. For example, it could be checked the proportion of the ingredients in a recipe, the temperature applied, the cooking time and even a possible synergism between them (Figure 2).

In the other side, foodomics is a new term introduced in 2009 by Cifuentes and co-workers[47,48] to describe "*a discipline that studies the Food and Nutrition domains through the application of advanced omics technologies to improve consumer's well-being, health and confidence*". Foodomics basic consist on the intent to perform a global approach integrating information from different areas where food, advanced analytical techniques and bioinformatics are involved[48,49]. This discipline is focused on analyzing hundreds of compounds found in a complex matrix and being able to find the perturbation in the compounds profile caused by an intervention, which could be provoked by a pathogen, a chemical contaminant or the effect of a process[49,50]. For that, foodomics tools have been used on food safety and quality[51], food authentication[52,53], to understand processing and matrix effects[54]. Therefore, the use of this tool in the study of culinary systems and typologies can reveal new information of how important are the proportion of ingredients in a recipe, influencing degradation of compounds and emergence of others and also reveal classes of chemical compounds not yet explored on some food that could show bioactivity (Figure 2).

3.3 Effect of cooking on health-related compounds

In this section it will be discussed how the culinary process can modify the structure of the food, being able to extract bioactive compounds from the matrix, which can result in an increase of the bioaccessibility and bioavailability of these compounds so that they can act in the organism promoting their bioactivity. Within the mentioned concepts, the cooking process mainly affects the bioaccessibility, which is defined as "*fraction of an ingested compounds which released or liberated from the food matrix on the gastrointestinal tract*". After liberate in the gastrointestinal tract the compounds must be bioavailable, in which is defined by "*fraction of a given compounds or its metabolites that reaches the system circulation*" and once the compounds reach a tissue and promote is effect its call bioactivity "*specific effect of a compound in the body- It include tissue uptake and the consequent physiological response"*[55–58].

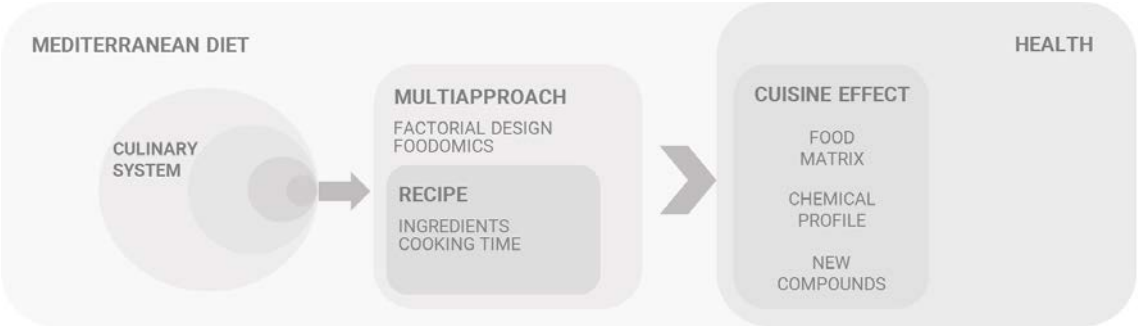

Figure 2. Proposal of a multiapproach for study Mediterranean cuisine and approximate of health.

3.3.1 Cooking and carotenoids

3.3.1.1 Carotenoids in food, absorption and bioavailability

Carotenoids are fat soluble pigments presents in plant, fungi and photosynthetic microorganism that participate in the photosynthesis light harvesting process, conferring photo-protection and antioxidant capacity. Carotenoids are classified based on their chemical structure as: carotenes, highly conjugated C40 hydrocarbons; xanthophylls, oxygenated carotenes; apo-carotenoids, shortened carotenes and xanthophylls resulting from carotenoids oxidation and metabolism; and C30 carotenoids, small class normally found in bacteria (Figure 3). Approximately 40-50 carotenoids are present in human food, in which some are important for the production of vitamin A. Provitamin A carotenoids contain at least one unsubstituted β-ionone ring (β-carotene, α-carotene and β-cryptoxanthin) that enzymatically can produce retinol, an essential vitamin for the human body[57,58].

The absorption and distribution of carotenoids in the body occurs similarly to other dietary fat soluble compounds. Firstly, carotenoids must be released from the food matrix and solubilized in oil drops. After that, carotenoids in the oil drops interact with bile salt micelles and

enzymes during lipids digestion like triglycerides, phospholipids and cholesterol esters that are cleavage to di-glycerides, mono-glycerides, lysophospholipids, free cholesterol and fatty acids. In this stage, carotenoids esters could also be deconjugated of fatty acids to their free form. These digested micelles are diffuse to the apical surface of enterocytes, interacting with bush border proteins for transfer to the cell interior. The absorption process can occur by two ways: passive diffusion and via transporters. The passive diffusion of carotenoids across the enterocyte it is believed to happen in supra-physiological concentrations. Internalization of carotenoids by transporters is facilitated by scavenger receptor class B type I (SR-B1), cluster determinant 36 (CD36) and Niemann-Pick C1 Like1 (NPC1L1), proteins that also act in the absorption of lipids compounds, however the process still unclear. Because these transporters also mediated dietary fat soluble compounds absorption, they may compete with carotenoids for binding and cellular uptake. Once in the cell, provitamin A carotenoids could be metabolize to retinal by β-carotene oxygenase 1 (BCO1), follow by reduction to retinol with retinol dehydrogenase (RDH) and esterification by lecithin-retinol acyltransferase (LRAT) to produce retinyl esters for be distribute to the body. Other enzyme such β-carotene oxygenase 2 (BCO2) also participate in metabolism process on carotenoids producing apo-carotenoids, however its participation is not yet fully understood[58,59].

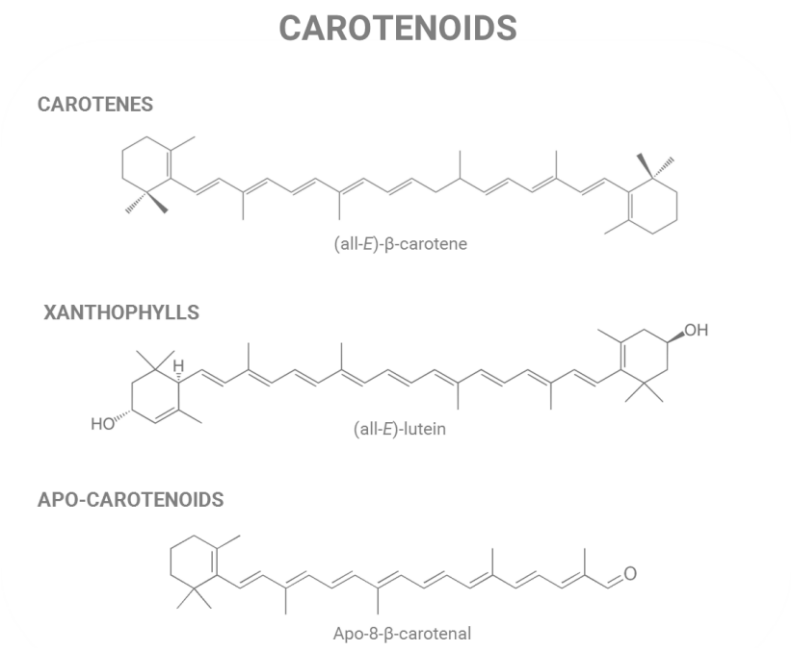

Figure 3. Carotenoids structures and classification.

After enterocyte metabolism, lipids species such as fatty acids, mono-acylglycerols are re-esterified to triacylglycerol that are used to produce chylomicrons, which are TAG-rich particles containing phospholipids, cholesterol and other lipophilic compounds with a single molecule of apo-B48 lipoprotein. Carotenoids and retinyl esters are also incorporated in the chylomicrons particles, that are released in plasma suffering hydrolysis by lipoprotein lipase that diminish the

particle size to be taken up by the liver. Within in the liver, the carotenoids are released from the remnant chylomicrons and incorporated in small particles such as very low density lipoprotein (VLDL) that again are secreted into plasma. The VLDL delivery triacylglycerol and some carotenoids to peripheral and adipose tissues, converting to a low-density lipoprotein (LDL), with less triacylglycerols. LDL continues to circulate thought the body, delivering carotenoids to peripheral tissues[57,58]. Xanthophylls and a small amount of carotenes are also associated with HDL, however some works have been suggesting that HDL only receive xanthophylls[60].

The absorption of carotenoids from ingested plant food in relatively inefficient and research has been done to increase the efficiency of carotenoids absorption due to its health properties. A lot of factors have been pointed out as causes of this inefficiency such as: carotenoids physico-chemical characteristics, natural barriers of the plant tissue, chemical barriers during food process that difficult carotenoid solubilization, food processing, dietary components interaction and other substances that difficult carotenoid partitioning into mixed micelles and competition with other substances for the cell transporter. Moreover, inter-individual variability such as polymorphism in genes responsible to codify carotenoids cleavage enzymes, enterocyte transporters and secretion of chylomicron could affect the bioavailability of carotenoids[57,58].

3.3.1.2 Factors that influence carotenoids content, bioaccessibility and bioavailability

In this section, it will describe how the different factors can interfere in the carotenoids bioaccessibility, giving basis to understand how the cooking process could help to release these compounds in the gastrointestinal system and improve bioavailability. In addition, a focus was given on carotenoids that are present in tomato *sofrito*, such as lycopene, α-carotene, β-carotene and lutein (Figure 4).

I) Molecular structure

Carotenoids chemical structure in food and after food process influence their bioaccessibility and bioavailability[57,58]. Among the carotene class, β-carotene and lycopene are abundantly present in carotenoids rich vegetables and are most studied in literature. These compounds in plant food are usually found in the form of large, tightly-packed crystalloid aggregates, especially, lycopene in *E*-form that has a planar structure that facilitates strong intermolecular interaction with other lycopene molecules[57]. These tightly crystal package presentation makes lycopene hardly solubilize, in which the presence of dietary lipids and thermal processing is necessary for solubilization. In addition, carotenes need to be incorporated in the center of the micelles by their high lipophilicity, in which is less spacious compared to the surface, which reduces their incorporation and bioaccessibility[57]. Therefore, carotenes are less favored to absorption compared to xanthophylls. However, the change in the structure conformation to *Z*-

form decreases aggregation and crystallization by not being more planar, which can increases its bioaccessibility[61].

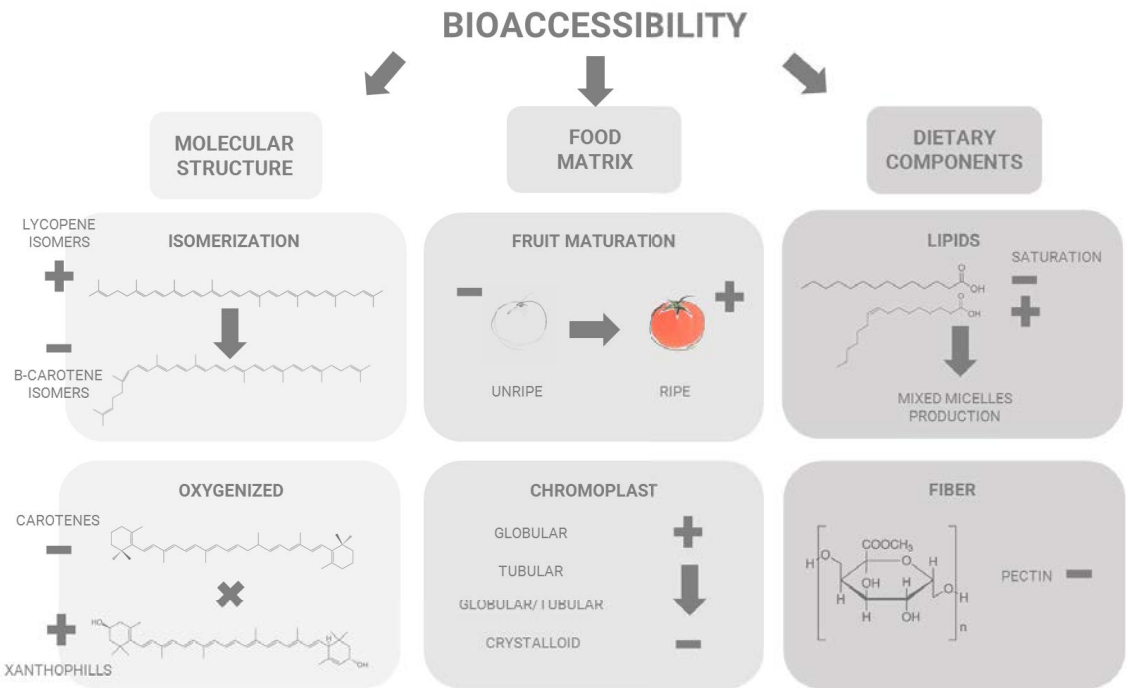

Figure 4. Factors that affect carotenoids bioaccessibility.

Xanthophylls contain at least one oxygen atom in their molecule structure with different types of substitute groups such as hydroxyl, methoxyl, carbonyl or epoxy groups. The presence of the oxygen increases the molecule polarity and improve their solubilization in aqueous system, especially in digestive phases with the presence of bile acids that are potent emulsifier. Different from carotenes, xanthophylls are able to form hydrogen bonds with other molecules, in which it facilitates their entry, stability and presence on the surface of the micelles[57,58,61].

In nature, the carotenoids all-*E*-configuration is the most commons and abundant, however, *Z*-isomer can be often found especially after food processing. The *Z*-form is characterized of at least one of the double bonds introduces a bend differentiating from the *E*-form geometric conformation[61]. This conformational change diminish the susceptibility to form crystal arrangements and improving their solubility in lipids[62]. Therefore, lycopene *Z*-isomers have been demonstrated to be significantly more bioavailable compared to the *E*-form, in which it was related to an increase in solubility in the micelles. In human blood and tissue, at least 18 different *E*/*Z*-isomers of lycopene have been found[63].

II) Food matrix

The food matrix shows important factors in the bioaccessibility of carotenoids, especially in fruits and vegetables. Literature have reported the main characteristics of the food matrix that affect the bioaccessibility and bioavailability of carotenoids are: fruit maturation, chromoplast morphology and cell wall structure and cluster [57,58].

During fruits ripening, large quantities of carotenoids are accumulated within specialized plastids: the chromoplast. These plastids are formed by chloroplast transformation into chromoplast during ripening by degradation of the photosynthetic system and accumulation of pigments. Chromoplast can be classified by their internal structure that directly influence the physical-chemical state and bioaccessibility of carotenoids[64]. A good review was recently published by Schweiggert about this topic [64]. Four class are described in literature such as: globular, characterized by the presence of plastoglobules bearing lipid-dissolved carotenoids; Tubular, that containing a tubular shape and carotenoids are presented in a liquid-crystalline form; globular-tubular, that show a mixed composition of the last two types; and crystalloid, that are characterized by the presence of crystalized carotenoids[64]. An *in vitro* bioaccessibility study comparing tomato, carrot, papaya and mango found that mango and papaya had higher β-carotene bioaccessibility, 10.1% and 5.3%, respectively, compared to carrot and tomato, 0.5% and 3.1 %, respectively. Mango and papaya are characterized by storage carotenoids in globular-tubular chromoplast and tomato and carrot in crystalloid chromoplast, indicating that crystalloid carotenoids were more difficult to extract from the matrix[65]. In addition, a human bioavailability study described an absorption 2.9 and 3.1 time higher for β-carotene from papaya than from carrot and tomato, respectively, and 2.6 higher for lycopene from papaya compared to tomato[66].

The cell wall and the cluster of adjacent cells represent another barrier for release carotenoid release from food matrix and the incorporation on lipid droplets during digestion[58]. For that, processing methods could disrupt cell cluster, walls and chromoplast being expected to increase bioaccessibility. Studies conducted by Hendrickx and co-workers developed a model to isolate chromoplasts and cell clusters from food to understand how these natural barriers could be affected by processing and *in vitro* digestion. Although heat and other mechanical methods induce the rupture of the structures, the bioaccessibility of β-carotene and lycopene was not significant greater compared to no-heated or non-processed controls for oranges, tomato and red carrot. This indicates that only cell wall disruption is not effective to increase carotenoids bioaccessibility.

III) Dietary components

Different dietary components such as lipids, fiber and also the synergism between liposoluble compounds like vitamin, multiple carotenoids, ω-3 and phytosterols can influence the carotenoids absorption and was put in evidence by a book chapter published by Cervantez-Paz and co-workers[57,58,67].

Dietary fiber, correspond to oligo and polymeric carbohydrates can be classified as a factor from the food matrix and also as a dietary component that influence carotenoids absorption. Among the different types of fiber, pectin is the one that influences the transference of carotenoids to oil droplets during digestion. Pectin has unique characteristics that may influence carotenoids bioaccessibility such as: degree on methyl-esterification, content in cell walls and pectin type[58,67]. For example, heat treatment of carrots increase the solubility,

depolymerizes and demethoxylates pectin which was correlated with an increase in carotene bioavailability in processed and digested carrot compared to raw digested carrot[68]. Moreover, emulsions model systems have been used to investigate the role of the pectin on carotenoid bioaccessibility, in which was inversely associated with the methyl-esterification degree, once with less esterification there is a greater formation of gel network forming structures that hinders the passage of lipid droplets[69]. Furthermore, the interaction of pectin with lipases, bile acids and the surface of the lipid droplets may affect the formation of the small lipid droplets during the digestion process, which makes it difficult to deliver to the apical membrane of enterocytes[58,67].

The consumption of fat and oil is the mandatory factor of the dietary components that influence the absorption of carotenoids and the most explored by literature. Fat acts in different ways to increase bioaccessibility and bioavailability of carotenoids. It starts with its ability to extract and solubilize carotenoids and other lipophilic compounds from the food matrix. Afterwards, the fat intake at the meals stimulate the secretion of lipases, bile salts and phospholipids into the intestinal lumen helping the formation of mixed micelles that solubilize carotenoids for absorption. In addition, fat slows gastric emptying and transit through the small intestine which provides more time for the production of fatty acids, mono-acylglycerides and other lipids that are responsible to produce the mixed micelles. Literature have suggests a minimum of 3-5 g per meal of fat to have a significant absorption effects. The type of fat also influences the bioaccessibility and bioavailability of carotenoids. Transference to mixed micelles showed to be greater to soybean, olive or canola oil compared to butter, indicating that unsaturated fatty acids improve carotenoids bioaccessibility. Moreover, when compared the type and ratios of fatty acids, olive oil showed better absorption in caco-2 cells model compared to soybean oil by the presence of more unsaturated fatty acids.

Phytosterols are correlated with a decrease in plasma cholesterol between 5 to 15% by inhibition of the micellarization of cholesterol that influence its absorption. For that, phytosterols influence part of the absorption process of vitamins, lipid and by consequence carotenoids absorption.

3.3.1.3 How cooking impact carotenoids on diet?

The main cooking techniques can impact the carotenoids content on food[13]. Home-cooking techniques are based in thermal process (boiling, steaming, microwaving, frying, grilling, baking and stir-frying) that provoke chemical modifications like isomerization and oxidation and losses of carotenoids[13,70–73]. Oxidation is the major cause of carotenoids loss. This process dependent of oxygen available and can be induced by heat, light, temperature and pH[74]. Cooking also improve bioaccessibility of carotenoids by overcoming the aforementioned factors. These changes are conditioned by cooking time and temperature on each cooking technique applied, which influences the profile and final content on food. However, there is still no consensus regarding the best way to cook to preserve these compounds[13].

I) Chemical stability of carotenoids on cooking

The conjugated polyene chains make carotenoids susceptible to isomerization and degradation from different agents. Boon and co-workers[74] published an excellent review about all aspect involving carotenoids degradation and pointed autoxidation, photodegradation, thermal degradation and free radicals reaction as the main causes. Oxidative damage after cooking process could results in loss of bioactivity. For that, understand detrimental aspect of carotenoids oxidation is important to have a critical view about the oxidation mechanism during cooking and how optimize the stability of these compounds[74]. Meanwhile, carotenoids degradation may generate novel compounds with bioactivity such as apocarotenoids and epoxides that are formed during thermal treatment and final reaction products such as volatiles that contribute to food flavor[75].

Carotenoids isomerization are currently gaining interest in food. Multiple isomeric forms of carotenoids can be found due to the long and conjugated double bonds carbon chain typical from this class of compounds. Electron transfer reactions was pointed as mediators in the isomerization process by the formation of carotenoid cations o dications by radicals, acids or metals like iron. Carotenoids cation formation can be a decisive step on isomerization, since the conformational change in cations require less energy compared to neutral molecules. Moreover, the synergism between neutral and radical carotenoids initiates a reaction chain that increases the number of isomers. However, Colle and co-workers[76] described mathematical models that explain the isomerization process, indicating that all isomers are produced from *E*-forms and tend to return for this configuration by being more stable. For that, after a certain process time the *Z*-isomers production will attain a maximum. However, the rate constant for *Z*-isomers formation and for come-back to *E*-form were significant lower in the presence of an oil matrix, indicating that isomerization reaction occur in the oil and lower activation energy is necessary[76,77]. The orientation of the double bounds on isomers influence the shape and length of the molecule that influence biological functions, improve antioxidant capacity and their bioaccessibility and bioavailability[61,74].Cooking process present three important factors for carotenoid degradation: presence of oxygen, temperature applied and time of processing. But also the same factors are important for heating, agitation and improving surface area help to breakdown cell walls and induce *Z* isomerization[13,74].

II) Effect of cooking techniques on carotenoids

A meta-analysis evaluating the effect of different cooking techniques on carotenoid content was published by Murador and co-wokers[13]. Using a response ratio (R) to see the influence of different cooking techniques, was indicated boiling (R = 1.01) and stewing (R = 1.33) as the techniques that improved carotenoid content. Steaming (R = 0.96), microwaving (R = 0.79) and stir-frying (R = 0.77) showed a decreased in the carotenoid content, being frying (R = 0.59) the technique that most affect carotenoids on food.

The boiling technique increase the content of carotenoid by promote structural changes on food matrix, softening vegetable tissues, which improve extractability[21]. However, some divergences are found in literature, indicating that a decrease could occur by water absorption by the food and may cause dilution on carotenoid content[78]. Moreover, structural specifies of carotenoids can influence in these processes, where more carotenes decrease with more time of processing compared to xanthophylls, probably by chromoplast internal structure differences[58,74]. The same reasoning line can be applied by steaming process, since it does not affect the carotenoid content either. Furthermore, carotenoid steam results are not affected by the gain of water in the food, which makes results more reliable[13]. Stewing technique showed to be the best way to improve carotenoids in cooked food[13]. This technique is characterized by an oil and water environment, in which water improve carotenoids extractability like in boiling process with an extra help of oil that improve carotenoid solubility[61]. Carotenoids are lipophilic molecules, in which are storage in hydrophobic structures of cells that are extracted by an oil matrix, however water is needed to soften the previous polar structures such as cell wall[61]. Stir-frying decrease the content of carotenoids by the high temperature used to process food, however it is may concentrate the carotenoids by loss of water. The most drastically methods for carotenoids are microwaving and frying. Microwaving is a technique used for the same purpose of boiling, while a longer processing time is necessary to achieve the same characteristics[21], which leads to a carotenoids oxidantion[13]. Frying, that are pointed as the mess aggressive process for carotenoids, decreases the content due to molecule instability in high temperature, presence of oxidative species generate by the lipid matrix and also by leaching carotenoids into the oil[13,73].

Mayeaux and co-workers[73] applied microwave, pan-frying and bake technique on tomatoes variating time and temperature, found that the less aggressive technique was microwave, in which a process with 1000 w for 60 s decrease in 35% the lycopene content. When bake technique was applied, lycopene degradation increases with time and temperature used, remaining 64% for 15 min, 45% for 30 min and 37% for 45 min at 177 ºC. With a higher temperature of 218 ºC, 51% for 15 min, 41% for 30 in and 25% for 45 min of lycopene was remained. Frying showed to be the most drastic technique with a lycopene degradation of 70% for 145 ºC and 75% for 165 ºC with 2 min of processing. Sahlin and co-workers[70] comparing boiling, baking and frying process on lycopene content for two varieties of tomato concluded that all cooking techniques diminish lycopene after process. Baked and boiled tomatoes showed no differences in lycopene content, however the frying process decreased almost the half.

Therefore, food matrix, chromoplast structure and molecular composition influences the effect of each cooking technique on carotenoids content in a unique way, which difficult stablish a rule for cooking effects on carotenoids. Literature regarding cooking process does not show the results on dry matter or equivalent to raw food, in which it is difficult to compare the effect of each technique on the carotenoid content. Moreover, industrial process and new technologies have been explored the presence of lipids to improve carotenoids extractability and bioaccessibility[79–81], but only a few studies can be seen using home-cooking techniques[82].

3.3.2 Cooking and phenolic compounds

3.3.2.1 Phenolic compounds in food and bioavailability

Polyphenol are structure characterized by a hydroxyl group on aromatic rings[83]. More than 8000 structures have been reported and several hundred of them have been identified on edible plants[83,84]. These compounds are classified into two major classes: flavonoids and non-flavonoids. Flavonoids share a common structure consisting of two benzenes rings (A and B) that are bound together through 3 carbon atoms that form an oxygenated heterocycle (ring C). They can be classified in main subclasses in function of the heterocyclic involved: flavones, flavonols, flavanols, isoflavones, flavanones and anthocyanidins (Figure 5). Other flavonoid groups that are minority dietary components are chalcones, dihydochalcones, coumarins and aurones. On the other hand, non-flavonoids are a heterogeneous group, in which can be simple such as phenolic acids (C6-C1), hydroxycinmates (C6-C3) (Figure 6) to a more complex like stilbenes (C6-C2-C6) and lignans[83,85]. Produced from secondary metabolism of plants, the phenolic compounds content can vary on food, since factors such as ripeness at the time of harvest, environmental (soil type, sun exposure, rainfall) and agronomic factors (greenhouse or field culture, biological culture, hydroponic culture, etc.) could influence its production[83]. Moreover, factors such as food processing and storage will also influence the final content of phenolic compounds in the food and as consequence its intake[83].

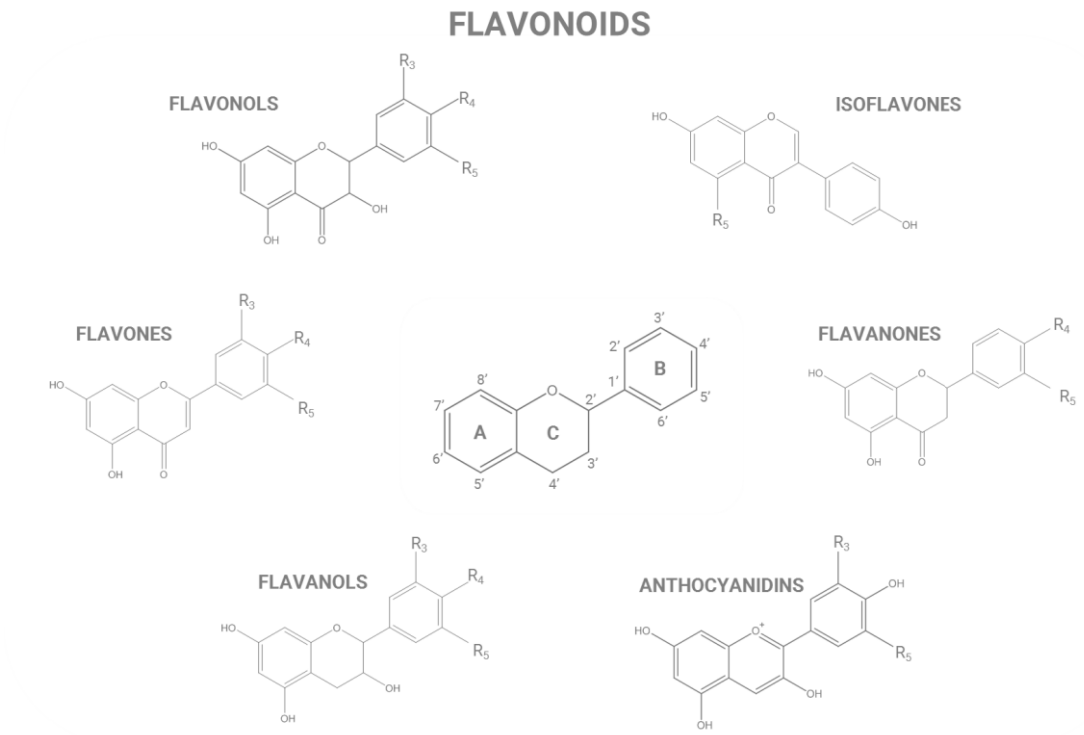

Figure 5. Structure of flavonoids found in food.

The absorption, distribution, metabolism and excretion of phenolic compounds after consumption have been explored by literature[83,85–89]. Follow ingestion, the first alteration on polyphenols begin with saliva contact, in which the first hydrolysis occurs to remove the sugar moiety by amylase[83]. However, this is a fast stage, with little interaction between enzymes and polyphenols, in which it does not contribute to the release of phenolic compounds[87,89]. After that, during the gastric phase, pepsin digestion with peristaltic movements and low pH diminish particle size that helps in the performance of enzymes. Moreover, the low pH increases liberation of polyphenols from food matrix to aqueous phase due to ionic interactions. Passing into the duodenum, the pH changes approximately to 7 and new enzymes are secreted by the pancreas and bile, with act in the digestion of sugars, protein and lipids[83,85]. The presence of lipases and bile salts are important for lipid digestion and results in the formation of mixed micelles, previously mentioned, in which are responsible for carotenoids absorption. Apolar polyphenols may also be micellarized, but there still unknown if this pathway improve or result in a lower bioaccessibility[83]. Upon reaching the intestine, polyphenols will be absorbed as aglycones, so the action of lactase-phlorizin hydrolase (LPH) enzymes found on the brush border of small intestine epithelial cells are necessary to remove the sugar moiety if it still persist[85,86]. LPH shows a specificity for flavonoid-*O*-*β*-D-glucosides, which after sugar hydrolysis, increases the lipophilicity of the polyphenol that would help to cross the cell membrane[83,87,90]. The absorption of a phenolic compound still conjugated with a sugar could accord by the sodium-dependent glucose transporter (SGLT-1), responsible for the glucose transport into the enterocyte. In this case, the sugar hydrolysis process would be carried out inside the cell, by the cytosolic *β*-glucosidase (GBC)[83,85,91]. Once inside the enterocyte, the aglycones can undergo into phase II metabolism forming sulfate by sulfotransferases (SULTs), glucuronide by uridin-5'-diphosphate glucosyltransferase (UGT) and/or methylated metabolites by catechol-*O*-methyltransferase (COMTs). Phenolic and possible metabolites are efflux from enterocyte by GLUT2 and MRP3 (ATP-dependent efflux transporters) transporters into the portal circulation to reach the liver, but some of these compounds also return to the intestinal lumen via MRP1/2 transporters. Once in the liver, their again suffer phase II metabolism, follow by enterohepatic recirculation and may result in some enterohepatic circulation to the small intestine by bile extraction[83,85].

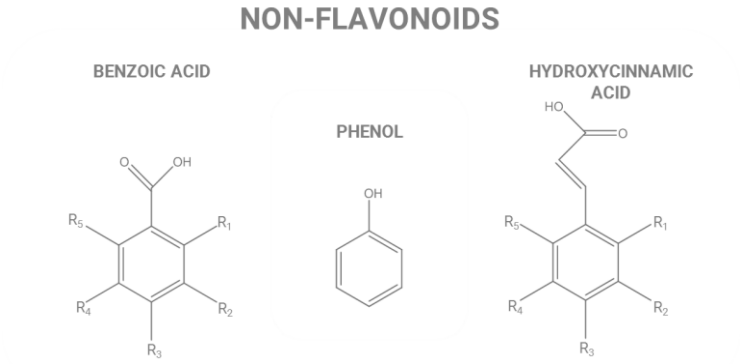

Figure 6. Structure of phenolic acids.

There is an increase evidence that a chronical intake of phenolic compounds may reduce the incidence of chronical diseases such as cancer, diabetes, cardiovascular and neurodegenerative diseases[85]. These compounds are responsible for approximately 90% of dietary antioxidants intake[92,93]. Follow a MD the consumption of polyphenols can reach more than 1g per day[94,95]. However, for these compounds to reach the target tissues and perform bioactivity it is necessary to overcome different factors that affect their bioaccessibility and bioavailability[88].

3.3.2.2 Factors that affect phenolic compounds bioaccessibility and bioavailability

As for carotenoid, this session will describe the main factors that affect the bioaccessibility of phenolic compounds and provide a basis for understand how the cooking process could improve it. Unlike carotenoids, polyphenols current a wide range of chemical structures, in which it is difficult to establish a common rule applied to all classes. Among phenolic compounds structures, glycosylation, acetylation/methylation and polymerization[88,93,96] showed to impact their bioaccessibility. Moreover, food matrix, the co-ingestion of dietary components and host variability could influence their absorption[83] (Figure 7).

I) Molecular structure

Polyphenols can exist in two forms: aglycones and glycosylates. The glycoside form can be as O-glycoside or as C-glycoside, being glucose the most common follow by galactose, rhamnose, xylose and arabinose. As previously stated, aglycones are absorbed and the glycosylated ones require the enzymatic process. However, some types of sugar, such as rahmnoses, are not hydrolysable is this process, requiring the microbiota enzymatic action for be absorbed[83]. Another molecular modification in polyphenols are the presence of acetylation and methylation. The first, usually found in nature, is the association of an organic acid or polyphenol with a flavonoid, such as catechin and epigallocatechin as an example. This modification does not interfere in the absorption of the polyphenols and is currently being studied as a tactic to improve biological activity, solubility and avoid oxidation[97]. Methylation process of flavonoids is a natural xenobiotic transformation by methyl transferases, especially promoted by gut microbiota. This process increase the ability to be transported across biological membranes, improving stability and reducing metabolic changes by decreasing the number of free hydroxyl groups that could suffer phase II conjugation[83,96,98]. Lastly, polyphenols that are polymerized, such as tannins, are not bioavailable and require gut microbiota metabolization to be released and absorbed[96].

II) Food matrix

Food matrix showed an important role in the bioaccessibility of phenolic compounds, since it is necessary to disrupt cell walls to improve digestibility and release them from

carbohydrates and proteins in which are trap to the food matrix. Phenolic compounds can be classified into two main classes by their solubility: extractable polyphenols and non-extractable polyphenols. When categorized as extractable, polyphenols have a low/intermediate molecular weight that can be extracted using water, methanol or hydroalcoholic solutions. On the other hand, the non-extractable polyphenols show a high molecular weight, since they are bound to fiber and protein, in which require a previous hydrolysis process before extraction to make them soluble and bioaccessible. Because of this need for hydrolysis, non-extractable polyphenols require an extra step to be bioavailable, in which they receive help from gut microbiota, which ferments these compounds releasing aglycones and other metabolites. Unfortunately, there is few literature about the health effects of these compounds on nutritional clinical trials[93,96].

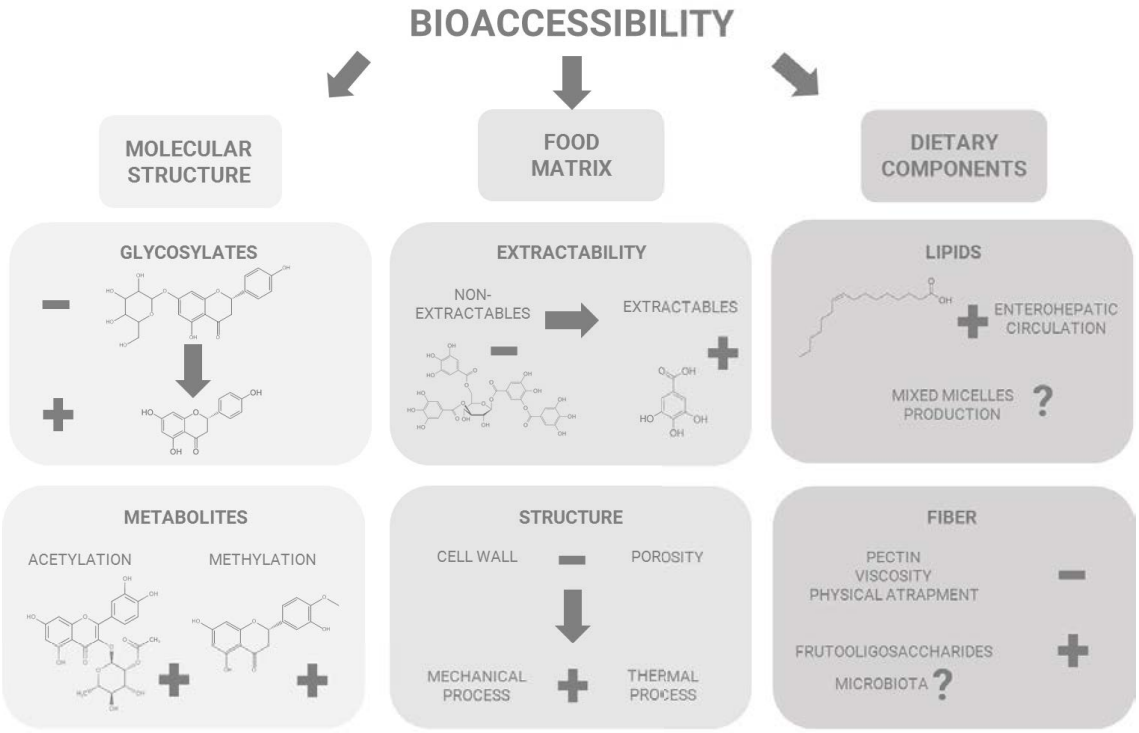

Figure 7. Factors that affect polyphenols bioaccessibility.

Another barrier associated with the solubility is in relation to the cell walls and porosity of the matrix. Mechanical processes are capable of disrupt cell walls and increase the bioaccessibility of polyphenols. In addition, they are able to increase the surface and porosity of the food matrix, in which allows bacteria and enzymes to enter and cleavage chemical bonds, liberating these compounds on the lumen. Therefore, foods in which matrices undergo a prior mechanical process and/or thermal process could increase polyphenols bioavailability[88,96].

III) Dietary components

The co-ingestion of dietary components such as fiber, lipids and proteins can change the digestive process, influencing the action of enzymes, delay gastric emptying, hindering

transporters performance and stimulate gut microbiota, which will impact the bioaccessibility of polyphenols.

Information on the effect of fiber consumption on the bioaccessibility of polyphenols is still scarce and studies have indicated that the presence of fiber impairs the absorption of these compounds[83,88]. Fiber promotes some processes in the gut that decrease the absorption of polyphenols as physical entrapment, viscosity increase, bulk increase and reduce transit time (depending of fiber type)[88]. Pectin consumption has been shown to be detrimental to the absorption of polyphenols, since pectin may form gels and meshes that impair enzyme and bacteria action due to surface decrease [88,99]. However, the presence of fermentable fibers, such as fructooligosaccharides, inulin and resistant starches, would help to stimulate gut microbiota on the production of enzymes and as consequence the liberation of more phenolic compounds[100,101]. With a greater microbial activity, the production of polyphenols metabolites could also be stimulated, in which the presence of native phenolic compounds are reduced. However, some studies with inulin have indicated that fiber enhancement decreases the bioaccessibiity of tomato polyphenols[102]. Dietary fiber also affects gastrointestinal transit time. Soluble and insoluble fibers increase the intestinal bulk, but only insoluble fiber reduces the transit time. With a shorter transit time it be expected less microbial activity, metabolites production and small intestinal uptake[88].

In relation to fat consumption, some studies were carried out in relation to aglycones and more apolar polyphenols[88]. The phenolic compounds appear to exert an effect on lipolysis during digestion, which decrease the lipid absorption and consequently increase the bulk. This increment on the bulk would result in a lower intestinal transit in which it could change the kinetics absorption of some polyphenols[88]. Recent studies have indicated that the use of olive oil in food preparation could increase the bioavailability of polyphenols, especially flavonoids. These compounds are also re-introduced into the gut by the enterohepatic cycle, which indicate that olive oil could increase the time that flavonoids remain in the body[103–105].

Proteins and carbohydrates are related to a lower absorption of polyphenols. Proteins are capable of forming complexes with polyphenols, which prevents their absorption. Polymerized polyphenols, such as tannins, are used for the precipitation of proteins. Carbohydrates, when hydrolyzed, release simple sugars that are a competitor for the uptake of polyphenols by SGLT-1 transporter, which hinders phenolic absorption. However, studies analyzing high simple sugar meals should be performed to confirm this hypothesis[88,96].

3.3.2.3 How cooking influence phenolic compounds on diet?

Cooking techniques impact the polyphenols content of food. The causes most pointed in the literature by the decrease in the content of polyphenols in cooked food are oxidation and leaching process[12,14,15]. The oxidation process considers many factors such as the cooking technique, degree of heating of the technique, pH of the medium and surface area exposes to

water and oxygen[12]. For leaching process, the polarity of the polyphenols is the decisive factor, in the majority polar by the presence of hydroxyls groups, in which it causes to be solubilized in aqueous medium and would remove them from the food matrix[12,14,21]. However, thermal cooking process are able to inactivate enzyme such as peroxidases, which use the polyphenols as a substrate for the enzymatic browning, avoiding the loss of these compounds[12,14]. In addition, thermal process softens vegetable tissues, in which it helps to break down cellular structure and cells, helping to release polyphenols for enzymatic action during gastrointestinal digestion[88].

I) Effect of cooking techniques on polyphenols

Another meta-analysis published by Murador and co-workers[12] evaluating the effect of different cooking techniques on total polyphenols content and antioxidant activity revealed that all cooking process diminish the content of phenolic compounds on food. Applying the same ratio cited on carotenoids section, the techniques steaming (R= 0.95) and blanching (R=0.94) were that most preserve polyphenols of food. Frying (R=0.72) decrease it content, but baking (R=0.51), boiling (R=0.62) and microwave (R=0.54) were the most impact the polyphenols content on food. It is important to note that the p-values for steaming and frying were not significant in the publication, indicating that the results for these techniques are not clear. It is worth remembering that even with these results performed by a metanalysis, depending on the food, processing conditions and extraction performed for analysis could modify the tendency showed for each technique. Moreover, this meta-analysis was performed for total phenolic compounds, in which it does not consider the variability of chemical structures of the polyphenols, in which it could change its behavior front each cooking technique.

Steaming process is characterized by the energy exchange of the water molecule in the vapor state, which is deposited on the surface of the food, promoting the softening of the vegetable tissue and their cooking[21]. This modification on the tissue helps to increase the bioaccessibility of polyphenols[12,14]. Moreover, this process maintains the temperature at approximately 100 °C, which is less drastic compared to other techniques and does not favor oxidation[21]. This process, different from boiling and blanching, the food does not come into contact of water, therefore it avoids the leaching process[12]. As describe by Murador and co-workers[12], the bleaching process does not decrease the polyphenols content compared to boiling, since it is characterized by a rapid passage through the boiling water, which decreases the contact time and avoid leaching[21]. In addition, in the end of this cooking process the food is immersing in water at low temperature that would help to stop oxidation reactions. For frying, baking and microwaving process, the degradation of polyphenols is carried out by the oxidation process[21]. Frying and baking use high temperatures on the food preparation that favors the appearance of oxidants species. In addition, the factor time is more relevant on baking than in the other cooking techniques, with reflects in a great decrease in the content of polyphenols[12].

The effect of different cooking techniques on tomatoes polyphenols content is divergent in the literature. Sahlin and co-workers[70] investigating the effect of boiling, baking and frying on

different tomato cultivars of tomato concluded that frying process was the factor that most affected the content of polyphenols, regardless of the tomato variety. The baking and boiling process showed similar polyphenols content reduction. Natella and co-workers[106] comparing microwave with traditional cooking methods found that microwave and pressure cooking increase total polyphenols content in 104% and 101%, respectively, but, boiling process showed a reduction of 95% on polyphenols content. Dolinsky and co-workers[107] quantifying total polyphenols after different cooking technique found only an increase on steaming process (25.50 mg GAE/100g) compared to raw tomato (24.81 mg GAE/100g) and a decrease for boiling (21.94 mg GAE/100g), microwave (18.18 mg GAE/100g)and pressure process (20.67 mg GAE/100g). However, no statistical differences was found between the processing techniques. Ramirez-Anaya and collaborators[32] studying Mediterranean vegetables prepared with olive oil in different cooking techniques found an increase in total polyphenols content in tomato for deep-fried and sautéed process compared to raw fruit and a reduction with boiling and boiling with water and olive oil. The increase in the polyphenols content was attributed to the presence of phenolic compound from olive oil, such as oleuropein, pinoresinol and hydroxytyrosol that was not present in tomato before cooking. An increase in typical tomato polyphenols such as chlorogenic acid and rutin were also detected.

In the production of homemade tomato sauce, Kamiloglu and co-workers[82] found a decrease on the content of total and some individual's phenolics with an increase only in the content of naringenin compared to raw tomato. Tomas and co-workers[34] compared industrial and home tomato sauce process and found a decrease on chlorogenic acid and rutin content compared with raw tomato, but an increase on naringenin, even higher than the industrial sauce. Searching for differences between tomato, tomato sauce and tomato sauce with 2% of olive oil, Martínez-Huélamo and co-workers[103] found that process increase the content of naringenin, rutin and also promote isomerization of dicaffeoylquinic acid.

Therefore, cooking process impacts the content of phenolic compounds on food. This process helps to break food matrix and release the polyphenols to make them more bioaccessible. However, the thermal and leaching process, depending on the technique, promote the oxidation and loss of polyphenols, respectively. Few studies in the literature have been found with a quantification of phenolic compounds individually, which makes it difficult to understand the differences in behavior between the different classes and structures in relation to the applied cooking technique. Some studies have attempted a more realistic approach of the culinary process by adding the lipid matrix. The presence of the oil matrix seems to help in the extraction of the phenolic compounds and consequently their absorption.

3.4 Cooking practice and the matrix effect on the health properties of Mediterranean diet: A study in tomato sauce

As described above, the cooking process and the presence of ingredients in a dish preparation may influence the bioaccessibility and bioavailability of bioactive compounds related to health-beneficial effects. Tomato and their derivate are one of the most consumed products in the MD, with a high daily consumption due to its affordable price and availability through the year. In addition, tomatoes are a great source of ascorbic acid, polyphenols and carotenoids, and epidemiological studies have related their consumption with a lower incidence of chronic-degenerative diseases, especially with processed tomato products.

A possible effect of cooking/food processing and the presence of ingredients may influence the food matrix and exert the beneficial effects of tomato products is described in the book chapter "*Cooking practice and the matrix effect on the health properties of Mediterranean diet: A study in tomato sauce*" (American Chemical Society, 2018, Advances in Plant Phenolics: From Chemistry to Human Health, eISBN: 9780841232952, DOI: 10.1021/bk-2018-1286.ch016).

Chapter 16

Cooking Practice and the Matrix Effect on the Health Properties of Mediterranean Diet: A Study in Tomato Sauce

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Culinary practices influence the content of bioactive compounds in food. Mediterranean diet and its key components have been inversely associated with cancer and cardiovascular diseases. However, cooking techniques and ingredients may modify the bioavailability of carotenoids and polyphenols, thus improving their absorption by the presence of an oil matrix with an optimum cooking process. The intake of cooked tomato-based products reduces the risk of chronic diseases compared to raw tomato. However, the bioavailability of each of these products should be taken into account to better understand the correlation between tomatoes and health.

Introduction

The Mediterranean diet (MD) is strongly recommended around the world as it has been associated with health benefits in several degenerative diseases such as cancer, neurodegenerative and cardiovascular diseases (*1*). This dietary pattern is characterized by a high consumption of fruits, vegetables, nuts, legumes, and non-refined cereals, moderate intake of fish, poultry and dairy products, and low consumption of sugar and red meat (*2*). This dietary pattern specifically involves the use of olive oil as the main source of fat and moderate alcohol intake (mainly in the form of red wine). Since the MD is mostly plant-based, its health claims have been associated with a high intake of phytochemicals. This correlation has been strengthened by the observational cohort PREDIMED (PREvención con DIeta MEDiterranea) study (*3*) which included 7447 individuals and was aimed at the primary prevention of cardiovascular diseases with the MD. The study concluded that an energy-unrestricted MD supplemented with extra-virgin olive oil or nuts can reduce the risk of cardiovascular events compared to a low fat diet in a high risk population (*3*).

Tomatoes and derivatives are one of the products most consumed in the MD, being rich in bioactive compounds such as ascorbic acid, polyphenols and carotenoids (*4*). Home cooked preparation of tomatoes is very prevalent in Mediterranean countries presenting a higher average daily consumption compared to other fruits and vegetables because of their accessible price and availability year round (*4*, *5*). Epidemiological studies have shown that the consumption of raw tomato and tomato products is associated with a low incidence of chronic-degenerative diseases, this correlation being greater for processed tomato-based products (*6*). In the MD vegetables and legumes are typically consumed after cooking which influences the intake of phytochemicals in the diet. Food preparation and cooking can positively influence the content of phytochemicals by improving bioavailability or may have negative effect due to thermal degradation, oxidation, nutrient loss or the formation of toxic products (*1*). Adherence to the MD has become popular in non-Mediterranean countries, but the cooking practices and products of Mediterranean countries may differ greatly from one region to another, and can have an important impact on the beneficial effects of this dietary pattern (*2*). This chapter aims to show how cooking and food preparation influence phytochemicals, and consequently, the effects on health benefits of the MD using the tomato sauce, *sofrito* and other tomato-based products as a basis for discussion.

Home Cooking and Phytochemicals

Phytochemicals are secondary plant metabolites with positive health effects. Among these compounds, carotenoids and polyphenols are of note. In the last years, polyphenols have received great attention by the health claims associated with their consumption. Since these compounds are susceptible to degradation and oxidation by heat, there is still debate as to whether or not the consumption of raw or processed fruits and vegetables is beneficial (*7*). Therefore, increased consumer information is needed on the health-related value of raw and processed foods as

well as research into the best methods for preserving the nutritional value under home cooking conditions (*7*, *8*).

The cooking process is essentially based on thermal processes that can induce the loss of bioactive compounds by oxidation, degradation or the formation of pro-oxidant compounds at high temperatures. However, there may also be beneficial aspects such as improving the bioavailability of several compounds by chemical structural changes and/or the release of phytochemicals from the food matrix. Carotenoids are sensitive to thermal processes, oxidation being the major cause of carotenoid loss, influenced by the presence of oxygen, heat and light. However, the cooking process can disrupt the carotenoid-protein complex and modify cellular matrix integrity, soften vegetable tissue, increase carotenoid extraction from food and improve bioaccessibility. Moreover, heating can promote carotenoid isomerization from the *E*-form to *Z*-form, which are known to be more bioavailable (*9*, *10*). Similar to carotenoids, polyphenols are also susceptible to lose by oxidation and leaching by water in cooking processes, but the application of high temperatures may lead to an inactivation of pro-antioxidant enzymes that are responsible for degrading phenolic compounds and reducing antioxidant activity (*11*).

The thermal cooking process presents different factors that can influence bioactive compound content such as the cooking technique, temperature, food matrix, leaching by the cooking medium (oil or water), pH and surface exposure to oxygen (*11*). The application of heat in the home cooking of food-stuffs includes different types of techniques, such as boiling, frying, steaming, baking, stewing and roasting, as well as the current new technologies such as microwave and steam ovens (*7*). These cooking techniques present different mechanisms of action, each of which has unique characteristics that can increase, decrease or change the bioactive compounds of food, completely transforming their potential benefits. Using a meta-analytical approach, Murador and co-workers (*11*) studied how cooking techniques influence the phenolic compound content and their antioxidant activity and found that the food matrix and leaching are the main factors that decrease the phenolic content in food. Polyphenols are water soluble compounds, and therefore cooking techniques such as boiling, in which food is in contact with water, drastically decrease their content. Frying and steaming have shown to be the best techniques for preserving phenolic compounds in food (*11*). In addition, the formation of Maillard reaction products with antioxidant activity and the synergism between other compounds present in the food matrix influence antioxidant activity after cooking. In another study on the effect of cooking techniques on carotenoids, Murador and co-workers (*9*) showed that frying decreases the content of carotenoids, while stewing most increased the carotenoid content. Frying decrease the carotenoid content by leaching because of their lipophilicity and stewing can concentrated carotenoid content by a loss of water. Here it is important to note that some studies are difficult to compare due to discrepancies in the cooking time, temperature and pH conditions employed and in the way of the study results are expressed (dry matter vs. raw vegetables).

There is a lack in the literature regarding the effect of different culinary techniques on the content of bioactive compounds in tomato, especially on the the content of individual polyphenols and carotenoids. Ramírez-Anaya and

co-wokers (*12*) studied polyphenols and antioxidant capacity in Mediterranean vegetables prepared with different domestic cooking techniques and reported that deep fried and sautéed techniques concentrated the total and individual polyphenol content in tomato by loss of water , while boiling decreased their content, compared to the raw vegetable. Indeed, the highest increase in polyphenol content was observed in deep fried compared to sautéed foods, probably because of the oxidation of phenolic compounds with the sautéed technique due to a higher exposure to air than when the food is totally submerged in oil. Gahler and co-workers (*4*) studied the effect of tomato products during cooking processing and reported that total phenolic compounds do not significantly change during processing except in baking which has been reported to increase the total phenolic compounds content of tomatoes. However, this value is highly dependent on the time and temperature used. On the other hand, Sahlin and co-workers (*13*) described a decrease in the total phenolic compounds, total lycopene and ascorbic acid in tomatoes after boiling, baking and frying processes.

Bioavailability of Bioactive Compounds of Tomato by Cooking and the Matrix Effect

In the last several years, epidemiological correlations between tomato consumption and the prevention of certain diseases have been demonstrated, especially in prostate cancer (*14*). However, assessment of tomato consumption in epidemiological studies is difficult due to a lack of nutritional information about the bioavailability of each type of tomato-based food which is affected by food processing and the matrix effect (*6*). Carotenoids are the main class of compounds related to the beneficial effect of tomato consumption, and their bioavailability is affected by different factors such as the food matrix, processing and cooking techniques as well as interaction with other dietary components such as fiber, lipids and other compounds (*10*, *15*, *16*). The structure of carotenoids plays an important role in their bioavailability, with 50% of plasma carotenoids being in the *Z*-form, whereas in nature they are predominantly found in the *E*-form. The major sources of carotenoids in the *Z*-form are from processed foods (*17*). Polyphenols are directly linked to health claims, but there is scarce information in the literature about the impact of the cooking process and the matrix effect on polyphenols content and their bioavailability in tomatoes and tomato products (*16*, *18*).

The effect of thermal treatments on the phenolic compounds and carotenoids of tomato sauce have been well studied in industrial processes, however, the preparation of tomato sauce and other homemade products has not been investigated. Indeed, the homemade process is characterized by the addition of ingredients that can influence the content of bioactive compounds and their bioavailability by a synergic effect. Searching for a combined effect of home cooking and ingredient addition, Vallverdú-Queralt and co-workers (*19*, *20*) studied tomato sauce processing with different cooking times of between 15 to 60 minutes and analyzed the influence of the addition of 5% and 10% of the extra virgin olive oil. They observed a decrease in phenolic content with

an increase in cooking time. However, in the first 30 minutes, there was an increase in polyphenol content which was attributed to the hydrolysis of flavonoid glycosides and a better extraction of polyphenols from the cell wall. On the other hand, carotenoid content increased with longer cooking time, especially the lycopene-*Z*-isomers, which are more bioavailable and possess more antioxidant activity compared to the *E*-form. The presence of an oil matrix such as olive oil improved the bioaccessibility of both carotenoids and polyphenols in tomato sauce. The addition of olive oil is of interest for improving the absorption of carotenoids and polyphenols, and consequently, enhancing their beneficial effects.

Sofrito, a typical tomato-based Mediterranean sauce, is characterized by containing olive oil, onion and garlic, and homemade sofrito is usually prepared by a stewing process (*21*). Vallverdu-Queralt and co-workers (*22*) characterized commercial Mediterranean *sofritos* and described that the presence and quality of the ingredients used to make the sauce influence the phenolic and carotenoid profile, with the presence of onion and extra virgin olive oil being of particular interest. In the search for a synergism effect between these ingredients in homemade *sofrito*, Alvarenga and co-workers (*21*) described that a long cooking time of extra virgin olive oil and onion is important for improving the isomerization of the *Z*-forms of lycopene from tomato, with onion indirectly improving the bioavailability of lycopene.

The increase in the bioavailability of tomato carotenoids after thermal treatment was determined by Unlu and co-wokers (*17*) who reported that heat-induced lycopene *Z*-isomer-rich tomato sauces are more easily absorbed by humans compared to *E*-form-rich tomato sauces. The presence of a lipid matrix enhances the content of lycopene in plasma (*23*) and also increases the maximum concentration (C_{max}) and area under the curve ($AUC_{0\text{-}24h}$) of all-*E*-lycopene and its isomers compared to samples without an oil matrix (*24*). Arranz and co-wokers (*24*) described that the bioavailability of carotenoids from tomato juice depends not only on the presence of an oil matrix but also on the geometrical isomerization and chemical structure, since the absorption of β-carotene was not influenced by the presence of olive oil in tomato juice but had a remarkable effect on lycopene.

A few studies have evaluated the bioavailability of phenolic compounds in tomato (*16*, *25*), their products and the effect of the addition of oil (*18*, *26*, *27*). Tulipani and co-workers (*26*) evaluated the effect of processing and the addition of oil on tomato sauce polyphenol bioavailability and found that thermal and mechanical processes increase the content of phenolic compounds, especially caffeoylquinic acid, rutin and naringenin, compared to raw tomato. After a single dose of tomato sauce, the phenolic compounds reached the maximum rate of excretion within 4 h after the intervention. However, the presence of an oil matrix in the sauce may stimulate re-absorption by enterohepatic circulation, increasing the plasma half-time of polyphenols, which is of particular interest for achieving better bioactivity. In a large number of volunteers, Martinez-Huélamo and co-workers (*27*) corroborated the results obtained by Tulipani and co-workers, demonstrating that the mechanical and thermal treatments carried out during tomato processing help to liberate phenolic compounds from the food matrix making them more bioavailable. In addition, they described that a presence of an oil matrix does not significantly change the absorption of polyphenols, with

the exception of naringenin and caffeoylquinic acid. Naringenin, caffeic acid, ferulic acid and chlorogenic acid are absorbed intact and undergo extensive biotransformation of phase II metabolism. Naringenin glucuronide metabolites demonstrate that the bioavailability of processed tomatoes is higher than in raw tomatoes. In a randomized feeding trial including 40 volunteers eating tomato, tomato sauce and tomato sauce with refined olive oil, Martinez-Huélamo and co-workers (*18*) identified 10 phenolic compounds in plasma and 93 in urine. The presence of the oil matrix improved the absorption of naringenin and quercetin. Moreover, a double peak in the plasma pharmacokinetics showed that naringenin glucuronide, quercetin and ferulic acid glucuronide underwent re-absorption by the enterohepatic circulation due to the addition of a fatty matrix. The cooking process and ingredients that influence the food matrix have a direct impact on the bioavailability of bioactive compounds, carotenoids and polyphenols, and consequently, favor their bioactivity of cells and tissues.

Cooking, Ingredients, and Health Effects

The intake of tomato and tomato-based products has been correlated with a low incidence of cancer (*14*, *28–30*), inflammation (*31*) and cardiovascular diseases (*30*, *32*, *33*). A large number of epidemiological studies have established the relationship between tomato products and the risk of cancer, especially prostate cancer (*7*). A Greek case-control study in 320 cases of prostate cancer and 246 controls reported that the intake of raw and cooked tomato reduced the risk of prostate cancer. The greatest reduction in risk was observed with cooked compared to raw tomatoes [odds ratio (OR) for lowest *versus* highest tertile intake 1.91, 95% confidence interval (CI): 1.20-3.04 for cooked tomatoes and 1.55, 95% CI: 1.00-2.52 for raw tomato], with cooked tomatoes being associated with a higher consumption of olive oil, the latter being the main source of lipids in this population (*28*). Giovannuci and co-wokers (*14*, *29*) performed a prospective cohort study of subjects followed for 12 years and concluded that the consumption of one or more portions of tomato sauce per week was associated with a statistically significant reduction in the incidence of prostate cancer (relative risk ratio: 0.80, 95% CI; 0.70-0.91). Higher correlations were observed with tomato products than with raw tomatoes, especially tomato sauce, suggesting that heat processes and the presence of an oil matrix improve the bioavailability of lycopene from tomatoes, being decisive for bioactivity in prostate cancer.

Recent clinical trials have studied the capacity of tomato products to reduce the risk of cardiovascular diseases and inflammation and have focused on the consumption of lycopene as a bioactive compound. Burton-Freeman and co-workers (*32*) compared the ingestion of high-fat meals with or without tomato products, and observed that the presence of tomato attenuates low density lipoprotein-oxidation (LDL-ox) and increases serum concentrations of interleukin-6, suggesting a protective effect against cardiovascular risk. Colmán-Martinez and co-wokers (*31*) evaluated the effect of tomato juice on inflammatory biomarkers in a 4-week dose-response clinical trial with a high cardiovascular risk population. A positive dose-response correlation with

E-lycopene plasma concentrations and the expression of ICAM-1 and VCAM-1 adhesion molecules was found, suggesting that tomato consumption may reduce the atherosclerotic plaque process. Valderas-Martinez and co-workers (*33*) carried out an acute clinical trial comparing the effect of the consumption of raw tomato, tomato sauce and tomato sauce with refined olive oil on inflammatory and atherosclerosis biomarkers and concluded that any tomato form was able to decrease total cholesterol, triglycerides and inflammatory biomarkers and increase high density lipoproteins (HDL) and antiinflammatory interleukin-10. The inflammatory response is modulated by bioactive compunds, such as carotenoids and polifenols, by gene regulation of NF-κB pathway (*33*, *34*). Moreover, biomarker modulation in the presence of an oil matrix was greater than after raw tomato and tomato sauce interventions, recommending cooked tomato intake enriched with oil. In a study on the possible correlation of lycopene intake, tomato products and the incidence of cardiovascular diseases, Jacques and co-workers (*35*) reported an inverse association between lycopene and the risk of cardiovascular diseases. However, almost all observational and human trials are performed with tomato-based products making it difficult to separate the potential contribution of lycopene or a synergism effect with other bioactive compounds on cardiovascular health.

Concluding Remarks

The culinary processes and cultural practices of the MD influence the content of bioactive compounds in tomato and tomato-based products. Studies on the different cooking techniques and the best way to use them should be carried out in order to encourage consumers to improve the nutritional value of homemade food. Exploring ingredients and the possible synergism between other bioactive compounds is necessary for understanding how to improve health with changing cooking techniques.

The matrix effect is a key factor that influences the bioavailability of bioactive tomato compounds. The presence of an oil matrix improves not only the absorption of lipophilic compounds but also some polar compounds such as polyphenols. More studies are necessary to determine how the cooking process and the addition of ingredients influence absorption, and consequently, their health effects.

Tomato and tomato-based products play an important role in reducing the risk of cancer and cardiovascular diseases. However, there is a great deal of work yet to be done. The presence of lipids in foods can be a confounding factor that influences the absorption of bioactive compounds from tomato and should be taken into account in relation to possible associations. Epidemiological studies have shown lycopene intake to be the principal factor of health claims, but other compounds such as polyphenols need further study, focusing on a possible synergism effect among them.

References

1. Hoffman, R.; Gerber, M. Food Processing and the Mediterranean Diet. *Nutrients* **2015**, *7* (9), 7925–7964.
2. Hoffman, R.; Gerber, M. Evaluating and Adapting the Mediterranean Diet for Non-Mediterranean Populations: A Critical Appraisal. *Nutr. Rev.* **2013**, *71* (9), 573–584.
3. Estruch, R.; Ros, E.; Salas-Salvadó, J.; Covas, M.-I.; Corella, D.; Arós, F.; Gómez-Gracia, E.; Ruiz-Gutiérrez, V.; Fiol, M.; Lapetra, J.; et al. Primary Prevention of Cardiovascular Disease with a Mediterranean Diet. *N. Engl. J. Med.* **2013**, *368* (14), 1279–1290.
4. Gahler, S.; Otto, K.; Böhm, V. Alterations of Vitamin C, Total Phenolics, and Antioxidant Capacity as Affected by Processing Tomatoes to Different Products. *J. Agric. Food Chem.* **2003**, *51* (27), 7962–7968.
5. Gómez-Romero, M.; Arráez-Román, D.; Segura-Carretero, A.; Fernández-Gutiérrez, A. Analytical Determination of Antioxidants in Tomato: Typical Components of the Mediterranean Diet. *J. Sep. Sci.* **2007**, *30* (4), 452–461.
6. Trumbo, P. R. Promises and Perils of Lycopene / Tomato Supplementation and Cancer Prevention Are There Adverse Effects of Lycopene Exposure? 1. *J. Nutr.* **2005**, *135* (5), 2060–2061.
7. van Boekel, M.; Fogliano, V.; Pellegrini, N.; Stanton, C.; Scholz, G.; Lalljie, S.; Somoza, V.; Knorr, D.; Jasti, P. R.; Eisenbrand, G. A Review on the Beneficial Aspects of Food Processing. *Mol. Nutr. Food Res.* **2010**, *54* (9), 1215–1247.
8. Taticchi, A.; Esposto, S.; Urbani, S.; Veneziani, G.; Selvaggini, R.; Sordini, B.; Servili, M. Effect of an Olive Phenolic Extract Added to the Oily Phase of a Tomato Sauce, on the Preservation of Phenols and Carotenoids during Domestic Cooking. *LWT - Food Sci. Technol.* **2017**, *84*, 572–578.
9. Murador, D. C.; da Cunha, D. T.; de Rosso, V. V. Effects of Cooking Techniques on Vegetable Pigments: A Meta-Analytic Approach to Carotenoid and Anthocyanin Levels. *Food Res. Int.* **2014**, *65* (PB), 177–183.
10. Cervantes-Paz, B.; Victoria-Campos, C. I.; Ornelas-Paz, J. de J. *Absorption of Carotenoids and Mechanisms Involved in Their Health-Related Properties*; 2016; Vol. 79, pp 415–454.
11. Murador, D.; Braga, A. R.; Da Cunha, D.; De Rosso, V. Alterations in Phenolic Compound Levels and Antioxidant Activity in Response to Cooking Technique Effects: A Meta-Analytic Investigation. *Crit. Rev. Food Sci. Nutr.* **2018**, *58* (2), 169–177.
12. Ramírez-Anaya, J. D. P.; Samaniego-Sánchez, C.; Castañeda-Saucedo, M. C.; Villalón-Mir, M.; De La Serrana, H. L. G. Phenols and the Antioxidant Capacity of Mediterranean Vegetables Prepared with Extra Virgin Olive Oil Using Different Domestic Cooking Techniques. *Food Chem.* **2015**, *188*, 430–438.
13. Sahlin, E.; Savage, G. P.; Lister, C. E. Investigation of the Antioxidant Properties of Tomatoes after Processing. *J. Food Compos. Anal.* **2004**, *17* (5), 635–647.

14. Giovannucci, E. A Prospective Study of Tomato Products, Lycopene, and Prostate Cancer Risk. *J. Natl. Cancer Inst.* **2002**, *94* (5), 391–398.
15. van het Hof, K. H.; de Boer, B. C.; Tijburg, L. B.; Lucius, B. R.; Zijp, I.; West, C. E.; Hautvast, J. G.; Weststrate, J. A. Carotenoid Bioavailability in Humans from Tomatoes Processed in Different Ways Determined from the Carotenoid Response in the Triglyceride-Rich Lipoprotein Fraction of Plasma after a Single Consumption and in Plasma after Four Days of Consumption. *J. Nutr.* **2000**, *130* (5), 1189–1196.
16. Bugianesi, R.; Salucci, M.; Leonardi, C.; Ferracane, R.; Catasta, G.; Azzini, E.; Maiani, G. Effect of Domestic Cooking on Human Bioavailability of Naringenin, Chlorogenic Acid, Lycopene and β-Carotene in Cherry Tomatoes. *Eur. J. Nutr.* **2004**, *43* (6), 360–366.
17. Unlu, N. Z.; Bohn, T.; Francis, D. M.; Nagaraja, H. N.; Clinton, S. K.; Schwartz, S. J. Lycopene from Heat-Induced Cis-Isomer-Rich Tomato Sauce Is More Bioavailable than from All-Trans-Rich Tomato Sauce in Human Subjects. *Br. J. Nutr.* **2007**, *98* (1), 140–146.
18. Martínez-Huélamo, M.; Vallverdú-Queralt, A.; Di Lecce, G.; Valderas-Martínez, P.; Tulipani, S.; Jáuregui, O.; Escribano-Ferrer, E.; Estruch, R.; Illan, M.; Lamuela-Raventós, R. M. Bioavailability of Tomato Polyphenols Is Enhanced by Processing and Fat Addition: Evidence from a Randomized Feeding Trial. *Mol. Nutr. Food Res.* **2016**, *60* (7), 1578–1589.
19. Vallverdú-Queralt, A.; Regueiro, J.; Rinaldi De Alvarenga, J. F.; Torrado, X.; Lamuela-Raventos, R. M. Home Cooking and Phenolics: Effect of Thermal Treatment and Addition of Extra Virgin Olive Oil on the Phenolic Profile of Tomato Sauces. *J. Agric. Food Chem.* **2014**, *62* (14), 3314–3320.
20. Vallverdú-Queralt, A.; Regueiro, J.; de Alvarenga, J. F. R.; Torrado, X.; Lamuela-Raventos, R. M. Carotenoid Profile of Tomato Sauces: Effect of Cooking Time and Content of Extra Virgin Olive Oil. *Int. J. Mol. Sci.* **2015**, *16* (5), 9588–9599.
21. de Alvarenga, J. F. R.; Tran, C.; Hurtado-Barroso, S.; Martinez-Huélamo, M.; Illan, M.; Lamuela-Raventos, R. M. Home Cooking and Ingredient Synergism Improve Lycopene Isomer Production in Sofrito. *Food Res. Int.* **2017**, 1–11.
22. Vallverdú-Queralt, A.; de Alvarenga, J. F. R.; Estruch, R.; Lamuela-Raventos, R. M. Bioactive Compounds Present in the Mediterranean Sofrito. *Food Chem.* **2013**, *141* (4), 3366–3372.
23. Fielding, J. M.; Rowley, K. G.; Cooper, P.; O'Dea, K. Increases in Plasma Lycopene Concentration after Consumption of Tomatoes Cooked with Olive Oil. *Asia Pac. J. Clin. Nutr.* **2005**, *14* (2), 131–136.
24. Arranz, S.; Martínez-Huélamo, M.; Vallverdu-Queralt, A.; Valderas-Martinez, P.; Illán, M.; Sacanella, E.; Escribano, E.; Estruch, R.; Lamuela-Raventos, R. M. A. Influence of Olive Oil on Carotenoid Absorption from Tomato Juice and Effects on Postprandial Lipemia. *Food Chem.* **2015**, *168*, 203–210.
25. Bugianesi, R.; Catasta, G.; Spigno, P.; D'Uva, A.; Maiani, G. Naringenin from Cooked Tomato Paste Is Bioavailable in Men. *J. Nutr.* **2002**, *132* (11), 3349–3352.

26. Tulipani, S.; Martinez Huelamo, M.; Rotches Ribalta, M.; Estruch, R.; Ferrer, E. E.; Andres-Lacueva, C.; Illan, M.; Lamuela-Raventós, R. M. Oil Matrix Effects on Plasma Exposure and Urinary Excretion of Phenolic Compounds from Tomato Sauces: Evidence from a Human Pilot Study. *Food Chem.* **2012**, *130* (3), 581–590.
27. Martínez-Huélamo, M.; Tulipani, S.; Estruch, R.; Escribano, E.; Illán, M.; Corella, D.; Lamuela-Raventós, R. M. The Tomato Sauce Making Process Affects the Bioaccessibility and Bioavailability of Tomato Phenolics: A Pharmacokinetic Study. *Food Chem.* **2015**, *173*, 864–872.
28. Bosetti, C.; Tzonou, A.; Lagiou, P.; Negri, E.; Trichopoulos, D.; Hsieh, C. C. Fraction of Prostate Cancer Incidence Attributed to Diet in Athens, Greece. *Eur. J. Cancer Prev.* **2000** *9*, 119−123.
29. Giovannucci, E.; Ascherio, A.; Rimm, E. B.; Stampfer, M. J.; Colditz, G. A.; Willett, W. C. Intake of Carotenoids and Retinol in Relation to Risk of Prostate Cancer. *J. Natl. Cancer Inst.* **1995**, *87* (23), 1767–1776.
30. Perveen, R.; Suleria, H. A. R.; Anjum, F. M.; Butt, M. S.; Pasha, I.; Ahmad, S. Tomato (Solanum Lycopersicum) Carotenoids and Lycopenes Chemistry; Metabolism, Absorption, Nutrition, and Allied Health Claims—A Comprehensive Review. *Crit. Rev. Food Sci. Nutr.* **2015**, *55* (7), 919–929.
31. Colmán-Martínez, M.; Martínez-Huélamo, M.; Valderas-Martínez, P.; Arranz-Martínez, S.; Almanza-Aguilera, E.; Corella, D.; Estruch, R.; Lamuela-Raventós, R. M. Trans-Lycopene from Tomato Juice Attenuates Inflammatory Biomarkers in Human Plasma Samples: An Intervention Trial. *Mol. Nutr. Food Res.* **2017**, *201600993*, 1–9.
32. Burton-Freeman, B.; Talbot, J.; Park, E.; Krishnankutty, S.; Edirisinghe, I. Protective Activity of Processed Tomato Products on Postprandial Oxidation and Inflammation: A Clinical Trial in Healthy Weight Men and Women. *Mol. Nutr. Food Res.* **2012**, *56* (4), 622–631.
33. Valderas-Martinez, P.; Chiva-Blanch, G.; Casas, R.; Arranz, S.; Martínez-Huélamo, M.; Urpi-Sarda, M.; Torrado, X.; Corella, D.; Lamuela-Raventós, R. M.; Estruch, R. Tomato Sauce Enriched with Olive Oil Exerts Greater Effects on Cardiovascular Disease Risk Factors than Raw Tomato and Tomato Sauce: A Randomized Trial. *Nutrients* **2016**, *8* (3), 1–14.
34. Libby, P. Inflammation and Atherosclerosis. *Circulation* **2002**, *105* (9), 1135–1143.
35. Jacques, P. F.; Lyass, A.; Massaro, J. M.; Vasan, R. S.; D'Agostino, R. B. Relationship of Lycopene Intake and Consumption of Tomato Products to Incident CVD. *Br. J. Nutr.* **2013**, *110* (3), 545–551.

RESULTS

4. RESULTS

The results are collected in eight manuscripts, five of them have been published in journals included in the Science Citation Index. All articles are published in journals ranked in the first quartile, except for one article that is published in *International Journal of Molecular Science* and *Molecules* which belongs to the second quartile. The remaining two papers have been submitted and are under revision to reference journals.

A brief summary containing objective of the study, methodology, results and conclusion is included prior each publication.

This section is further organized in three parts:

I) Home cooking and bioactive compounds (publication 1 and 2)

II) *Sofrito* cooking process and the ingredients impact bioactive compounds (publication 3, 4, 5, 6 and 7).

III) Health effect of tomato *sofrito* intake (publication 8).

4.1 Section I: Home cooking and bioactive compounds

4.1.1 Publication 1. Carotenoid profile of tomato sauces: effect of cooking time and content of extra virgin olive oil

Anna Vallverdú-Queralt, Jorge Regueiro, **José Fernando Rinaldi de Alvarenga**, Xavier Torrado, Rosa Maria Lamuela-Raventós. *International Journal of Molecular Sciences*, 2015, 16, 9588-9599

The consumption of carotenoid-rich vegetables is associated with a reduction of the risk of several chronical diseases. Tomato products has been call attention by *Z*-isomers formation during thermal process and by the addition of an oil matrix, which are related with better bioavailability. The aim of this study was to verify the effect of thermal treatment and the addition of EVOO on the carotenoid content of homemade tomato sauces. Tomato sauce were prepared following a regular homemade process. A 4x2 mixed level factorial design was applied, which the 8 different formulation were processed in triplicate, involving a total of 24 randomized runs. The factor EVOO had 2 levels (5% and 10%) and the factor time of process had 4 levels (15, 30 45 and 60 min). A targeted quantification of carotenoids were performed using a HPLC-DAD and identification was confirmed by HPLC-ESI-QqQ-MS/MS. The antioxidant capacity were measured by $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assay. The antioxidant capacity increased with a lower percentage of EVOO and a higher time of cooking for both methods. The content all-E-α-carotene and all-E-β-carotene increased during cooking process with a maximum levels in 60 min, with an increase of 25% and 40%, respectively. All-*E*-lycopene content increased during the first 30 min and 45 min of cooking, for 10% and 5% of EVOO, respectively, and after that a decreased was observed. Different from all-*E*-lycopene, the isomers 13, 9 and 5-*Z*-lycopene slightly increased with longer cooking time by 31%, 15% and 18%, respectively, indicating an isomerization process of all-*E*-lycopene by the cooking process. Tomato sauces prepared with 5% of EVOO showed a higher content of total carotenoids and individual carotenoids compared to 10% of EVOO. However, no statistically differences were found between samples containing 5% or 10% of EVOO at the same time of cooking. As a general pattern, longer time of cooking increased the content of carotenoids in tomato sauce and improve *Z*-lycopene isomers production that could contribute to improve bioavailability of carotenoids.

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Article

Carotenoid Profile of Tomato Sauces: Effect of Cooking Time and Content of Extra Virgin Olive Oil

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Abstract: The consumption of carotenoid-rich vegetables such as tomatoes and tomato sauces is associated with reduced risk of several chronic diseases. The predominant carotenoids in tomato products are in the (*all-E*) configuration, but (*Z*) isomers can be formed during thermal processing. The effect of cooking time (15, 30, 45 and 60 min) and the addition of extra virgin olive oil (5% and 10%) on the carotenoid extractability of tomato sauces was monitored using liquid chromatography-tandem mass spectrometry (LC-ESI-MS/MS) and LC-ultraviolet detection (LC-UV). The thermal treatment and the addition of extra virgin olive oil increased the levels of antioxidant activity, total carotenoids, *Z*-lycopene isomers, α-carotene and β-carotene. These results are of particular nutritional benefit since higher lycopene intake has been associated with a reduced risk of lethal prostate and a reduction

of prostate-specific antigen (PSA) levels. Moreover, β-carotene has been reported to suppress the up-regulation of heme oxygenase-1 gene expression in a dose dependent manner and to suppress UVA-induced *HO-1* gene expression in cultured FEK4.

Keywords: LC-ESI-MS/MS; LC-UV; C30 column; β-carotene; α-carotene; lycopene; antioxidant capacity

1. Introduction

The traditional Mediterranean diet is characterized by a high intake of olive oil, fruit, nuts, vegetables, and cereals [1,2]. Tomato and tomato sauces are typical components of the Mediterranean diet and are of great interest because of their high content of bioactive compounds, including carotenoids, polyphenols, and vitamin C. Regular consumption of tomato products has been related to a decrease in the incidence of chronic degenerative diseases [3,4]. Some carotenoids, such as β-carotene and (*all-E*)-α-carotene, may exhibit provitamin A activity [5]. Studies have demonstrated that consumption of lycopene decreases the risk of degenerative diseases, for example, certain kinds of cancer and cardiovascular diseases [6]. Another major characteristic of the Mediterranean diet is a high consumption of olive oil, which is associated with numerous health benefits [7,8]. Oil added to tomato sauce has been reported to improve the accessibility and extractability of bioactive compounds in tomato [9]. Gärtner *et al.* [10] reported that with a constant content of fat and other ingredients, lycopene bioavailability from tomato paste was significantly higher than from fresh tomatoes [10].

Thermal treatments are the main cause of the depletion of natural antioxidants in food. Browning and oxidation reactions are responsible for the degradation of naturally occurring antioxidants during the processing of tomato products [11,12]. The highly unsaturated nature of carotenoids makes them susceptible to isomerization, oxidation and breakdown of the carotenoid molecule during thermal processes, especially under severe processing conditions. The oxidation products formed are a mixture of epoxides, apocarotenals and hydroxy compounds [13]. During thermal treatments, (*Z*)-isomers are formed. Studies have proven that >50% of the carotenoids present in the human body are in the (*Z*) isomeric configuration, suggesting that this is the most bioavailable form [14].

The effects of industrial processing on the lycopene content of tomatoes have been extensively studied. However, little is known about the impact of cooking time or added extra virgin olive when tomato products are prepared at home. Thus, the aim of this study was to examine how the duration of cooking (15, 30, 45 and 60 min) and the addition of extra virgin olive oil (5% and 10%) affected the carotenoid content of tomato sauces.

2. Results and Discussion

While some studies have reported a loss of lycopene in tomato-based foods undergoing thermal processing (bleaching, retorting and freezing) [15], others have shown that processing tomatoes may increase the levels of some bound antioxidants [9,16,17]. As carotenoids are widely present in food, the assessment of their stability in food systems is of major importance.

Analysis of the tomato sauces during the home-cooking processes showed that the antioxidant level measured by the $ABTS^+$ assay increased from 106.68 mmol/g DW at 15 min to 124.57 mmol TE/g DW at 60 min or from 96.91 mmol TE/g DW at 15 min to 119.08 mmol TE/g DW at 60 min in sauces containing 5% or 10% extra virgin olive oil, respectively (Figure 1). The same pattern of antioxidant capacity was observed in the DPPH assay. Food processing may improve carotenoid bioavailability by breaking down cell walls, which weakens the bonding forces between carotenoids and the plant tissue matrix. Piga *et al.* [18] reported an increase in the DPPH content of mandarin juices over time, and attributed this increase in antioxidant capacity to the formation of Maillard's reaction products [18]. The food matrix in which the bioactive compounds are contained also plays a crucial role in determining their accessibility and extractability from food [9].

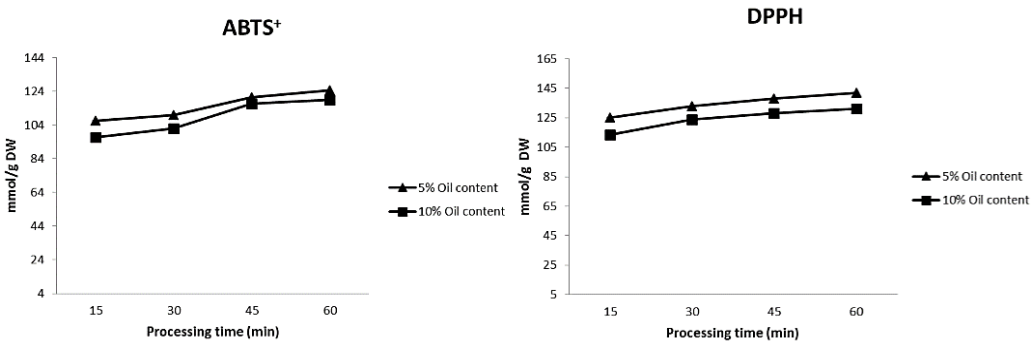

Figure 1. Changes in antioxidant capacity during processing time.

Figures 2 and 3 show the evolution of the carotenoid content in the studied tomato sauces during the cooking process. The concentration after 15 min of processing of (*all-E*)-lycopene was 573.76 ± 45.83 mg/kg·DW, whereas those of (*all-E*)-α-carotene and (*all-E*)-β-carotene were 4.99 ± 0.13 and 4998.5 ± 268.95 mg/kg DW, respectively (Figures 2 and 3). Between 15 and 30 min of cooking, (*all-E*)-lycopene increased from 573.76 to 659.27 mg/kg·DW in tomato sauces containing 5% olive oil and from 503.37 to 643.46 mg/kg DW in those with 10% olive oil. Between 30 and 60 min, the (*all-E*)-lycopene content decreased from 659.27 to 597.11 mg/kg DW in tomato sauces containing 5% olive oil, with a similar pattern being observed in sauces containing 10% olive oil. In contrast, (*all-E*)-α-carotene and (*all-E*)-β-carotene increased over the cooking period (15 to 60 min) from 4.99 to 6.50 mg/kg DW and from 4998.5 to 8185.12 mg/kg DW, respectively. No statistically significant differences were found between samples containing 5% or 10% olive oil at the same time of processing. Gärtner *et al.* [10] reported that tomato juice cooked in oil medium resulted in a two- to three-fold increase in carotenoid serum concentrations one day after ingestion, while no rise was found after an equivalent consumption of unprocessed tomato juice [10].

Our results are in line with those reported by Lin and Chen [19], who attributed the lower stability of (*all-E*)-lycopene to its coplanar structure with 11 conjugated double bonds, which results in a higher reactivity in comparison with (*all-E*)-β-carotene [19]. In contrast, tomato processing may activate enzymes involved in the synthesis of β- and α-carotene. The carotenoid biosynthetic pathway in plants has two main branches after lycopene, distinguished by different cyclic end-groups. Two β rings

lead to the β,β branch (β-carotene and its derivatives) through β-carotene cyclase, while one beta and one epsilon ring define the β,ε branch (α-carotene and its derivatives) through ε- and β-carotene cyclase. Thermal processing may activate ε- and β-carotene cyclase and, thus stimulate α- and β-carotene production.

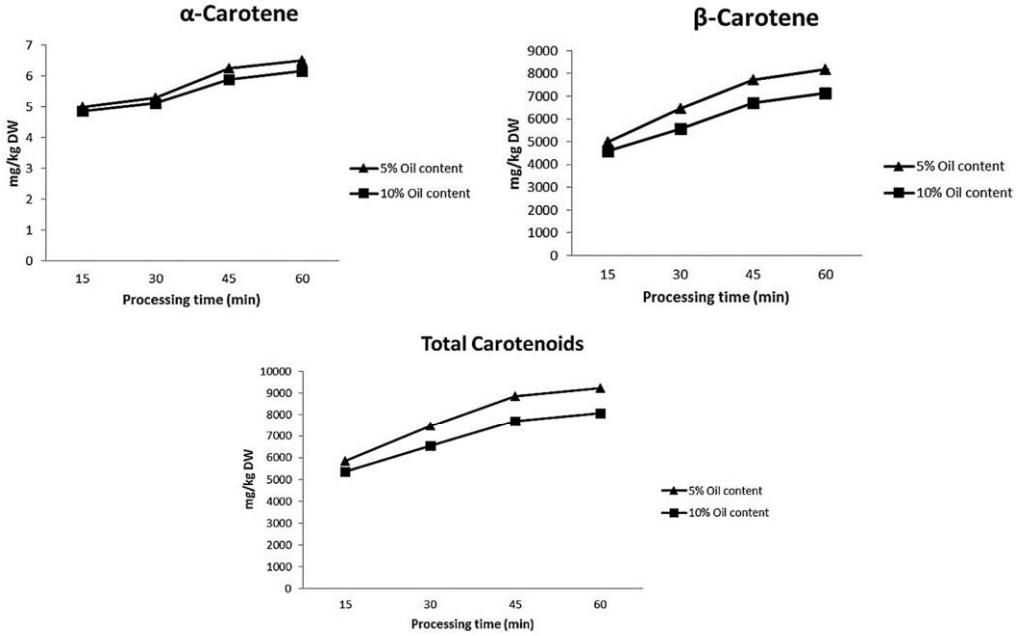

Figure 2. Changes in total carotenoids, α-carotene and β-carotene during processing time.

The (*Z*)-isomers of lycopene during processing were also studied since these compounds are of particular nutritional benefit due to their ready absorption in the human intestine [20]. We tentatively identified three isomers in tomato sauces: 5-, 9- and 13-(*Z*)-lycopene (Figure 3). This lycopene isomer profile is in accordance with that reported by other authors in different tomato products, in which (*all-E*)-lycopene represents the most abundant lycopene isomer, varying from 35% to 96% of total lycopene, and 5-, 9-, 13- and 15-(*Z*)-lycopene are the main (*Z*)-isomers detected [21,22]. We observed higher levels of (*Z*)-lycopene in sauces containing less virgin olive oil. For instance, at 45 min of cooking the concentration of 5-(*Z*)-lycopene was 79.35 mg/kg DW with 5% olive oil as opposed to 72.06 mg/kg DW with 10% olive oil.

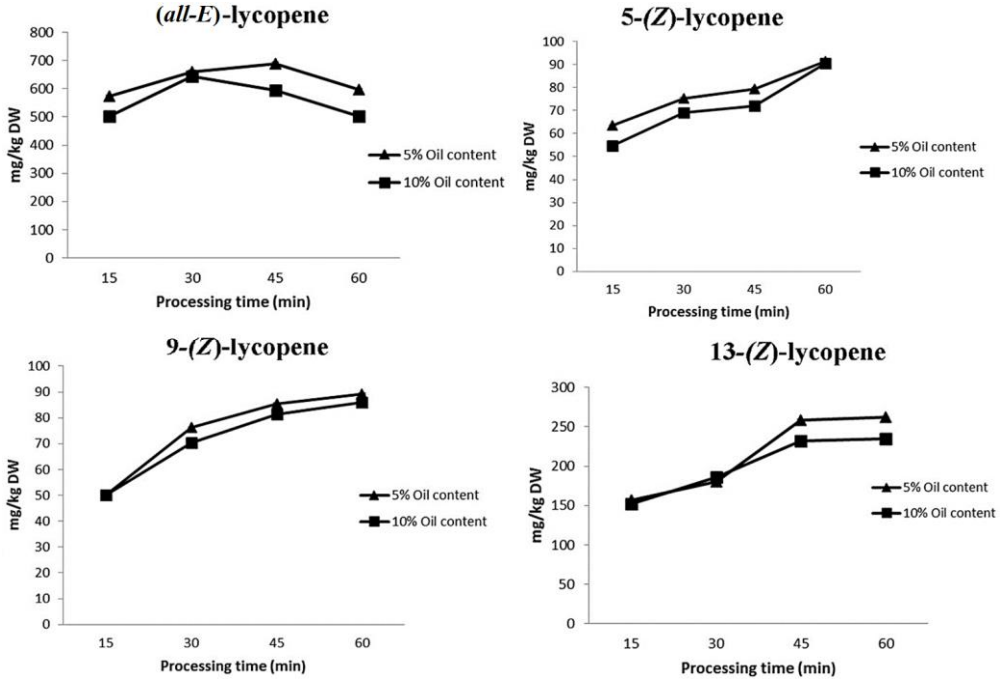

Figure 3. Changes in (*all-E*)-lycopene and 5-(*Z*), 9-(*Z*) and 13-(*Z*)-lycopene during processing time.

It has been shown that during the cooking process lycopene undergoes (*all-E*)/(*Z*)-isomerization, leading to an increase in the proportion of (*Z*)-isomers [19]. As mentioned above, (*all-E*)-lycopene underwent a loss of 9% during the preparation of tomato sauces, in contrast with (*Z*)-lycopene isomers, which increased slightly, by 31%, 18% and 15% in the case of 13-, 5- and 9-(*Z*)-lycopene, respectively (Figure 3). These results are in agreement with those reported by Lin and Chen [19], who observed the formation of (*Z*)-lycopene isomers in tomato juice [19]. This could be explained by the isomerization phenomenon: (*all-E*)-lycopene can be converted to 13-(*Z*)-lycopene, which can then undergo subsequent conversion into other (*Z*)-isomers [23]. Similar lycopene degradation patterns have been described by Giovanelli and Paradiso [24] during the processing and storage of tomato paste. The biosynthetic enzymes involved in carotenoid biosynthesis in plants are encoded by nuclear genes, and precursor proteins are post-translationally imported into plastids, where carotenoid biosynthesis takes place. Disruption of tissue by wounding could promote the transcription of the genes or the transport of the mRNA related to the synthesis of carotenoids [25]. The production of (*Z*)-lycopene isomers due to thermal treatments could be explained by the activation of enzymes such as phytoene synthase and ζ-carotene desaturase (ZDS). Phytoene undergoes a set of desaturation reactions, each of which creates a new double bond and extends the chromophore by two conjugated double bonds; the end product is lycopene [26]. Controversially, García Alonso *et al.* [27] found that the total lycopene content remained quite stable throughout the storage trial and varied from 99 to 120 mg/kg in tomato juice packaged in Tetra Paks and from 96 to 115 mg/kg in samples stored in glass bottles. For both types of

samples, no clear temperature dependency was revealed regarding the rate of total lycopene loss, which was affected only by storage time [27].

The thermal treatment increased the levels of antioxidant activity and total carotenoids by 15% and 30%, respectively. As mentioned above, 13-, 5- and 9-(*Z*)-lycopene increased by 31%, 18% and 15%, respectively. The (*Z*)-isomers of lycopene during processing are of particular nutritional benefit due to their ready absorption in the human intestine [10]. Higher lycopene intake is associated with a reduced risk of lethal prostate [28] and an increase of levels of lycopene in sauce may help to reduce PSA [29]. Moreover, (*all-E*)-α-carotene and (*all-E*)-β-carotene increased over the cooking period by 25% and 40%, respectively. Dietary β-carotene has been reported to decrease (distal) colon cancer [30] and moreover, to suppress the up-regulation of heme oxygenase-1 gene expression in a dose dependent manner and to suppress UVA-induced *HO-1* gene expression in cultured FEK4 [31]. Moreover, it seems that these compounds may act synergically increasing their anticancer activity [32].

3. Experimental Section

3.1. Standards

All samples and standards were handled without exposure to light. β-carotene, α-carotene, lycopene, methyl tert-butyl ether (MTBE), ABTS: 2,2'azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), Trolox: (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid 97% and manganese dioxide and hexane were purchased from Sigma® (St. Louis, MO, USA); ethanol and methanol HPLC grade were obtained from Scharlau (Barcelona, Spain); DPPH: 2,2-diphenyl-1-picrylhydrazyl from Extrasynthèse (Genay, France); and ultrapure water (Milli-Q) from Millipore (Bedford, MA, USA).

3.2. Tomato Sauce Material

A commercial tomato (*Licopersicon esculentum* Mill. c.v. Daniella), suitable for tomato sauce making, was used for the study. Virgin olive oil was kindly furnished by Manuel Heredia Halcón (Cortijo De Suerte Alta, Albendin-Baena-Cordoba). The tomato sauces were prepared at Torribera Campus, University of Barcelona (UB, Barcelona, Spain), following a conventional home cooking method. Fruits were washed, chopped in a breaker unit, weighed and crushed with a Thermomix®. Two variables were evaluated: virgin olive oil addition at two different concentrations and the cooking time. The olive oil (5% and 10% of oil, *w*/*w*) was added to the chopped tomatoes, and the mixture was cooked at 95–96 °C for 15, 30, 45 or 60 min. The tomato sauces were aliquoted and stored in vacuum bags at −20 °C until the day of the analysis. A 4 × 2 mixed-level factorial design was performed, involving a total of 8 randomized runs. For the reproducibility, each process was evaluated three times. The carotenoids profile of tomato sauce control without addition virgin olive oil are reported in Table 1.

Table 1. The carotenoids profile of tomato sauce control without addition virgin olive oil.

Compounds	Tomato Sauce Control (mg/kg DW)
α-carotene	4.3 ± 0.3
β-carotene	3266.9 ± 53.1
(*all-E*)-Lycopene	178.9 ± 10.1
5-(*Z*)-Lycopene	20.3 ± 1.1
9-(*Z*)-Lycopene	26.2 ± 0.9
13-(*Z*)-Lycopene	33.6 ± 2.7
Total Carotenoids	3530.1

3.3. Extraction and Analysis of Carotenoids

3.3.1. Extraction of Carotenoid Compounds

The extraction of carotenoids was carried out in darkness using dry ice in order to minimize autoxidation and (*Z*)-(*all-E*) isomerization, and to avoid exposure to light, oxygen and high temperatures.

Tomato sauces (0.5 gram) were weighed and homogenized with 5 mL ethanol:n-hexane (4:3, *v*/*v*) following a procedure described in the literature [33]; they were then sonicated for 5 min and centrifuged (4000 rpm at 4 °C) for 15 min. The supernatant was transferred into a flask and extraction was repeated. The supernatants were combined and evaporated under nitrogen flow; finally, the residue was reconstituted with MTBE up to 1 mL and filtered through a 25 mm, 0.45 μm PTFE filter (Waters, Mildford, MA, USA).

3.3.2. Analysis of Carotenoid Compounds

Chromatographic analysis was performed using the HPLC system previously described by Vallverdu-Queralt *et al.* [28]. The analytes were separated on a reversed-phase C30 column YMC30 (250 × 4.6 mm, 5 μm) from YMC (Dinslaken, Germany) and kept at 20 °C. The injection volume was 20 μL and flow rate 1 $mL \cdot min^{-1}$. The mobile phase consisted of two different solvent mixtures: A—water: MTBE:methanol (4:26:70, *v*/*v*/*v*)—and B, with the same solvents but other proportions (4:90:6, *v*/*v*/*v*). The linear gradient was 26% B to 90% B in 23 min. The column was equilibrated for 10 min prior to each analysis. MTBE was used as a modifier to facilitate the elution of lycopene, which is strongly retained in a methanol environment [34].

3.4. Identification and Quantification of Carotenoids

3.4.1. Diode Array Detector

Commercially available carotenoid standards ((*all-E*)-α-carotene, (*all-E*)-β-carotene and (*all-E*)-lycopene) were used to identify analytes by retention times and UV-VIS spectra. The LC-DAD chromatograms were acquired by selecting the 450 nm wavelength; in addition, the UV-VIS spectra were recorded in the range of 350–550 nm for the tentative identification of carotenoids and their geometrical isomers ((*Z*)-lycopene isomers), on the basis of the retention times and absorption spectrum characteristics described in the literature [21,34].

3.4.2. Mass Spectrometry

The API 3000 (PE Sciex, Concord, ON, Canada) triple quadrupole mass spectrometer in positive-ion mode was used to obtain MS/MS data for carotenoid analysis. Turbo Ionspray source settings were the same as previously described by our group [33]. Post-column addition of a solution of LiCl (500 $mg \cdot L^{-1}$) was performed using an isocratic pump Agilent 1100 Series (Agilent Technologies, Palo Alto, CA, USA) at a flow rate of 100 $\mu L \cdot min^{-1}$ via a zero-volume mixing T-piece.

Carotenoids were quantified with respect to their corresponding standard according to the internal standard method using *trans*-β-apo-8'-carotenal. When standards were not available, as in the case of (*Z*) isomers of lycopene, they were quantified on the basis of the peak area of the (*all-E*)-lycopene standard. Results are expressed as mg/kg dry weight (DW).

3.5. Antioxidant Capacity

The antioxidant capacity of tomato sauces was measured using $ABTS^+$ and DPPH assays reported in the literature [33].

3.5.1. $ABTS^+$ Assay

One mM Trolox (antioxidant standard) was prepared in methanol once a week. Working standards were prepared daily by diluting 1 mM Trolox with methanol.

An $ABTS^+$ radical cation was prepared by passing a 5 mM stock solution of ABTS (in methanol) through manganese dioxide powder. Excess manganese dioxide was filtered through a 13 mm 0.45 µm filter PTFE (Waters). Before analysis, the solution was diluted in methanol pH 7.4 to give an absorbance at 734 nm of 1.0 ± 0.1, and pre-incubated in ice. Then, 245 µL of $ABTS^+$ solution was added to 5 µL of Trolox or to tomato extracts and the solutions were stirred for 30 s. The absorbance was recorded continuously every 30 s with a UV/VIS spectrophotometer Thermo Multiskan Spectrum from Thermo Fischer Scientific (San Jose, CA, USA) for 1 h and methanol blanks were run in each assay.

The working range for Trolox (final concentration 0–750 µmol/L) was based on triplicate determinations and consisted of plotting the absorbance as a percentage of the absorbance of the uninhibited radical cation (blank). The activities of the tomato extracts were assessed at four different concentrations, which were within the range of the dose-response curve. Each sample was analyzed in triplicate at each concentration. Results were expressed as mmol Trolox equivalent (TE)/g DW.

3.5.2. DPPH Assay

The antioxidant capacity was also studied through the evaluation of the free radical-scavenging effect on the DPPH radical. Solutions of known Trolox were used for calibration. 5 µL of tomato extracts or Trolox were mixed with 250 µL of methanolic DPPH (0.025 g/L). The homogenate was shaken vigorously and kept in darkness for 30 min. Absorption of the samples was measured on a UV/VIS Thermo Multiskan Spectrum spectrophotometer at 515 nm. The percentage of inhibition of the DPPH activity was calculated and plotted as a function of the Trolox concentration for the standard reference data. The final DPPH values were calculated using a regression equation between the Trolox concentration and the percentage of DPPH inhibition and results were expressed as mmol TE/100 g DW.

3.6. Statistical Treatments

Treatments of tomato sauces were carried out in triplicate and each replicate was analyzed three times. Significance of the results and statistical differences were analyzed using Statgraphics plus v. 5.1 software (Manugistics, Rockville, MA, USA). Data were analyzed by multifactor analysis of variance and a Duncan multiple range test was applied to determine differences among means, with a significance level of $p = 0.05$.

4. Conclusions

In this work, the effects of cooking time (15, 30, 45 and 60 min) and the amount of added virgin olive oil (5% and 10%) on the carotenoid profile of tomato sauces were studied using LC-ESI-MS/MS and LC-UV. During the thermal treatment, levels of antioxidant activity, total carotenoids, α-carotene, β-carotene and (*Z*)-lycopene increased. This information may be used for industries to develop new treatments so as to obtain products with high carotenoid content and higher physiological effects. This increase in carotenoids and antioxidant capacity may reduce lipid peroxidation, increase HDL and decrease the incidence of chronic degenerative diseases, such as cancer and cardiovascular diseases.

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Author Contributions

Anna Vallverdu-Queralt participated in the study design, carried out the analysis and wrote the manuscript. Jorge Regueiro participated in the study design and in the revision of manuscript content. José Fernando Rinaldi de Alvarenga participated in the HPLC-DAD/MS analyses. Xavier Torrado participated in the study design. Rosa M. Lamuela-Raventos designed and supervised the study. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Estruch, R.; Martinez-Gonzalez, M.A.; Corella, D.; Salas-Salvado, J.; Ruiz-Gutierrez, V.; Covas, M.I.; Fiol, M.; Gomez-Gracia, E.; Lopez-Sabater, M.C.; Vinyoles, E.; *et al*. Effects of a mediterranean-style diet on cardiovascular risk factorsa randomized trial. *Ann. Intern. Med.* **2006**, *145*, 1–11.
2. Estruch, R.; Ros, E.; Salas-Salvado, J.; Covas, M.I.; Pharm, D.; Corella, D.; Aros, F.; Gomez-Gracia, E.; Ruiz-Gutierrez, V.; Fiol, M.; *et al*. Primary prevention of cardiovascular disease with a mediterranean diet. *N. Engl. J. Med.* **2013**, *368*, 1279–1290.
3. Grieb, S.M.D.; Theis, R.P.; Burr, D.; Benardot, D.; Siddiqui, T.; Asal, N.R. Food groups and renal cell carcinoma: Results from a case-control study. *J. Am. Diet. Assoc.* **2009**, *109*, 656–667.
4. Silaste, M.L.; Alfthan, G.; Aro, A.; Kesaniemi, Y.A.; Horkko, S. Tomato juice decreases LDL cholesterol levels and increases LDL resistance to oxidation. *Br. J. Nutr.* **2007**, *98*, 1251–1258.
5. Olson, J.A. Biological actions of carotenoids—Introduction. *J. Nutr.* **1989**, *119*, 94–95.
6. Agarwal, S.; Rao, A.V. Tomato lycopene and its role in human health and chronic diseases. *CMAJ* **2000**, *163*, 739–744.
7. Torre-Carbot, K.; Jauregui, O.; Gimeno, E.; Castellote, A.; Lamuela-Raventos, R.M.; Lopez-Sabater, M.C. Characterization and quantification of phenolic compounds in olive oils by solid-phase extraction, HPLC-DAD, and HPLC-MS/MS. *J. Agric. Food Chem.* **2005**, *53*, 4331–4340.
8. Visioli, F.; Galli, C. Olive oil phenols and their potential effects on human health. *J. Agric. Food Chem.* **1998**, *46*, 4292–4296.
9. Vallverdu-Queralt, A.; Odriozola-Serrano, I.; Oms-Oliu, G.; Lamuela-Raventos, R.M.; Elez-Martinez, P.; Martin-Belloso, O. Impact of high-intensity pulsed electric fields on carotenoids profile of tomato juice made of moderate-intensity pulsed electric field-treated tomatoes. *Food Chem.* **2013**, *141*, 3131–3138.
10. Gärtner, C.; Stahl, W.; Sies, H. Lycopene is more bioavailable from tomato paste than from fresh tomatoes. *Am. J. Clin. Nutr.* **1997**, *66*, 116–122.
11. Aguilo-Aguayo, I.; Soliva-Fortuny, R.; Martin-Belloso, O. Volatile compounds and changes in flavour-related enzymes during cold storage of high-intensity pulsed electric field- and heat-processed tomato juices. *J. Sci. Food Agric.* **2010**, *90*, 1597–1604.
12. Odriozola-Serrano, I.; Soliva-Fortuny, R.; Martin-Belloso, O. Changes of health-related compounds throughout cold storage of tomato juice stabilized by thermal or high intensity pulsed electric field treatments. *Innov. Food Sci. Emerg. Technol.* **2008**, *9*, 272–279.
13. Bonnie, T.Y.P.; Choo, Y.M. Oxidation and thermal degradation of carotenoids. *J. Oil Palm Res.* **1999**, *2*, 62–78.
14. Boileau, A.C.; Merchen, N.R.; Wasson, K.; Atkinson, C.A.; Erdman, J.W. (*cis*)-Lycopene is more bioavailable than (*trans*)-lycopene *in vitro* and *in vivo* in lymph-cannulated ferrets. *J. Nutr.* **1999**, *129*, 1176–1181.
15. Shi, J.; Le Maguer, M. Lycopene in tomatoes: Chemical and physical properties affected by food processing. *Crit. Rev. Biotechnol.* **2000**, *20*, 293–334.

16. Abushita, A.A.; Daood, H.G.; Biacs, P.A. Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. *J. Agric. Food Chem.* **2000**, *48*, 2075–2081.
17. Dewanto, V.; Wu, X.; Adom, K.K.; Liu, R.H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **2002**, *50*, 3010–3014.
18. Piga, A.; Agabbio, M.; Gambella, F.; Nicoli, M.C. Retention of antioxidant activity in minimally processed mandarin and satsuma fruits. *LWT Food Sci. Technol.* **2002**, *35*, 344–347.
19. Lin, C.H.; Chen, B.H. Stability of carotenoids in tomato juice during storage. *Food Chem.* **2005**, *90*, 837–846.
20. Takeoka, G.R.; Dao, L.; Flessa, S.; Gillespie, D.M.; Jewell, W.T.; Huebner, B.; Bertow, D.; Ebeler, S.E. Processing effects on lycopene content and antioxidant activity of tomatoes. *J. Agric. Food Chem.* **2001**, *49*, 3713–3717.
21. Fröhlich, K.; Conrad, J.; Schmid, A.; Breithaupt, D.E.; Böhm, V. Isolation and structural elucidation of different geometrical isomers of lycopene. *Int. J. Vitam. Nutr. Res.* **2007**, *77*, 369–375.
22. Schierle, J.; Bretzel, W.; Bühler, I.; Faccin, N.; Hess, D.; Steiner, K.; Schuep, W. Content and isomeric ratio of lycopene in food and human blood plasma. *Food Chem.* **1997**, *59*, 459–465.
23. Vallverdu-Queralt, A.; Arranz, S.; Casals-Ribes, I.; Lamuela-Raventos, R.M. Stability of the phenolic and carotenoid profile of gazpachos during storage. *J. Agric. Food Chem.* **2012**, *60*, 1981–1988.
24. Giovanelli, G.; Paradiso, A. Stability of dried and intermediate moisture tomato pulp during storage. *J. Agric. Food Chem.* **2002**, *50*, 7277–7281.
25. Bartley, G.E.; Scolnik, P.A. Plant carotenoids: Pigments for photoprotection, visual attraction, and human health. *Plant Cell* **1995**, *7*, 1027–1038.
26. DellaPenna, D.; Pogson, B.J. Vitamin synthesis in plants: Tocopherols and carotenoids. *Annu. Rev. Plant Biol.* **2006**, 57, 711–738.
27. Garcia-Alonso, F.J.; Bravo, S.; Casas, J.; Perez-Conesa, D.; Jacob, K.; Periago, M.J. Changes in antioxidant compounds during the shelf life of commercial tomato juices in different packaging materials. *J. Agric. Food Chem.* **2009**, *57*, 6815–6822.
28. Zu, K.; Mucci, L.; Rosner, B.A.; Clinton, S.K.; Loda, M.; Stampfer, M.J.; Giovannucci, E. Dietary lycopene, angiogenesis, and prostate cancer: A prospective study in the prostate-specific antigen era. *J. Natl. Cancer Inst.* **2014**, *106*, djt430.
29. Edinger, M.S.; Koff, W.J. Effect of the consumption of tomato paste on plasma prostate-specific antigen levels in patients with benign prostate hyperplasia. *Braz. J. Med. Biol. Res.* **2006**, *39*, 1115–1119.
30. Leenders, M.; Leufkens, A.M.; Siersema, P.D.; van Duijnhoven, F.J.; Vrieling, A.; Hulshof, P.J.; van Gils, C.H.; Overvad, K.; Roswall, N.; Kyrø, C.; *et al.* Plasma and dietary carotenoids and vitamins A, C and E and risk of colon and rectal cancer in the European Prospective Investigation into Cancer and Nutrition. *Int. J. Cancer* **2014**, *135*, 2930–2939.
31. Wei, S.; Wang, Y.; Chai, Q.; Fang, Q.; Zhang, Y.; Wang, J. *In vivo* and *in vitro* effects of heme oxygenase-1 silencing on the survival of acute myelocytic leukemia-M2 cells. *Exp. Ther. Med.* **2015**, *9*, 931–940.

32. Linnewiel-Hermoni, K.; Khanin, M.; Danilenko, M.; Zango, G.; Amosi, Y.; Levy, J.; Sharoni, Y. The anti-cancer effects of carotenoids and other phytonutrients resides in their combined activity. *Arch. Biochem. Biophys.* **2015**, *572*, 28–35.
33. Vallverdu-Queralt, A.; Martinez-Huelamo, M.; Arranz-Martinez, S.; Miralles, E.; Lamuela-Raventos, R.M. Differences in the carotenoid content of ketchups and gazpachos through HPLC/ESI(Li^+)-MS/MS correlated with their antioxidant capacity. *J. Sci. Food Agric.* **2012**, *92*, 2043–2049.
34. Matea, C.; Soran, M.; Pintea, A.; Bele, C. Analytical determination of carotenoids in inland *Ocimum basilicum* L. *Bull. UASVM Agric.* **2010**, *67*, 298–302.

4.1.2 Publication 2. Home cooking and phenolics: effect of thermal treatment and addition of extra virgin olive oil on the phenolic profile of tomato sauces

Anna Vallverdú-Queralt, Jorge Regueiro, **José Fernando Rinaldi de Alvarenga**, Xavier Torrado, Rosa Maria Lamuela-Raventós. *Journal of Agricultural and Food Chemistry*, 2014, 62, 3314-3320

Tomato products and olive oil are keys components of the Mediterranean diet which are strongly related to health benefits. The use of olive oil in tomato sauces, typically from home-made sauces, could improve the bioaccessibility and bioavailability of phenolic compounds. The aim of this study was to verify the effect of thermal treatment and the addition of EVOO on the phenolic content of homemade tomato sauces. Tomato sauce were prepared following a regular homemade process. A 4x2 mixed level factorial design was applied, which the 8 different formulation were processed in triplicate, involving a total of 24 randomized runs. The factor EVOO had 2 levels (5% and 10%) and the factor time of process had 4 levels (15, 30 45 and 60 min). A targeted quantification of phenolic compounds were performed using a HPLC-ESI-QqQ-MS/MS and the antioxidant capacity were measured by DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assay. The antioxidant capacity decreased with time of cooking for both methods. Among hydroxycinnamic and phenolic acids, *p*-coumaric, protocatechuic and chlorogenic acids showed the maximum levels at 30 min of cooking and then decreased. Ferulic acid was stable during up to the first 30 min of processing and slighty decreased after this time. Unlike the others, caffeic acid increased up to 60 min of thermal treatment. Among flavonoids, rutin and quercetin decreased during the cooking process, in which this decrease was more abruptly for quercetin. Naringenin showed a slight increase in the first 15 min up to 45 min of cooking, after that a decreased in its content was observed. Tomato sauces prepared with 10% of EVOO showed a higher content of all phenolic compounds compared to 5% of EVOO. As a general pattern, longer time of cooking decreased the content of phenolic compounds and antioxidants, except for caffeic acid. Tomato sauces prepared with higher percent of EVOO showed higher levels of phenolic compounds, which could improve bioacessibility and bioavailability of polyphenols in culinary process.

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Home Cooking and Phenolics: Effect of Thermal Treatment and Addition of Extra Virgin Olive Oil on the Phenolic Profile of Tomato Sauces

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ABSTRACT: Tomato products are a key component of the Mediterranean diet, which is strongly related to a reduced risk of cardiovascular events. The effect of cooking time (15, 30, 45, and 60 min) and the addition of extra virgin olive oil (5 and 10%) on the phenolic content of tomato sauces was monitored using liquid chromatography coupled to tandem mass spectrometry. Concentration of phenolics in the tomato sauces decreased during the cooking process, with the exception of caffeic acid and tyrosol. The main degradation observed was the oxidation of quercetin, since the hydroxy-function at the *C*-ring of this flavonoid is not blocked by a sugar moiety, unlike rutin. Higher levels of virgin olive oil in tomato sauce seemed to enhance the extraction of phenolic compounds from the tomato, leading to higher phenolic contents in the sauces. Thus, the food matrix containing the phenolic compounds plays a crucial role in determining their accessibility.

KEYWORDS: *thermal treatments, cooking effects, polyphenols, food matrix, tomato sauces, extra virgin olive oil, HPLC−MS/MS*

INTRODUCTION

Tomatoes have been attracting renewed interest due to their high content of phenolic compounds with multiple biological effects.[1,2] A high consumption of virgin olive oil (VOO), another main feature of the Mediterranean diet, has long been reported to exhibit numerous biological health benefits.[3,4] Thus, the typical daily habit of consuming meals that combine tomatoes and olive oil may improve several health outcomes. It has also been suggested that VOO added to tomato sauce plays a crucial role in the accessibility and bioavailability of phenolic compounds from food.[5] Moreover, the addition of VOO to tomato sauces enriches meals with other polyphenols not present in tomatoes such as tyrosol and hydroxytyrosol.[3,6,7]

The effects of technological processing on the lycopene content of tomatoes have been exhaustively examined.[8,9] However, the impact of these treatments on phenolic compounds is still not fully understood. Thus, some studies indicate that industrial processing results in a considerable loss of hydrophilic antioxidants. Vallverdu-Queralt et al.[10] reported that heat treatment of tomatoes and, to a lesser extent, the sterilization stage during the paste-making process negatively affect the phenolic content. In contrast, other studies have reported that processing enhances the total phenolic content. In experiments carried out by Chang et al.,[11] six tomato varieties (Japan-Golden, Chin-Yan, Holland-Golden, Holland-Red, Sheng-Neu, and Taur-Tay-Lang) air-dried at 80 °C for 2 h and then at 60 °C for 6 h had higher total flavonoid and total phenolic contents than fresh tomatoes.

Although the effects of thermal treatments on the phenolic content of tomato sauces has been well studied in industrial manufacturing processes,[10,12] little is known about the influence of home cooking time and the addition of extra virgin olive in the preparation of this kind of product at home. Thus, the aim of this study was to examine the effect of the length of thermal treatment (15, 30, 45, and 60 min) and the addition of VOO (5 and 10%) on the phenolic content of homemade tomato sauces.

MATERIALS AND METHODS

Standards. All samples and standards were handled without exposure to light. Caffeic, ferulic, *p*-coumaric, protocatechuic, and chlorogenic acids, rutin, quercetin, ABTS (2,2′azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid 97%), manganese dioxide, and glacial acetic acid (100%) were purchased from Sigma (Madrid, Spain). Tyrosol, hydroxytyrosol, naringenin, and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Extrasynthèse (Genay, France). Acetonitrile, ethanol, *n*-hexane, and methanol, all of HPLC grade, and formic acid (≥98%) were acquired from Scharlau (Barcelona, Spain). Ultrapure water was obtained from a Milli-Q Gradient water purification system (Millipore, Bedford, MA, USA).

Tomato Sauce Material. A commercial tomato (*L. esculentum* L.) variety (cv Pera), suitable for tomato sauce preparation, was used for

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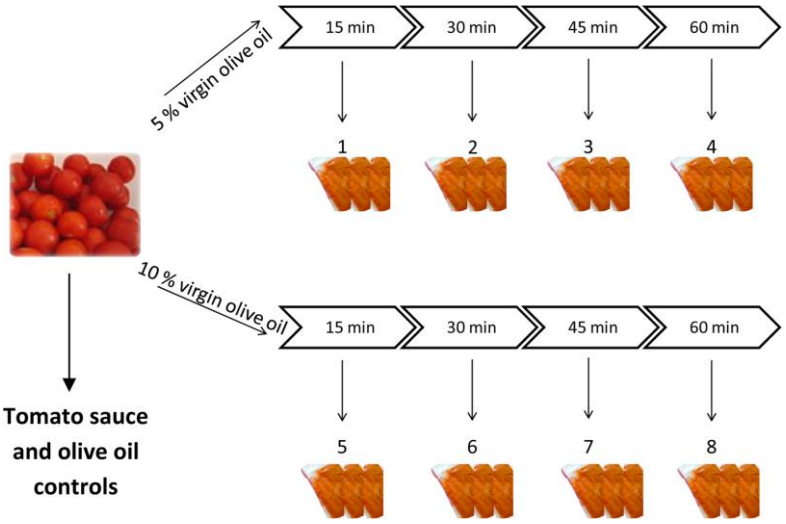

Figure 1. Diagram flowchart of the processes.

Table 1. Levels of Polyphenols in Tomato Sauce Control

chlorogenic acid (μg/g FW)	caffeic acid (μg/g FW)	ferulic acid (μg/g FW)	*p*-coumaric acid (μg/g FW)	protocatechuic acid (μg/g FW)	quercetin (μg/g FW)	rutin (μg/g FW)	naringenin (μg/g FW)	tyrosol (μg/g FW)	hydroxytyrosol (μg/g FW)
2.61 ± 0.03	2.55 ± 0.06	7.05 ± 0.43	0.15 ± 0.03	1.19 ± 0.08	3.02 ± 0.12	28.30 ± 0.41	4.97 ± 0.39	BDL[a]	BDL

[a]BDL: below detection limit.

Table 2. Levels of Polyphenols in VOO

chlorogenic acid (μg/g FW)	caffeic acid (μg/g FW)	ferulic acid (μg/g FW)	*p*-coumaric acid (μg/g FW)	protocatechuic acid (μg/g FW)	quercetin (μg/g FW)	rutin (μg/g FW)	naringenin (μg/g FW)	tyrosol (μg/g FW)	hydroxytyrosol (μg/g FW)
BDL[a]	BDL	0.41 ± 0.03	BDL	BDL	BDL	BDL	BDL	12.13 ± 0.67	8.15 ± 0.44

[a]BDL: below detection limit.

the study. VOO was kindly furnished by Manuel Heredia Halcón (Cortijo de la Suerte Alta, Albendin-Baena-Cordoba). The tomato sauces were prepared following a regular home cooking process at the Torribera Campus, University of Barcelona (Barcelona, Spain). Tomatoes were washed, chopped in a breaker unit, weighed, and crushed with a Thermomix. Two variables were evaluated: VOO addition at two different concentrations and thermal treatment time. The VOO (5 and 10% of oil, w/w) was added to the chopped tomatoes, and the mixture was cooked at 95−96 °C for 15, 30, 45, or 60 min. The tomato sauces were aliquoted and stored in vacuum bags at −20 °C until the day of the analysis. A 4 × 2 mixed-level factorial design was performed, involving a total of 8 randomized runs. For the reproducibility, each process was evaluated three times (Figure 1). The phenolic profile of tomato sauce control without addition of VOO and the content of polyphenols in VOO are reported in Tables 1 and 2.

Extraction and Analysis of Polyphenols. *Extraction of Polyphenols from Tomato Sauces.* Extraction and analysis of each replicate of the treatment was carried out in triplicate in a darkened room with a red safety light to prevent analyte oxidation, following the procedure of Vallverdu-Queralt et al.[13] with some modifications.

Tomato sauces (0.5 g) were weighed and homogenized with 80% ethanol in Milli-Q water (4 mL); the homogenate was sonicated for 5 min and centrifuged (4000 rpm at 4 °C) for 15 min. The supernatant was transferred into a flask and extraction was repeated. Both supernatants were combined and evaporated under nitrogen flow; finally, the residue was reconstituted up to 2 mL with Milli-Q water containing 0.1% of formic acid and filtered through a 0.45-μm polytetrafluoroethylene (PTFE) filter from Waters (Milford, USA) into an insert-amber vial.

Solid phase extraction (SPE) was carried out to eliminate interferences such as ascorbic acid, amino acids, and reducing sugars. For this purpose, Oasis MAX cartridges with 30 mg of mixed-mode anion-exchange and reversed-phase sorbent from Waters (Milford, USA) were used following the procedure of Vallverdu-Queralt et al.[14] The eluted fractions were evaporated under nitrogen flow and the residue was reconstituted up to 500 μL with Milli-Q water containing 0.1% formic acid and filtered through a 0.45-μm PTFE filter into an insert-amber vial for HPLC analysis.

Extraction of Polyphenols from VOO. The extraction of phenolic compounds from VOO was performed following the procedure previously described by García-Villalba et al.[15] Briefly, diol cartridges Supelclean LC-Diol (500 mg, 3 mL) from Sigma (Madrid, Spain) were placed in a SPE vacuum manifold and conditioned passing 5 mL of methanol and subsequently 5 mL of *n*-hexane without vacuum. About 3 g of VOO was accurately weighted and then thoroughly mixed with 3 mL of *n*-hexane and loaded onto the SPE cartridge under vacuum. The cartridge was then washed with 5 mL of *n*-hexane to remove the nonpolar fraction of the oil and then dried with nitrogen to remove the *n*-hexane. Finally, the phenolic compounds were eluted with 10 mL methanol under vacuum and the eluate was evaporated to dryness. The dried residue was then reconstituted in 1 mL of methanol/water (7:3, v/v) and filtered through a 0.45-μm PTFE filter for HPLC analysis.

Analysis of Polyphenols. Liquid chromatography coupled to electrospray ionization tandem mass spectrometry (HPLC−ESI-MS/

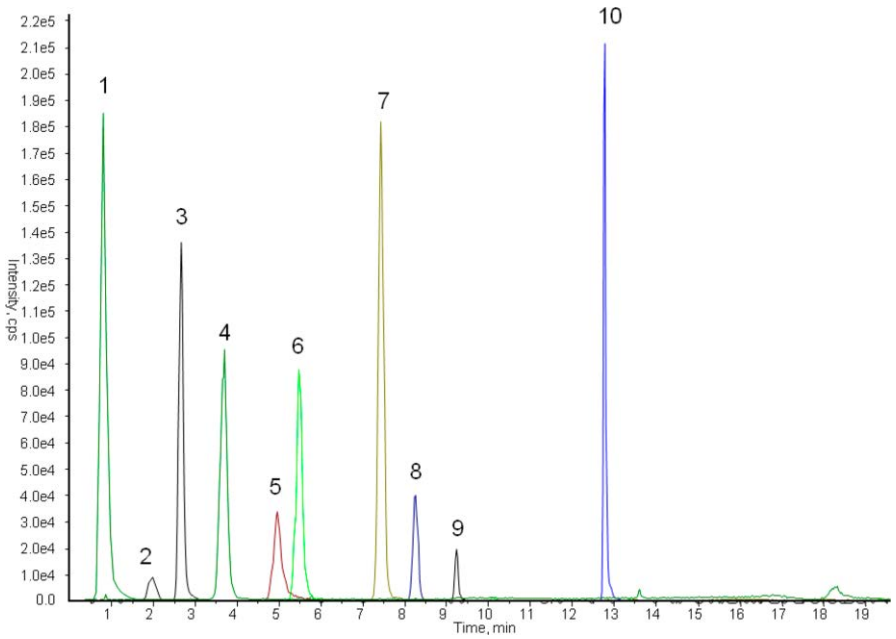

Figure 2. HPLC Chromatogram of tomato sauce treated during 15 min at 5% of VOO. 1. Hydroxytyrosol; 2. tyrosol; 3. protocatechuic acid; 4. chlorogenic acid; 5. caffeic acid; 6. *p*-coumaric acid; 7. rutin; 8. ferulic acid; 9. quercetin; 10. naringenin.

MS) was used to evaluate the content of flavonols, flavanones, and hydroxycinnamic acids in VOO and tomato sauces following the procedure of Vallverdu-Queralt et al.[13]

Liquid chromatography was performed with an Agilent series 1100 HPLC instrument (Agilent, Waldbronn, Germany) equipped with a quaternary pump, an autosampler, and a column oven set to 30 °C. A Luna C_{18} column 50 × 2.0 mm i.d., 5 μm (Phenomenex, Torrance, CA, USA) was used. The injection volume was 20 μL and the flow rate was 0.4 mL/min. Mobile phase consisted of Milli-Q water containing 0.5% acetic acid (A) and acetonitrile (B). Separation was carried out in 15 min under the following conditions: 0 min, 5% B; 10 min, 18% B; 13 min, 100% B; 14 min, 100% B; 15 min, 5% B. The column was equilibrated for 5 min prior to each analysis.

An API 3000 (PE Sciex, Concord, ON, Canada) triple quadrupole mass spectrometer equipped with a Turbo Ionspray source in negative-ion mode was used to obtain MS/MS data. Turbo Ionspray source settings were as follows: capillary voltage, −3500 V; nebulizer gas (N_2), 10 au (arbitrary units); curtain gas (N_2), 12 au; collision gas (N_2), 4 au; focusing potential, −200 V; entrance potential, −10 V; drying gas (N_2), heated to 400 °C and introduced to a flow rate of 6000 cm^3/min. The declustering potential and collision energy were optimized for each compound by infusion experiments: individual standard solutions (10 μg/mL) dissolved in 50:50 (v/v) mobile phase were infused at a constant flow rate of 5 μL/min using a model syringe pump (Harvard Apparatus, Holliston, MA, USA). Full-scan data acquisition was performed scanning from m/z 100 to 800 in profile mode and using a cycle time of 2 s with a step size of 0.1 u and a pause between each scan of 2 ms. For quantification purposes, data were collected in the multiple reaction monitoring (MRM) mode, tracking the transition of parent and product ions specific for each compound.

Quantification of polyphenols was performed by the internal standard method. Polyphenols were quantified related to their corresponding standard and results were expressed as μg/g fresh weight (FW).

Antioxidant Capacity. The antioxidant capacity of tomato sauces was measured using $ABTS^+$ and DPPH assays.[12]

$ABTS^+$ assay. One mM Trolox (antioxidant standard) was prepared in methanol once a week. Working standards were prepared daily by diluting 1 mM Trolox with methanol.

An $ABTS^+$ radical cation was prepared by passing a 5 mM aqueous stock solution of ABTS (in methanol) through manganese dioxide powder. Excess manganese dioxide was filtered through a 0.45-μm filter PTFE (Waters). Before analysis, the solution was diluted in methanol pH 7.4 to give an absorbance at 734 nm of 1.0 ± 0.1, and preincubated in ice. Then, 245 μL of $ABTS^+$ solution was added to 5 μL of Trolox or to tomato extracts and the solutions were stirred for 30 s. The absorbance was recorded continuously every 30 s with a UV/vis Multiskan Spectrum spectrophotometer from Thermo Fisher Scientific (San Jose, CA, USA) for 1 h and methanol blanks were run in each assay.

The working range for Trolox (final concentration 0−750 μM) was based on triplicate determinations and consisted of plotting the absorbance as a percentage of the absorbance of the uninhibited radical cation (blank). The activities of the tomato extracts were assessed at four different concentrations within the range of the dose−response curve. Each sample was analyzed in triplicate at each concentration. Results were expressed as mmol Trolox equivalent (TE)/100 g FW.

DPPH Assay. Antioxidant capacity was also studied by evaluating the free radical-scavenging effect on the DPPH radical. Solutions of known Trolox concentrations were used for calibration. Five μL of tomato extracts or Trolox was mixed with 250 μL of methanolic DPPH (0.025 g/L). The homogenate was shaken vigorously and kept in darkness for 30 min. Absorption of the samples was measured on a UV/vis Thermo Multiskan Spectrum spectrophotometer at 515 nm. The percentage of inhibition of the DPPH was calculated and plotted as a function of the concentration of Trolox for the standard reference data. The final DPPH values were calculated using a regression equation between the Trolox concentration and the percentage of DPPH inhibition and results were expressed as mmol TE/100 g FW.

Statistical Treatments. Treatments of tomato sauces were carried out in triplicate and each replicate was analyzed three times. Significance of the results and statistical differences were analyzed

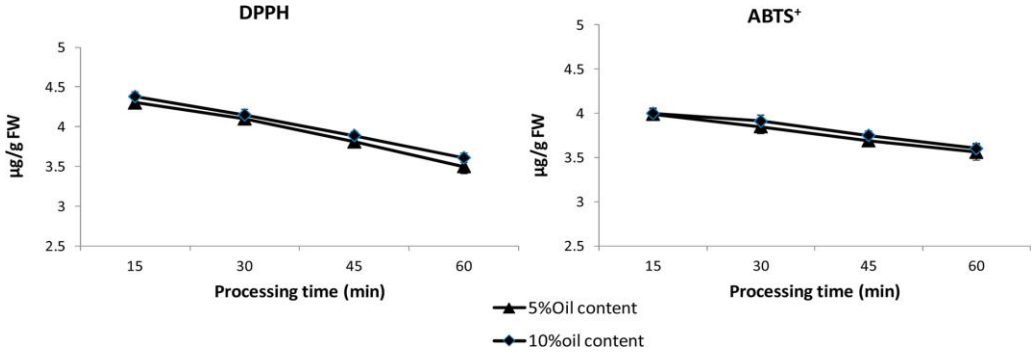

Figure 3. Changes in antioxidant capacity during processing time.

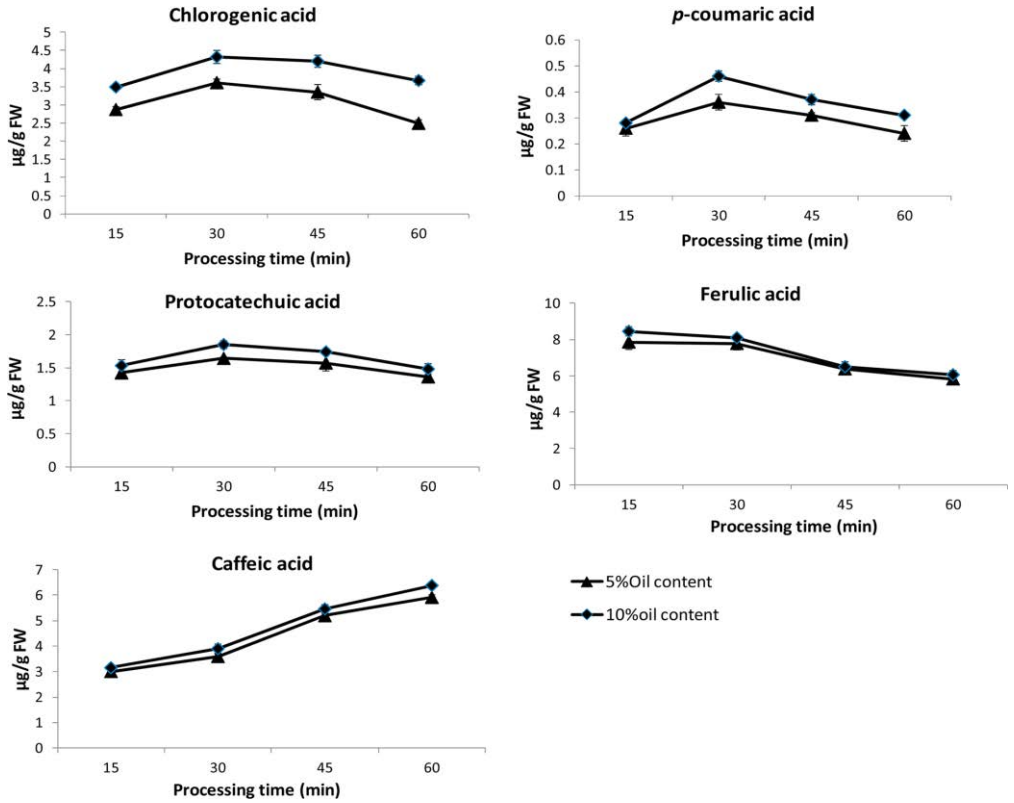

Figure 4. Changes in hydroxycinnamic and phenolic acids during processing time.

using Statgraphics plus v. 5.1 software (Manugistics, Inc., Rockville, MA, USA). Data were analyzed by multifactor analysis of variance and a Duncan multiple range test was applied to determine differences among means, with a significance level of $P = 0.05$.

RESULTS AND DISCUSSION

Changes in Antioxidant Capacity during Processing Time. It has been reported that some labile antioxidants are degraded during food processing, but other studies have shown that industrial-scale processing of tomatoes involving heat treatments may increase the levels of some bound antioxidants.[16,17] Figure 2 shows a chromatogram of tomato sauce treated during 15 min at 5% of VOO. Our analysis of antioxidant capacity showed that from 15 to 60 min of cooking, the level of antioxidants measured by the DPPH assay decreased from 4.3 to 3.5 μg/g FW in sauces containing 5% VOO and from 4.4 to 3.6 μg/g FW in sauces containing 10% extra VOO (Figure 3). The same pattern was observed in the

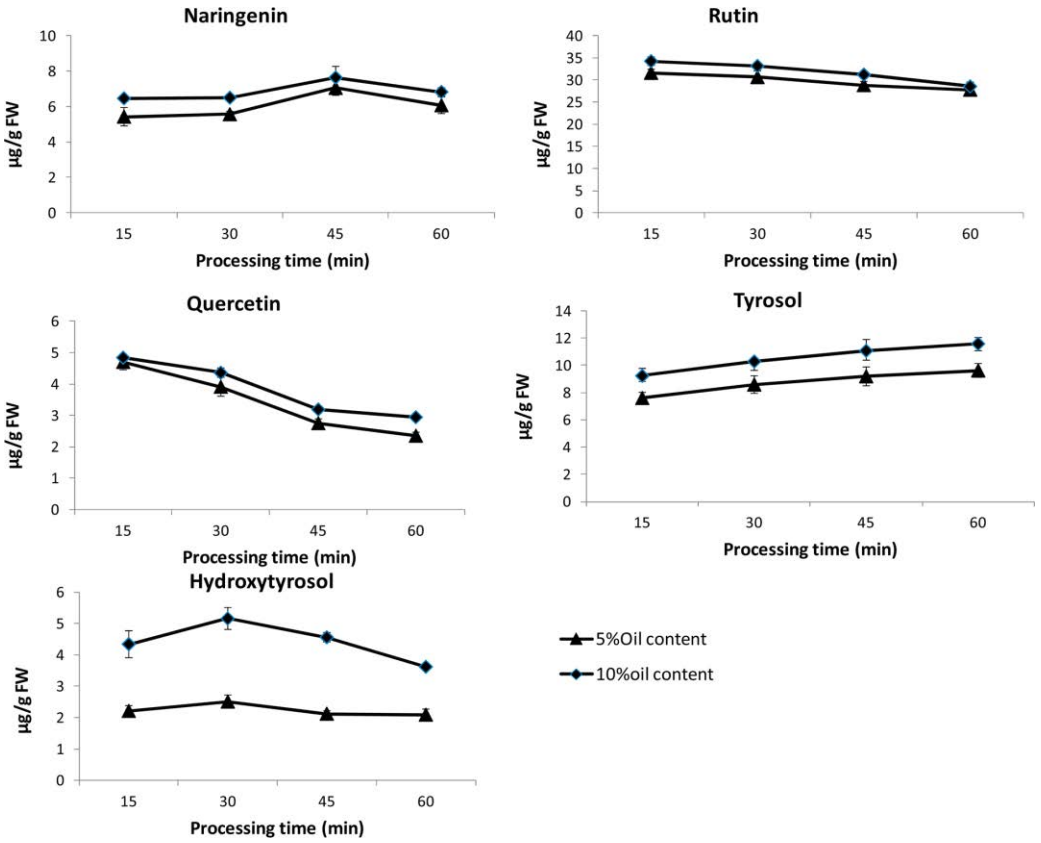

Figure 5. Changes in flavonols, flavanones, and other phenols.

ABTS^{+} assay. Thermal processing of sauces might be expected to have an effect on heat-labile and oxidizable compounds. However, Dewanto et al.[18] reported that total polyphenols did not change during tomato processing.

The phenolic profile of tomato sauce control without addition of VOO and the content of polyphenols in VOO are reported in Tables 1 and 2. For all polyphenols, levels in tomato sauce control are lower than in samples containing 5% or 10% VOO (Figures 4 and 5). Probably, VOO enhances the extractability of phenolic compounds.[5] Moreover, tomato sauces with 5 and 10% VOO contain tyrosol and hydroxytyrosol due to the addition of VOO. It has previously been reported that the addition of olive oil to tomato sauces enriches meals with other polyphenols not present in tomatoes such as tyrosol and hydroxytyrosol.[3,6,7]

Changes in Hydroxycinnamic and Phenolic Acids during Processing Time. In our study, hydroxycinnamic and phenolic acids were also analyzed (Figure 4). *p*-Coumaric, protocatechuic, and chlorogenic acids achieved their maximum levels (0.3−4 μg/g FW) at 30 min of cooking, regardless of the amount of VOO added (5 or 10%) and then decreased slightly (at 45 and 60 min) (Figure 4). Higher levels of these compounds were found when sauces were supplemented with 10% rather than 5% of VOO. For example, at 30 min of cooking, sauces with 10% of VOO contained 4 μg/g FW of chlorogenic acid, whereas in those containing 5% of VOO, levels of chlorogenic acid were 3 μg/g FW (Figure 4). The same behavior was exhibited by protocatechuic and *p*-coumaric acids. The food matrix containing phenolic compounds plays a crucial role in determining their accessibility and bioavailability from food.[5] Thus, higher amounts of VOO could promote the extraction of phenolic compounds from tomato, leading to a higher phenolic content in the sauce.

Through the catalytic oxidation process, hydroxycinnamic acids can undergo oxidation to reactive *O*-quinones, which can interact with NH_2 and SH groups of amino acids.[19] However, in this work caffeic acid increased from 3 to 6 μg/g FW at 15−60 min of thermal treatment, irrespective of the amount of VOO added (5 or 10%) (Figure 4). It has been reported that air-drying tomatoes at 80 °C increases the number of free hydroxyl phenol groups owing to the hydrolysis of flavonoid glycosides and/or the release of cell wall polyphenols.[20] The 2-fold increase in caffeic acid we observed is in accordance with the results of Re et al.,[21] who reported a similar effect of heat treatment. In contrast, it should be noted that we observed the maximum concentration of ferulic acid at 15 min, after which it decreased slightly (at 30, 45, and 60 min). Values for ferulic acid at 15 min were 7.8 μg/g FW and 8.4 μg/g FW in sauces containing 5% and 10% of VOO, respectively (Figure 4). The addition of 10% of VOO slightly increased the amount of all

studied polyphenols, supporting the likelihood that olive oil added in the culinary process is an important factor increasing phenolic extractability.[5]

Changes in Flavonols, Flavanones, and Other Phenols. On the basis of average yearly consumption, red tomatoes contain 15 μg/g FW of flavonoids, determined as aglycones, according to the USDA flavonoid database. Naringenin (45%) is reported to be the main flavonoid in tomatoes, followed by quercetin (39%), myricetin (10%), and kaempferol (5%).[22] Other studies report rutin as the main flavonoid in several tomato cultivars.[14,22] In our work, rutin was the dominant flavonol in all the treatments, followed by naringenin. Naringenin contents increased slightly from 5.4 μg/g FW at 15 min of cooking to 6 μg/g FW at 45 min when sauces contained 5% of VOO. The same pattern was observed in sauces containing 10% olive oil. Longer cooking periods of up to 60 min resulted in a decrease in naringenin, irrespective of the content of added VOO (5 or 10%) (Figure 5). Our results are in accordance with Re et al.,[21] who analyzed the effects of three different methods for processing tomato sauces. The effect of processing on naringenin could be attributed to the formation of reactive quinones and the extensive oxidation process created during thermal processing.[23]

Similarly, rutin decreased from 35 μg/g FW in sauces cooked for 15 min with 5% of VOO to 28.5 μg/g FW after 60 min of cooking. The same pattern was observed in sauces containing 10% of VOO, with rutin decreasing from 30 to 27 μg/g FW during the thermal treatment (Figure 5). Capanoglu et al.[24] found that rutin decreased after heat treatments of up to 80 °C. These results are in agreement with those described previously.[25]

Quercetin decreased more abruptly during the cooking process than rutin, being reduced from 5 to 3 μg/g FW in sauces containing 5% VOO and from 4.7 to 2.30 μg/g FW in sauces containing 10% VOO (Figure 5). The acceleration of quercetin and rutin degradation may be caused by reactive oxygen species that are captured by flavonols. During thermal processing the flavonols are able to transfer one or two hydrogen atoms, forming quinonoid structures, which leads to the formation of quinones.[26] In the case of rutin, the 3-hydroxy-function at the *C*-ring is blocked by a sugar moiety, whereas in quercetin it remains unoccupied, thus explaining the higher stability of rutin toward oxidation.[27] Naringenin and rutin levels were slightly increased by the addition of 10% of VOO, once again suggesting that oil added in the culinary process may enhance the extractability of phenolic compounds from food.[5]

Finally, tyrosol and hydroxytyrosol from tomato sauces were also evaluated. Hydroxytyrosol increased during the cooking process from 15 to 30 min, decreasing thereafter (at 45 and 60 min), whereas tyrosol continued to increase slightly throughout (at 15, 30, 45, and 60 min) (Figure 5). These results are in line with those of Cheikhousman et al.,[28] who reported that during thermal oxidation hydroxytyrosol derivatives are the first antioxidants to be lost, which means they are the first compounds to be oxidized, while tyrosol derivatives seem to be the most stable compounds. This observed pattern is also in agreement with the high antioxidant activity of hydroxytyrosol and its secoiridoid derivatives in VOO compared with tyrosol derivatives.[29] Polyphenols from VOO are effective stabilizers of alpha-tocopherol during heating, thus contributing to the nutritional value of cooked foods.[30] The addition of VOO (5 or 10%) clearly enhanced the levels of both tyrosol and hydroxytyrosol, since both compounds are the main phenolic acids in VOO.[31]

In summary, in this work the effects of cooking time (15, 30, 45, and 60 min) and the amount of added VOO (5 and 10%) on the phenolic profile of tomato sauces were evaluated using HPLC–MS/MS. As a general pattern, the longer the cooking process, the greater the decrease in levels of polyphenols and antioxidants, with the exception of caffeic acid and tyrosol. However, during thermal treatments, free hydroxyl phenol groups may increase due to the hydrolysis of flavonoid glycosides and/or the release of cell wall polyphenols. Tomato sauces prepared with a higher percentage of VOO contained higher levels of polyphenols. Thus, the use of VOO in the culinary process may increase the range of phenolic compounds in the meal, and can also enhance phenolic bioavailability. There is not an increase in the antioxidant capacity with the oil addition, however, this does not mean that the functional capacity or the nutritional properties of these sauces with virgin olive oil have not increased; since a previous human intervention study from our group (data not yet published) demonstrated that the addition of refined oil in tomato sauce increased the antinflammatory properties of the sauce.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Cassidy, A. n.; Mukamal, K. J.; Liu, L.; Franz, M.; Eliassen, A. H.; Rimm, E. B. High anthocyanin intake is associated with a reduced risk of myocardial infarction in young and middle-aged women. *Circulation* **2013**, *127* (2), 188–196.

(2) Kay, C. D.; Hooper, L.; Kroon, P. A.; Rimm, E. B.; Cassidy, A. n. Relative impact of flavonoid composition, dose and structure on vascular function: A systematic review of randomised controlled trials of flavonoid-rich food products. *Mol. Nutr. Food Res.* **2012**, *56* (11), 1605–1616.

(3) Martin-Pelaez, S.; Covas, M. I.; Fito, M.; Kusar, A.; Pravst, I. Health effects of olive oil polyphenols: Recent advances and possibilities for the use of health claims. *Mol. Nutr. Food Res.* **2013**, *57* (5), 760–771.

(4) Visioli, F.; Galli, C. Olive oil phenols and their potential effects on human health. *J. Agric. Food Chem.* **1998**, *46* (10), 4292–4296.

(5) Tulipani, S.; Martínez-Huélamo, M.; Rotchés-Ribalta, M.; Estruch, R.; Ferrer, E.; Andres-Lacueva, C.; Illan, M.; Lamuela-Raventós, R. M. Oil matrix effects on plasma exposure and urinary excretion of phenolic compounds from tomato sauces: Evidence from a human pilot study. *Food Chem.* **2011**, *130*, 581–590.

(6) Torre-Carbot, K.; Jauregui, O.; Gimeno, E.; Castellote, A. I.; Lamuela-Raventos, R. M.; Lopez-Sabater, M. C. Characterization and quantification of phenolic compounds in olive oils by solid-phase extraction, HPLC-DAD, and HPLC-MS/MS. *J. Agric. Food Chem.* **2005**, *53* (11), 4331–4340.

(7) Miro-Casas, E.; Covas, M. I.; Fito, M.; Farre-Albadalejo, M.; Marrugat, J.; de la Torre, R. Tyrosol and hydroxytyrosol are absorbed

from moderate and sustained doses of virgin olive oil in humans. *Eur. J. Clin. Nutr.* **2003**, *57* (1), 186−190.
(8) Sharma, S. K.; LeMaguer, M. Lycopene in tomatoes and tomato pulp fraction. *Ital. J. Food Sci.* **1996**, *8* (2), 107−113.
(9) Goula, A. M.; Adamopoulos, K. G.; Chatzitakis, P. C.; Nikas, V. A. Prediction of lycopene degradation during a drying process of tomato pulp. *J. Food Eng.* **2006**, *74* (1), 37−46.
(10) Vallverdu-Queralt, A.; Medina-Remon, A.; Casals-Ribes, I.; Andres-Lacueva, C.; Waterhouse, A. L.; Lamuela-Raventos, R. M. Effect of tomato industrial processing on phenolic profile and hydrophilic antioxidant capacity. *LWT−Food Sci. Technol.* **2012**, *47* (1), 154−160.
(11) Chang, C. H.; Liu, Y. C. Study on lycopene and antioxidant contents variations in tomatoes under air-drying process. *J Food Sci.* **2007**, *72* (9), E532−E540.
(12) Vallverdú-Queralt, A.; Medina-Remón, A.; Andres-Lacueva, C.; Lamuela-Raventos, R. M. Changes in phenolic profile and antioxidant activity during production of diced tomatoes. *Food Chem.* **2011**, *126* (4), 1700−1707.
(13) Vallverdú-Queralt, A.; Rinaldi de Alvarenga, J. F.; Estruch, R.; Lamuela-Raventos, R. M. Bioactive compounds present in the Mediterranean sofrito. *Food Chem.* **2013**, *141*, 3366−3372.
(14) Vallverdu-Queralt, A.; Medina-Remon, A.; Martinez-Huelamo, M.; Jauregui, O.; Andres-Lacueva, C.; Lamuela-Raventos, R. M. Phenolic profile and hydrophilic antioxidant capacity as chemotaxonomic markers of tomato varieties. *J. Agric. Food Chem.* **2011**, *59* (8), 3994−4001.
(15) Garcia-Villalba, R.; Carrasco-Pancorbo, A.; Oliveras-Ferraros, C.; Vazquez-Martin, A.; Menendez, J. A.; Segura-Carretero, A. Characterization and quantification of phenolic compounds of extra-virgin olive oils with anticancer properties by a rapid and resolutive LC-ESI-TOF MS method. *J. Pharm. Biomed. Anal.* **2010**, *51*, 416−429.
(16) Abushita, A. A.; Daood, H. G.; Biacs, P. A. Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. *J. Agric. Food Chem.* **2000**, *48* (6), 2075−2081.
(17) Dewanto, V.; Wu, X.; Adom, K. K.; Liu, R. H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **2002**, *50* (10), 3010−3014.
(18) Dewanto, V.; Wu, X.; Adom, K. K.; Liu, R. H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **2002**, *50* (10), 3010−3014.
(19) Friedman, M. Dietary impact of food processing. *Annu. Rev. Nutr* **1992**, *12*, 119−137.
(20) Lavelli, V.; Hippeli, S.; Peri, C.; Elstner, E. F. Evaluation of radical scavenging activity of fresh and air-dried tomatoes by three model reactions. *J. Agric. Food Chem.* **1999**, *47* (9), 3826−3831.
(21) Re, R.; Bramley, P. M.; Rice-Evans, C. Effects of food processing on flavonoids and lycopene status in a Mediterranean tomato variety. *Free Radical Res.* **2002**, *36* (7), 803−810.
(22) Slimestad, R.; Fossen, T.; Verheul, M. J. The flavonoids of tomatoes. *J. Agric. Food Chem.* **2008**, *56* (7), 2436−2441.
(23) Singleton, V. L. Oxygen with phenols and related reactions in musts, wines, and model systems - Observations and practical implications. *Am. J. Enol. Vitic.* **1987**, *38* (1), 69−77.
(24) Capanoglu, E.; Beekwilder, J.; Boyacioglu, D.; Hall, R.; de Vos, R. Changes in antioxidant and metabolite profiles during production of tomato paste. *J. Agric. Food Chem.* **2008**, *56* (3), 964−973.
(25) Lavelli, V.; Peri, C.; Rizzolo, A. Antioxidant activity of tomato products as studied by model reactions using xanthine oxidase, myeloperoxidase, and copper-induced lipid peroxidation. *J. Agric. Food Chem.* **2000**, *48* (5), 1442−1448.
(26) Hvattum, E.; Ekeberg, D. Study of the collision-induced radical cleavage of flavonoid glycosides using negative electrospray ionization tandem quadrupole mass spectrometry. *J. Mass Spectrom.* **2003**, *38* (1), 43−49.
(27) Makris, D. P.; Rossiter, J. T. Heat-induced, metal-catalyzed oxidative degradation of quercetin and rutin (quercetin 3-O-rhamnosylglucoside) in aqueous model systems. *J. Agric. Food Chem.* **2000**, *48* (9), 3830−3838.
(28) Cheikhousman, R.; Zude, M.; Bouveresse, D. R.; Léger, C.; Rutledge, D.; Birlouez-Aragon, I. s. Fluorescence spectroscopy for monitoring deterioration of extra virgin olive oil during heating. *Anal. Bioanal. Chem.* **2005**, *382* (6), 1438−1443.
(29) Baldioli, M.; Servili, M.; Perretti, G.; Montedoro, G. F. Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *J. Am. Oil Chem. Soc.* **1996**, *73* (11), 1589−1593.
(30) Pellegrini, N.; Visioli, F.; Buratti, S.; Brighenti, F. Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. *J. Agric. Food Chem.* **2001**, *49* (5), 2532−2538.
(31) Carrasco-Pancorbo, A. a.; Cerretani, L.; Bendini, A.; Segura-Carretero, A.; Lercker, G.; Fernández-Gutiérrez, A. Evaluation of the influence of thermal oxidation on the phenolic composition and on the antioxidant activity of extra-virgin olive oils. *J. Agric. Food Chem.* **2007**, *55* (12), 4771−4780.

4.2 Section II: *Sofrito* cooking process and the ingredients impact bioactive compounds

4.2.1 Publication 3. Bioactive compounds present in the Mediterranean *sofrito*

Anna Vallverdú-Queralt, **José Fernando Rinaldi de Alvarenga**, Ramon Estruch, Rosa Maria Lamuela-Raventós. *Food Chemistry*, 141 (2013) 3365-3372

The aim of this study was to characterize the profile of bioactive compounds presents in commercial tomato *sofrito*. Ten different brands of tomato *sofritos* were analyzed to measure the content of polyphenols and carotenoids. The phenolic profile was identified using a HPLC-LQT-Orbitrap-MS/MS and the content of each polyphenol by UPLC-QqQ-MS/MS and the total polyphenols by Folin-Ciocalteu reaction. The carotenoid content was determinate by HPLC-DAD and the identification confirmed by UPLC-QqQ-MS/MS. Forty phenolic compounds were identified in Mediterranean *sofrito* using high resolution mass spectrometry. Some of these polyphenols were founded in tomato products, but luteolin-*O*-hexoside, sinapic acid-*O*-hexoside, sinapic acid, quercetin-*O*-dihexoside and isoharmnetin-*O*-hexoside were reported for the first time, due to the presence of different ingredients such as onion, garlic and olive oil. The total polyphenols content ranges from 13.20 to 20.23 mg GAE/100 g fresh weight. Among phenolic acids, chlorogenic acid was the major compound, with a content ranging from 4.43 to 5.50 μg/g FW, probably due to presence of onion and garlic as ingredients. Other major phenolic acids were caffeic acid and sinapic acid with levels of 2.01-3.54 and 3.11-4.72 μg/g FW, respectively. Higher levels of ferulic, *p*-hydroxybenzoic and protocatechuic acid were found in garlic contaning *sofritos*. Among flavonols a high content of rutin, quercertin-3-*O*-glucoside and quercetin were found in *sofritos* and correlated with the presence of onion and garlic. Naringenin, the main flavonone in tomato, and naringenin-7-*O*-glucoside were detected at levels of 10.20-13.05 μg/g FW and 3.78-4.23 μg/g FW, respectively, without differences related to the presence of garlic or another ingredient. The content of total carotenoid ranges from 53.10 to 81.49 μg/g FW. The major carotenoid found in *sofrito* was lycopene, especially the isomer trans, at levels of 29.32 to 40.50 μg/g FW, and other isomers such as 13-*Z*-lycopene, 9-*Z*-lycopene and 5-*Z*-lycopene were detected. The higher content of carotenoids was detected in *sofrito* with higher levels of extra virgin olive oil. A PCA analysis was performed to discriminate among *sofritos* according to their phenolics and carotenoids profiles. The metabolites chlorogenic, ferulic and *p*-coumaric acids and rutin, and also *E*-lycopene, β-carotene and lutein had higher discriminant power. The presence of extra virgin olive oil, onion and garlic influenced the phenolic and carotenoid profile of tomato *sofrito.*

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Bioactive compounds present in the Mediterranean *sofrito*

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ABSTRACT

Sofrito is a key component of the Mediterranean diet, a diet that is strongly associated with a reduced risk of cardiovascular events. In this study, different Mediterranean *sofritos* were analysed for their content of polyphenols and carotenoids after a suitable work-up extraction procedure using liquid chromatography/electrospray ionisation-linear ion trap quadrupole-Orbitrap-mass spectrometry (LC/ESI-LTQ-Orbitrap-MS) and liquid chromatography/electrospray ionisation tandem triple quadrupole mass spectrometry (LC/ESI-MS-MS). In this way, 40 polyphenols (simple phenolic and hydroxycinnamoylquinic acids, and flavone, flavonol and dihydrochalcone derivatives) were identified with very good mass accuracy (<2 mDa), and confirmed by accurate mass measurements in MS and MS^2 modes. The high-resolution MS analyses revealed the presence of polyphenols never previously reported in Mediterranean *sofrito*. The quantification levels of phenolic and carotenoid compounds led to the distinction of features among different Mediterranean *sofritos* according to the type of vegetables (garlic and onions) or olive oil added for their production.

1. Introduction

Recent evidence from the PREDIMED (PREvención con DIeta MEDiterránea) study confirms the association of Mediterranean diet and low incidence of cardiovascular events (Estruch et al., 2006; Estruch et al., 2013). The health-promoting effects of this diet have been mainly attributed to a high intake of fruit, vegetables, olive oil, nuts, and cereals; a moderate intake of fish, poultry and wine; and a low intake of dairy products, meat and sweets. In the PREDIMED study, a validated 14-item questionnaire was used to evaluate the adherence of traditional Mediterranean diet (Martinez-Gonzalez et al., 2012; Schroder et al., 2011). One of these 14 items was "how many times per week do you consume vegetables, pasta, rice, or other dishes seasoned with tomato-based *sofrito*?" (Estruch et al., 2006, appendix). An increase of two or more points in this score has been related to a lower risk of cardiovascular events (Estruch et al., 2013). Nevertheless, the bioactive compounds present in commercial *sofrito* have not been previously defined. Tomato consumption has greatly increased worldwide over the past 2 decades, mostly due to a growing demand for products like *sofritos*, which also contain onions and garlic. Regular consumption of tomato-based products has been consistently associated with a reduction in the incidence of chronic degenerative diseases (Grieb et al., 2009; Silaste, Alfthan, Aro, Kesaniemi, & Horkko, 2007). This protective effect has long been attributed to the predominant bioactive compounds in tomato, such as lycopene and other carotenoids (Agarwal & Rao, 2000). Recently, the high content of phenolic compounds in tomato, such as flavonoids and hydroxycinnamic acids, has been attracting interest because of the multiple biological effects that it possesses (Cassidy et al., 2013; Kay, Hooper, Kroon, Rimm, & Cassidy, 2012; Medina-Remon, Estruch Ramon, & Vallverdú-Queralt Anna, 2012). Moreover, onions, the second most important component of *sofritos*, are known to be a very rich source of flavonol compounds that exhibit relatively high scavenging properties compared to those of other vegetables (Griffiths, Trueman, Crowther, Thomas, & Smith, 2002), and are linked to a lower incidence of the development of cancer (Grieb et al., 2009). Another ingredient of some *sofritos* is garlic, which contains phytochemicals that act against the formation of lipid peroxides and scavenging superoxide radicals (Dillon, Burmi, Lowe, Billington, & Rahman, 2003). Protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric and ferulic acids, quercetin and kaempferol have been identified in onion and garlic species (Gorinstein et al., 2008). Moreover, oil added to sofritos may be an important factor governing the *in vivo* bioavailability

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of phenolic compounds, playing a crucial role in their accessibility and extractability from food (Tulipani et al., 2011).

The objective of this study was to extensively examine the polyphenol profile of different Mediterranean *sofritos* through a combination of mass spectrometry (MS) techniques: liquid chromatography/electrospray ionisation-linear ion trap quadrupole-Orbitrap-MS (LC/ESI-LTQ-Orbitrap-MS) and liquid chromatography/electrospray ionisation tandem triple quadrupole MS (LC/ESI-MS-MS). The high-resolution MS analyses allowed the identification of *sofritos* for the first time, as well as the quantification of seven carotenoids: lutein, α-carotene, β-carotene, *trans*-lycopene, 5-, 9- and 13-*cis*-lycopene.

2. Materials and methods

2.1. Standards and samples

All samples and standards were handled without exposure to light. Caffeic, ferulic, *p*-coumaric, protocatechuic, sinapic, *p*-hydroxybenzoic and chlorogenic acids, rutin, quercetin, β-carotene, α-carotene, *trans*-lycopene and lutein, and methyl tert-butyl ether (MTBE) were purchased from Sigma® (Madrid, Spain); naringenin, naringenin-7-*O*-glucoside, quercetin-3-*O*-glucoside and hexane from Extrasynthèse (Genay, France). Ethanol, methanol and HPLC grade formic acid were obtained from Scharlau (Barcelona, Spain) and ultrapure water (Milli-Q) from Millipore (Bedford, USA). Samples were stored at 15 °C and protected from light before analysis. The expiry dates of all samples were at least three months after the analysis. The information contained in the labels is summarised in Table 1.

2.2. Extraction and analysis of polyphenols

2.2.1. Extraction of polyphenols

Each sample was extracted and analysed four times in a darkened room with a red safety light to avoid oxidation of the analytes, following a previously reported procedure (Vallverdú-Queralt, Jáuregui, Medina-Remón, Andrés-Lacueva, & Lamuela-Raventós, 2010) with some modifications.

Sofritos (0.5 g) were weighed and homogenised with 80% ethanol in Milli-Q water (4 mL). The homogenate was sonicated for 5 min and centrifuged (4000 rpm at 4 °C) for 15 min. The supernatant was transferred into a flask and extraction was repeated. Both supernatants were combined and evaporated under nitrogen flow. Finally, the residue was reconstituted with up to 2 mL Milli-Q water containing 0.1% of formic acid and filtered through a 13 mm, 0.45 μm Polytetrafluoroethylene (PTFE) filter from Waters (Milford, USA) into an insert-amber vial.

Solid phase extraction (SPE) was carried out to eliminate interferences such as ascorbic acid, amino acids and reducing sugars. For this procedure, Oasis® MAX cartridges with 30 mg of mixed-mode anion-exchange and reversed-phase sorbent from Waters (Milford, USA) were used following a previously reported procedure (Vallverdú-Queralt et al., 2010). The eluted fractions were evaporated under nitrogen flow and the residue was reconstituted with up to 500 μL Milli-Q water containing 0.1% formic acid and filtered through a 13 mm, 0.45 μm PTFE filter into an insert-amber vial for HPLC analysis.

2.2.2. HPLC-LTQ-Orbitrap-MS and HPLC-MS/MS analysis

For accurate mass measurements, an LTQ Orbitrap Velos mass spectrometer (Thermo Scientific, Hemel Hempstead, UK) equipped with an ESI source in negative mode was used to acquire mass spectra in profile mode with a setting of 30,000 resolution at *m/z* 400. Operation parameters were as follows: source voltage, 4 kV; sheath gas, 20 (arbitrary units); auxiliary gas, 10 (arbitrary units); sweep gas, 2 (arbitrary units); and capillary temperature, 275 °C. Default values were used for most other acquisition parameters (FT Automatic gain control (AGC) target $5{\cdot}10^5$ for MS mode and $5{\cdot}10^4$ for MS^n mode). *Sofritos* were first analysed in full MS mode with the Orbitrap resolution set at 30,000 at *m/z* 400. The successive analyses were done in MS^n mode with the Orbitrap resolution set at 15,000 at *m/z* 400. An isolation width of 100 amu was used and precursors were fragmented by high-energy C-trap dissociation (HCD) with normalised collision energy of 2% and an activation time of 10 ms. The maximum injection time was set to 100 ms with two micro scans for MS mode and to 1000 ms with one micro scan for MS^n mode. The mass range was from *m/z* 100 to 1000. The data analyses were performed using XCalibur software. The liquid chromatograph was an Accela (Thermo Scientific, Hemel Hempstead, UK) equipped with a quaternary pump, a photodiode array detector (PDA) and a thermostated autosampler. A Luna C_{18} column 50 × 2.0 mm i.d, 5 μm (Phenomenex, Torrance, CA, USA) was used. Gradient elution was performed with water/0.1% formic acid (solvent A) and acetonitrile/0.1% formic acid (solvent B) at a constant flow rate of 0.4 mL/min, and injection volume was 5 μL. An increasing linear gradient of solvent B was used. Separation was carried out in 15 min under the following conditions: 0 min, 5% B; 10 min, 18% B; 12 min, 100% B; 13 min, 100% B; 15 min, 5% B. The column was equilibrated for 5 min prior to each analysis. These conditions were adapted from a previous study with some modifications (Vallverdú-Queralt et al., 2010).

The elemental composition of each polyphenol was selected according to the accurate masses and isotopic pattern (through the Formula Finder feature in Analyst QS 2.0) and searched for in the Dictionary of Natural Products (Chapman & Hall/CRC), the MOTO database (http://appliedbioinformatics.wur.nl/moto) and the Plant Metabolic Network. The interpretation of the observed MS/MS spectra in comparison with those found in the literature was the main tool for putative identification of polyphenols.

HPLC–ESI-MS/MS was used to evaluate the content of flavonols, flavanones and hydroxycinnamic acids following the procedure of

Table 1
Sample information reported on the labels.

Sample code	Ingredients	Packaging
Sofrito A	Tomato, sunflower oil, fresh onion, fresh garlic, corn flour, natural flavours	Tetra pak
Sofrito B	Tomato and tomato concentrate, sunflower oil, sugar, corn flour, salt, onion, garlic, white pepper, natural flavours	Clear glass bottle
Sofrito C	Tomato, water, onion, oil, sugar, garlic, salt, almond	Clear glass bottle
Sofrito D	Tomato, onion, sunflower oil, corn flour, sugar, garlic, salt	Tetra pak
Sofrito E	Tomato concentrate, extra-virgin olive oil, sugar, corn flour, onion, garlic, salt	Clear glass bottle
Sofrito F	Tomato concentrate, sunflower oil, corn flour modify, onion, garlic, sugar, salt, citric acid	Tetra pak
Sofrito G	Tomato, onion, sugar, sunflower oil, salt, corn flour, citric acid, spice	Clear glass bottle
Sofrito H	Tomato concentrate, sugar, sunflower oil, salt, corn flour, onion	Clear glass bottle
Sofrito I	Tomato, onion, sunflower oil, salt, corn flour, salt	Clear glass bottle
Sofrito J	Tomato, onion, olive oil, sugar, salt, corn flour	Clear glass bottle

other studies (Vallverdú-Queralt, Jáuregui, Medina-Remon, & Lamuela-Raventos, 2012; Vallverdú-Queralt et al., 2011).

Liquid chromatography was performed with an Agilent series 1100 HPLC instrument (Agilent, Waldbronn, Germany) equipped with a quaternary pump, an autosampler and a column oven set to 30 °C. Mobile phase consisted of Milli-Q water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The injection volume was 20 μL and the flow rate was 0.4 mL/min. Separation was carried out in 15 min under the conditions described above.

An API 3000 (PE Sciex, Concord, Ontario, Canada) triple quadrupole mass spectrometer equipped with a Turbo Ionspray source in negative-ion mode was used to obtain MS/MS data. Turbo Ionspray source settings were as follows: capillary voltage, −3500 V; nebulizer gas (N_2), 10 a.u. (arbitrary units); curtain gas (N_2), 12 a.u.; collision gas (N_2), 4 a.u.; focusing potential, −200 V; entrance potential, −10 V; drying gas (N_2), heated to 400 °C and introduced to a flow rate of 6000 cm^3/min. The declustering potential and collision energy were optimised for each compound in infusion experiments: individual standard solutions (10 μg/mL) dissolved in 1:1 (v/v) mobile phase were infused at a constant flow rate of 5 μL/min using a model syringe pump (Harvard Apparatus, Holliston, MA, USA). For quantification purposes, data were collected in the multiple reaction monitoring (MRM) mode, tracking the transition of parent and product ions specific for each compound.

Quantification of polyphenols was performed by the internal standard method and results were expressed as μg/g fresh weight (FW). The internal standard used was ethyl gallate.

2.2.3. Analysis of total polyphenols (TP)

For the TP assay, each sample was analysed four times; 20 μL of the eluted fractions was mixed with 188 μL of Milli-Q water in a thermo microtiter 96-well plate (nunc™, Roskilde, Denmark), and 12 μL of F–C reagent and 30 μL of sodium carbonate (200 g/L) was added following the procedure described by Vallverdú-Queralt, Medina-Remon, et al. (2011). The mixtures were incubated for 1 h at room temperature in the dark. After the reaction period, 50 μL of Milli-Q water was added and the absorbance was measured at 765 nm in a UV/Vis Thermo Multiskan Spectrum spectrophotometer (Vantaa, Finland). This spectrophotometer allowed the absorbance of a 96-well plate to be read in 10 s. Results were expressed as mg of gallic acid equivalents (GAE)/100 g FW.

2.3. Extraction and analysis of carotenoid compounds

2.3.1. Extraction of carotenoids

Each sample was extracted and analysed four times in a darkened room with a red safety light to avoid oxidation of the analytes, according to a previously reported method with minor modifications (Vallverdú-Queralt, Martinez-Huelamo, Arranz-Martinez, Miralles, & Lamuela-Raventos, 2012).

Sofritos (0.5 g) were weighed and homogenised with 5 mL EtOH: Hexane (4:3 v/v); the homogenate was sonicated for 5 min and centrifuged (4000 rpm at 4 °C) for 15 min. The supernatant was transferred into a flask and extraction was repeated. Both supernatants were combined and evaporated under nitrogen flow. Finally, the residue was reconstituted with up to 1 mL of MTBE and filtered through a 25 mm, 0.45 μm PTFE filter into an insert-amber vial for HPLC analysis.

2.3.2. Analysis of carotenoids

Chromatographic analysis was performed using the HPLC system described above for polyphenol analysis. The analytes were separated on a C_{30} column 250×4.6 mm i.d, 5 μm (YMC™, Waters Co., Milford, MA, USA) and kept at 20 °C. The injection volume was 20 μL and flow rate 1 mL/min. Mobile phases consisted of water (A), methanol (B) and MTBE (C). Separation was carried out in 23 min under the following conditions: 0 min, 70% B; 10 min, 20% B; 20 min, 6% B; 21 min, 6% B; 23 min, 70% B. Water was kept constant at 4% throughout the analysis. The column was equilibrated for 10 min prior to each analysis. The analysis was carried out following the procedure of other authors (Matea, Soran, Pintea, & Bele, 2010). MTBE was used as a modifier to facilitate elution of lycopene, which is strongly retained in a methanol environment.

Commercially available carotenoid standards (lutein, α-carotene, β-carotene and *trans*-lycopene) were used to identify analytes by retention times and ultraviolet–visible (UV–VIS) spectra. The HPLC-UV chromatograms were acquired selecting the 450 nm wavelength. Afterwards, the UV–VIS spectra were recorded in the range of 350–550 nm for the tentative identification of carotenoids and their geometrical isomers on the basis of the retention times and absorption spectrum characteristics described in the literature (Frohlich, Conrad, Schmid, Breithaupt, & Bohm, 2007; Matea et al., 2010).

The API 3000 (PE Sciex, Concord, Ontario, Canada) triple quadrupole mass spectrometer in positive-ion mode was used to obtain MS/MS data for carotenoid analysis. Turbo Ionspray source settings were as follows: capillary voltage, 4500 V; nebulizer gas (N_2), 10 a.u. (arbitrary units); curtain gas (N_2), 12 a.u.; collision gas (N_2), 4 a.u.; focusing potential, 200 V; entrance potential, 10 V; drying gas (N_2), heated to 400 °C and introduced to a flow rate of 6000 cm^3/min. The declustering potential and collision energy were optimised for each compound in infusion experiments. A solvent delivery system connected post-column was used for the delivery of 100 μL/min of a LiCl solution at a concentration of 500 ppm.

Carotenoids were quantified related to their corresponding standards and results were expressed as μg/g FW. When standards were not available, as in the case of 5-, 9–13-*cis*-lycopene, the carotenoids were quantified related to *trans*-lycopene. The total carotenoid content was obtained by the sum of each individual carotenoid.

3. Results and discussion

3.1. Phenolic profile of Mediterranean sofritos

From a technological point of view, the main difficulty in the production of these Mediterranean *sofritos* is choosing the right variety of tomatoes with suitable characteristics. Good commercial practices can maintain the physicochemical parameters of tomatoes, onions, garlic and other ingredients and provide optimum products. Tomato varieties and agricultural conditions, such as cultivar selection, temperature, light exposure, fertilisation and season, may have an influence on phenolic accumulation (Slimestad & Verheul, 2009).

Table 2 shows the list of 40 compounds identified in Mediterranean *sofritos* by HPLC-ESI-LTQ-Orbitrap-MS along with their retention times (rt), accurate mass (acc. mass), molecular formula (MF), mDa of error between the mass found and the accurate mass of each polyphenol and the MS–MS ions used for identification. To our knowledge, this research constitutes the first comprehensive identification of phenolic compounds in Mediterranean *sofritos* by HPLC coupled to high-resolution mass spectrometry. Some compounds have been previously identified in raw tomatoes (Vallverdú-Queralt et al., 2010), but luteolin-*O*-hexoside, sinapic acid-*O*-hexoside, sinapic acid, quercetin *O*-dihexoside and isoharmnetin-*O*-hexoside are reported for the first time in Mediterranean *sofritos*, their presence probably due to the use of additional ingredients like garlic or onions for the production of these tomato-based products. Onions are rich in bioactive compounds such as the flavonol quercetin (Rohn, Buchner, Driemel, Rauser, & Kroh,

Table 2
List of polyphenols tentatively identified in Mediterranean *sofritos*.

Compound	rt	$[M-H]^-$	MS/MS ions	Acc Mass	Mda	MF
Protocatechuic acid*	2,08	153	153 (40), 109 (90)	153,0193	0,18	C7H6O4
Caffeic acid-*O*-hexoside 1	2,98	341	179 (100), 135 (10)	341,0877	0,52	C15H18O9
p-hydroxibenzoic acid*	3,29	137	137 (20), 93 (100)	137,0244	1,08	C7H6O3
Neochlorogenic acid	3,36	353	191 (100), 179 (70), 135 (20)	353,0877	0,32	C16H18O9
Luteolin-*O*-hexoside	3,43	447	447 (30), 285 (100)	447,0933	0,63	C21H20O11
Caffeic acid-*O*-hexoside 2	3,81	341	179 (100), 135 (10)	341,0877	0,62	C15H18O9
Vanillic acid-*O*-hexoside	4,05	329	329 (10), 167 (100)	329,0877	0,13	C14H18O9
Homovanillic acid-*O*-hexoside 1	4,25	343	181 (100), 137 (10)	343,1034	1,13	C15H20O9
Homovanillic acid-*O*-hexoside 2	4,56	343	181 (100), 137 (10)	343,1034	0,92	C15H20O9
Chlorogenic acid*	4,69	353	191 (100)	353,0877	0,24	C16H18O9
Caffeic acid-*O*-hexoside 3	4,95	341	179 (100)	341,0877	1,44	C15H18O9
Cryptochlorogenic acid	5,15	353	191(50), 173 (100), 135 (20)	353,0877	0,19	C16H18O9
Sinapic acid-*O*-hexoside	5,19	385	385 (20), 223 (100)	385,1139	0,22	C17H22O10
Ferulic acid-*O*-hexoside	5,22	355	355 (20), 193 (100), 178 (30)	355,1034	0,08	C16H20O9
Chlorogenic acid isomer	5,96	353	191 (100)	353,0877	0,29	C16H18O9
Coumaroylquinic acid	6,21	337	191 (100), 163 (30)	337,0930	0,28	C16H18O8
Caffeic acid*	6,85	179	179 (10), 135 (100)	179,0349	1,36	C9H8O4
p-Coumaric acid*	7,12	163	163 (20), 119 (100)	163,0400	0,43	C9H8O3
Quercetin-*O*-dihexoside	7,69	625	463 (100), 301 (20)	625,1410	1,16	C27H30O17
Naringenin-*C*-hexoside	8,35	433	433 (20), 343(50), 313 (100)	433,1140	0,15	C21H22O10
Sinapic acid*	8,40	223	223 (10), 208 (10), 179 (100)	223,0611	0,08	C11H12O5
Rutin-*O*-hexoside	8,97	771	609 (100), 301 (20)	771,1989	1,23	C33H40O21
Eriodictyol-*O*-hexoside 1	9,02	449	449 (10), 287 (100)	449,1089	0,18	C21H22O11
Ferulic acid*	9,56	193	193 (10), 178 (70), 149 (30), 134 (100)	193,0506	0,25	C10H10O4
Quercetin-3-*O*-glucoside*	10,02	463	463 (30), 301 (100)	463,0881	0,15	C21H20O12
Eriodictyol-*O*-hexoside 2	10,25	449	449 (10), 287 (100)	449,1089	0,14	C21H22O11
Rutin-*O*-pentoside	10,93	741	609 (100), 301 (30)	741,1883	0,46	C32H38O20
Naringenin-*O*-hexoside	11,01	433	433 (10), 271 (100)	433,1140	0,28	C21H22O10
Rutin*	11,16	609	609 (15), 301 (100)	609,1460	1,15	C27H30O16
Dicaffeoylquinic acid 1	11,20	515	515 (70), 353 (50), 191 (100)	515,1194	0,09	C25H24O12
Phloretin-*C*-diglycoside	11,25	597	597 (20), 477 (40), 417 (60), 387 (90), 357 (100)	597,1819	0,21	C27H34O15
Kaempferol-3-*O*-rutinoside	11,35	593	593 (10), 285 (100)	593,1511	0,16	C27H30O15
Naringenin-7-*O*-glucoside (prunin)*	11,45	433	433 (20), 271 (100)	433,1140	0,33	C21H22O10
Quercetin-*O*-hexoside	11,52	463	463 (20), 301 (100)	463,0881	0,19	C21H20O12
Eriodictyol-*O*-hexoside 3	11,59	449	449 (10), 287 (100)	449,1089	0,23	C21H22O11
Dicaffeoylquinic acid 2	11,73	515	515 (70), 353 (50), 191 (100)	515,1194	0,12	C25H24O12
Isoharmnetin-*O*-hexoside	11,80	477	477 (10), 315 (100)	477,1038	0,67	C22H22O12
Eriodictyol	11,89	287	287(15), 151 (100)	287,0560	1,08	C15H12O6
Naringenin*	12,07	271	271 (15), 151 (100)	271,0611	0,29	C15H12O5
Quercetin*	13,63	301	301 (10), 151 (100)	301,0353	0,49	C15H10O7

* Comparison with standard. rt, retention time; Acc. mass, accurate mass; MF, molecular formula; mDa, millidaltons of error between the mass found and the accurate mass of each polyphenol.

2007; Santas, Carbo, Gordon, & Almajano, 2008), while garlic species have been reported to contain *p*-coumaric, ferulic and sinapic acids, isoquercitrin, rutin, quercitrin, quercitin, luteolin, kaempferol and apigenin (Parvu, Toiu, Vlase, & Alina Parvu, 2010). Additionally, Gorinstein et al. detected protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric and ferulic acids, quercetin and kaempferol in onion and garlic species (Gorinstein et al., 2008). It is known that the processing of garlic and onions may influence their content of bioactive compounds (Gorinstein et al., 2005; Gorinstein et al., 2007). Olive oil added to *sofritos* could be an important factor governing the *in vivo* bioavailability of phenolic compounds, playing a crucial role in their accessibility and extractability from food (Tulipani et al., 2011). Luteolin, apigenin, ferulic, caffeic, vanillic, gallic, *p*-hydroxybenzoic and *p*-coumaric acids have been previously identified in extra-virgin olive oils (Bakhouche et al., 2013; Ballus, Meinhart, Bruns, & Godoy, 2011).

Table 3
Total Polyphenols of Mediterranean *sofritos* (mg GAE/100 g FW) expressed as mean ± SD ($n = 4$). Different Letters in the Columns Represent Statistically Significant Differences ($P < 0.05$).

	Total polyphenols(mg GAE/100 g FW)
Sofrito A	17.43 ± 0.43^{a}
Sofrito B	17.02 ± 0.31^{a}
Sofrito C	18.30 ± 0.30^{b}
Sofrito D	17.72 ± 0.34^{a}
Sofrito E	20.23 ± 0.21^{c}
Sofrito F	$17.99 \pm 0.12^{a,b}$
Sofrito G	14.80 ± 0.08^{d}
Sofrito H	14.55 ± 0.15^{d}
Sofrito I	13.20 ± 0.29^{e}
Sofrito J	16.65 ± 0.16^{f}

SD: standard deviation; GAE: gallic acid equivalent; FW: fresh weight.

Consistent differences ($P < 0.05$) in TP content were observed among the different Mediterranean *sofritos* (Table 3), ranging from 13.20 to 20.23 mg GAE/100 g FW. These values are higher than the TP content in raw tomatoes reported by Vallverdú-Queralt et al., which ranged from 8.60 to 13.32 mg of GAE/100 g FW (Vallverdú-Queralt, Medina-Remon, et al., 2011). This difference in TP levels could be partially explained by the lower water content of *sofritos* in comparison with raw tomatoes, but our results also suggest that the addition of ingredients such as garlic, onion or olive oil could increase TP content.

3.2. Quantification of individual polyphenols in Mediterranean sofritos

The results of the quantitative determination of the target polyphenols are summarised in Table 4. The statistically significant differences ($P < 0.05$) found between the Mediterranean *sofritos* for

each polyphenol analysed are highlighted with different superindices.

Chlorogenic acid was found to be the main phenolic acid in *sofritos*, varying from 4.43 to 5.50 μg/g FW, compared with 0.36 to 0.46 μg/g FW in raw tomatoes (Vallverdú-Queralt, Medina-Remon, et al., 2011). The higher chlorogenic acid levels found in this work are probably due to the onion and garlic ingredients typical of Mediterranean *sofritos*. Sinapic and caffeic acid levels were 2.01–3.54 μg/g FW and 3.11–4.72 μg/g FW, respectively. Caffeic acid is found in very low concentrations in free forms only, and sinapic acid is predominantly found in ester-bound forms (Gorinstein et al., 2004). It should be noted that sinapic and caffeic acid concentrations were significantly ($P < 0.05$) higher in *sofritos* A–F, which contained garlic as a main ingredient, suggesting that these differences could be attributed to the polyphenols found in garlic. Gorinstein et al. reported that the major phenolic compound in garlic was caffeic acid (Gorinstein et al., 2008), and that the sum of hydroxycinnamic acids (caffeic, *p*-coumaric, ferulic, and sinapic acids) was two times higher in garlic than in onions (Gorinstein et al., 2005).

Higher levels of ferulic and protocatechuic acids were also found in garlic-containing *sofritos* A–F. Ferulic acid ranged from 2.40 to 3.89 μg/g FW in *sofritos* A–F, and 1.99 to 2.33 μg/g FW in *sofritos* G–J. The same pattern was followed by protocatechuic acid, which is a major component in red onions and has also been detected in garlic (Gorinstein et al., 2008), while ferulic acid has been detected in garlic species (Beato, Orgaz, Mansilla, & Montao, 2011). The enhanced ferulic and protocatechuic acid concentration was highly reproducible in garlic-containing *sofritos* A–F, which also showed higher values of *p*-coumaric acid, previously detected in both garlic and virgin olive oil (El Riachy, Priego-Capote, Leon, Luque de Castro, & Rallo, 2012; Gorinstein et al., 2008).

Another phenolic acid, *p*-hydroxybenzoic acid, was detected at relatively high levels in *sofritos* A–F, ranging from 2.98 to 3.33 μg/g FW, whereas in garlic-free sofritos G–J, values were between 2.33 and 2.69 μg/g FW. *p*-Hydroxybenzoic acid has been previously detected in garlic species (Beato et al., 2011; Gorinstein et al., 2008).

Among the flavonols present in Mediterranean *sofritos*, rutin was predominant, ranging from 12.60 to 21.30 μg/g FW. Rutin has been previously detected as the main polyphenol in tomatoes, tomato sauces and diced tomatoes (Vallverdú-Queralt, Medina-Remón, Andres-Lacueva, & Lamuela-Raventos, 2011; Vallverdú-Queralt et al., 2012; Vallverdú-Queralt, Medina-Remon, et al., 2011). There was no marked tendency for rutin content to be higher in *sofritos* containing garlic (A–F). Quercetin and quercetin-3-*O*-glucoside were also detected, ranging from 8.05 to 14.50 μg/g FW, and from 3.49 to 4.20 μg/g FW, respectively, which are higher values than those reported in raw tomatoes (Vallverdú-Queralt, Medina-Remon, et al., 2011). The main polyphenols in onions are flavonoids such as quercetin (Rohn et al., 2007; Santas et al., 2008), but the higher concentration of quercetin and its glucoside form in *sofritos* A–F might also have been due to the additional ingredient of garlic. This would be in agreement with Miean and Mohamed (2001), who reported relatively high amounts of quercetin in garlic (Miean & Mohamed, 2001).

Lastly, naringenin, which is the main flavanone in tomato and tomato sauces (Tulipani et al., 2011; Vallverdú-Queralt, Medina-Remon, et al., 2012) and naringenin-7-*O*-glucoside were also detected in the *sofritos*, at levels of 10.20–13.05 μg/g FW and 3.78–4.23 μg/g FW, respectively, without differences related to the presence of garlic or other ingredients. Naringenin (45%) is reported to be the main flavonoid in tomatoes, followed by quercetin (39%), myricetin (10%) and kaempferol (5%) (Slimestad, Fossen, & Verheul, 2008).

Table 4
Quantification of individual polyphenols of Mediterranean *sofritos* (μg/g FW) expressed as mean ± SD ($n = 4$). Different letters in the columns represent statistically significant differences ($P < 0.05$).

	Ferulic acid	Chlorogenic acid	Caffeic acid	Sinapic acid	Protocatechuic acid	*p*-Coumaric acid	*p*-Hydroxybenzoic acid	Rutin	Quercetin	Quercetin-3-*O*-glucoside	Naringenin	Naringenin-7-*O*-glucoside
Sofrito A	3.02 ± 0.12^{a}	5.16 ± 0.15^{a}	3.88 ± 0.18^{a}	2.91 ± 0.09^{a}	1.84 ± 0.05^{a}	3.40 ± 0.06^{a}	3.15 ± 0.14^{a}	15.40 ± 0.65^{a}	12.55 ± 0.21^{a}	4.07 ± 0.12^{a}	10.20 ± 0.40^{a}	4.10 ± 0.12^{a}
Sofrito B	2.40 ± 0.08^{b}	4.80 ± 0.16^{b}	3.98 ± 0.21^{b}	$2.94 \pm 0.07^{a,b}$	1.90 ± 0.08^{b}	3.30 ± 0.09^{b}	3.24 ± 0.21^{b}	12.60 ± 0.60^{b}	10.10 ± 0.33^{b}	4.10 ± 0.10^{a}	11.30 ± 0.35^{b}	4.23 ± 0.08^{b}
Sofrito C	$3.10 \pm 0.11^{a,c}$	5.11 ± 0.21^{a}	3.90 ± 0.23^{a}	2.99 ± 0.05^{b}	1.83 ± 0.06^{a}	$3.35 \pm 0.05^{a,b}$	3.33 ± 0.13^{c}	21.30 ± 0.53^{c}	14.50 ± 0.42^{c}	3.78 ± 0.13^{b}	10.30 ± 0.25^{a}	3.87 ± 0.05^{c}
Sofrito D	2.55 ± 0.14^{d}	4.43 ± 0.19^{c}	4.10 ± 0.14^{c}	3.12 ± 0.04^{c}	1.73 ± 0.04^{c}	3.28 ± 0.09^{b}	3.10 ± 0.10^{d}	17.50 ± 0.43^{d}	12.90 ± 0.38^{a}	3.69 ± 0.12^{b}	12.60 ± 0.41^{c}	$4.05 \pm 0.09^{a,d}$
Sofrito E	3.89 ± 0.16^{e}	5.50 ± 0.21^{d}	4.72 ± 0.12^{d}	3.54 ± 0.10^{d}	2.26 ± 0.07^{d}	4.01 ± 0.07^{c}	3.01 ± 0.08^{e}	16.66 ± 0.67^{e}	13.77 ± 0.25^{d}	4.20 ± 0.09^{c}	10.33 ± 0.44^{a}	4.21 ± 0.05^{b}
Sofrito F	3.16 ± 0.13^{c}	5.01 ± 0.13^{e}	3.87 ± 0.15^{a}	2.88 ± 0.08^{a}	2.15 ± 0.09^{e}	3.43 ± 0.10^{a}	2.98 ± 0.13^{e}	18.40 ± 0.55^{f}	13.01 ± 0.31^{a}	3.79 ± 0.07^{b}	11.99 ± 0.32^{d}	3.97 ± 0.06^{d}
Sofrito G	2.30 ± 0.18^{f}	$4.90 \pm 0.12^{b,c}$	3.11 ± 0.13^{e}	2.19 ± 0.15^{e}	1.15 ± 0.04^{f}	2.67 ± 0.05^{d}	2.51 ± 0.22^{f}	15.61 ± 0.61^{a}	9.33 ± 0.26^{e}	3.50 ± 0.15^{d}	11.71 ± 0.36^{d}	3.78 ± 0.03^{e}
Sofrito H	2.08 ± 0.08^{g}	$5.06 \pm 0.10^{a,e}$	3.20 ± 0.22^{f}	2.14 ± 0.09^{e}	1.00 ± 0.06^{g}	2.89 ± 0.07^{e}	2.33 ± 0.33^{g}	20.50 ± 0.43^{g}	8.90 ± 0.21^{f}	3.45 ± 0.17^{d}	10.24 ± 0.21^{a}	3.86 ± 0.04^{e}
Sofrito I	1.99 ± 0.05^{g}	4.80 ± 0.19^{b}	3.40 ± 0.17^{g}	2.01 ± 0.12^{f}	1.01 ± 0.07^{g}	2.91 ± 0.11^{e}	2.44 ± 0.31^{h}	15.70 ± 0.29^{a}	8.21 ± 0.27^{g}	3.49 ± 0.08^{d}	12.94 ± 0.29^{c}	3.94 ± 0.06^{d}
Sofrito J	2.33 ± 0.09^{f}	5.20 ± 0.18^{a}	3.48 ± 0.14^{g}	2.08 ± 0.16^{g}	1.09 ± 0.05^{h}	2.95 ± 0.04^{f}	2.69 ± 0.14^{i}	18.20 ± 0.37^{f}	8.05 ± 0.25^{g}	3.47 ± 0.10^{d}	13.05 ± 0.44^{c}	$4.01 \pm 0.08^{a,d}$

Table 5
Quantification of individual polyphenols of Mediterranean *sofritos* (μg/g FW) expressed as mean ± SD ($n = 4$). The superscript letters in the columns represent statistically significant differences ($P < 0.05$).

	trans-α-Carotene	*trans*-β-Carotene	*trans*-Lutein	*trans*-Lycopene	5-*cis*-Lycopene	9-*cis*-Lycopene	13-*cis*-Lycopene	Total carotenoid content
Sofrito A	0.83 ± 0.02^{a}	18.12 ± 0.13^{a}	2.96 ± 0.02^{a}	33.40 ± 0.12^{a}	2.05 ± 0.04^{a}	1.44 ± 0.03^{a}	1.99 ± 0.10^{a}	60.79^{a}
Sofrito B	1.01 ± 0.01^{b}	25.40 ± 0.15^{b}	2.85 ± 0.03^{b}	35.60 ± 0.16^{b}	2.20 ± 0.05^{b}	1.22 ± 0.02^{b}	1.76 ± 0.11^{b}	70.04^{b}
Sofrito C	0.79 ± 0.02^{a}	21.20 ± 0.11^{c}	3.01 ± 0.02^{a}	31.80 ± 0.11^{c}	2.44 ± 0.08^{c}	1.39 ± 0.05^{c}	1.83 ± 0.09^{c}	62.46^{a}
Sofrito D	0.85 ± 0.01^{a}	19.40 ± 0.12^{d}	3.10 ± 0.05^{c}	30.40 ± 0.18^{d}	3.01 ± 0.09^{d}	0.99 ± 0.01^{d}	2.01 ± 0.08^{a}	59.76^{a}
Sofrito E	1.10 ± 0.03^{c}	29.69 ± 0.11^{e}	3.42 ± 0.04^{d}	40.50 ± 0.33^{e}	2.81 ± 0.07^{e}	1.88 ± 0.10^{e}	2.09 ± 0.07^{a}	81.49^{c}
Sofrito F	0.81 ± 0.02^{a}	21.20 ± 0.13^{f}	2.91 ± 0.04^{a}	30.20 ± 0.16^{d}	3.08 ± 0.08^{d}	1.81 ± 0.08^{e}	1.72 ± 0.04^{b}	61.73^{a}
Sofrito G	0.73 ± 0.01^{d}	15.60 ± 0.11^{g}	2.55 ± 0.03^{f}	29.32 ± 0.13^{d}	2.50 ± 0.04^{c}	1.21 ± 0.02^{b}	1.76 ± 0.04^{b}	53.67^{d}
Sofrito H	0.69 ± 0.02^{d}	13.30 ± 0.12^{h}	2.41 ± 0.05^{g}	31.35 ± 0.18^{c}	2.20 ± 0.03^{b}	1.33 ± 0.04^{c}	1.82 ± 0.05^{c}	53.10^{d}
Sofrito I	0.68 ± 0.02^{d}	17.80 ± 0.19^{i}	2.58 ± 0.03^{f}	30.13 ± 0.28^{d}	2.99 ± 0.01^{d}	1.41 ± 0.09^{a}	1.83 ± 0.06^{c}	57.42^{e}
Sofrito J	0.71 ± 0.01^{d}	16.50 ± 0.21^{j}	2.51 ± 0.02^{f}	29.86 ± 0.14^{d}	3.01 ± 0.09^{d}	1.39 ± 0.05^{c}	1.93 ± 0.05^{a}	55.91^{d,e}

3.3. Quantification of individual carotenoids in Mediterranean sofritos

The results of the quantitative determination of the target carotenoids are summarised in Table 5. The statistically significant differences ($P < 0.05$) found among the Mediterranean *sofritos* for each carotenoid analysed are highlighted with different superindices.

β-carotene levels in Mediterranean *sofritos* ranged from 13.30–17.80 μg/g FW in *sofritos* G–J to 18.12–29.69 μg/g FW in *sofritos* A–F, with α-carotene following the same pattern. β-carotene has been detected in onions at levels of around 112 μg/100 g DW (Hart & Scott, 1995). Lutein was also detected at significantly ($P < 0.05$) higher levels (2.85–3.42 μg/g FW) in garlic-containing *sofritos* A–F than in *sofritos* G–J (2.41–2.58 μg/g FW). Lutein has previously been detected in garlic (Aruna & Baskaran, 2010), although otherwise there is little information available in the literature on lutein and α-carotene in onion and garlic species.

trans-Lycopene was detected in Mediterranean *sofritos* at levels of 29.32–40.50 μg/g FW (Table 5), which is in line with results reported by other studies (Lenucci et al., 2009; Lenucci, Cadinu, Taurino, Piro, & Dalessandro, 2006), who found that tomatoes are characterised by a high lycopene content (>200 mg kg^{-1} fresh weight). The differences in the lycopene content among the Mediterranean *sofritos* could be mainly due to genotypic factors and to an enhanced enzymatic activity of phytoene synthase I, which causes a high production of lycopene precursors in ripening grade tomatoes (Fraser, Enfissi, & Bramley, 2009). It should be noted that *sofrito* E contained higher levels of lycopene than the other *sofritos*, possibly due to its virgin olive oil content. The combination of tomatoes and tomato-based products with a lipid matrix has been reported to favour the extractability and bioaccessibility of tomato carotenoids (Fielding, Rowley, Cooper, & O'Dea, 2005; Graziani et al., 2003).

The Mediterranean *sofritos* were also analysed for their content of *cis*-lycopene isomers (Table 5), which are believed to be more bioavailable (Boileau, Boileau, & Erdman, 2002) than *trans*-isomers, at least partially because of increased micellarization and uptake by the enterocyte (Failla, Chitchumroonchokchai, & Ishida, 2008). Therefore, human studies have been focused on the nutritional benefits of consuming tomato products with a high percentage of *cis*-isomers, 5-, 9-, 13-, and 15-*cis*-lycopene being predominant (Frohlich et al., 2007). It has been shown that in the cooking process, lycopene undergoes *trans/cis*-isomerization, increasing the proportion of *cis*-isomers (Lin & Chen, 2005). In this work, there were no marked differences among the *cis*-lycopene isomers found in the *sofritos:* 5-*cis*-lycopene levels ranged from 2.05 to 3.08 μg/g

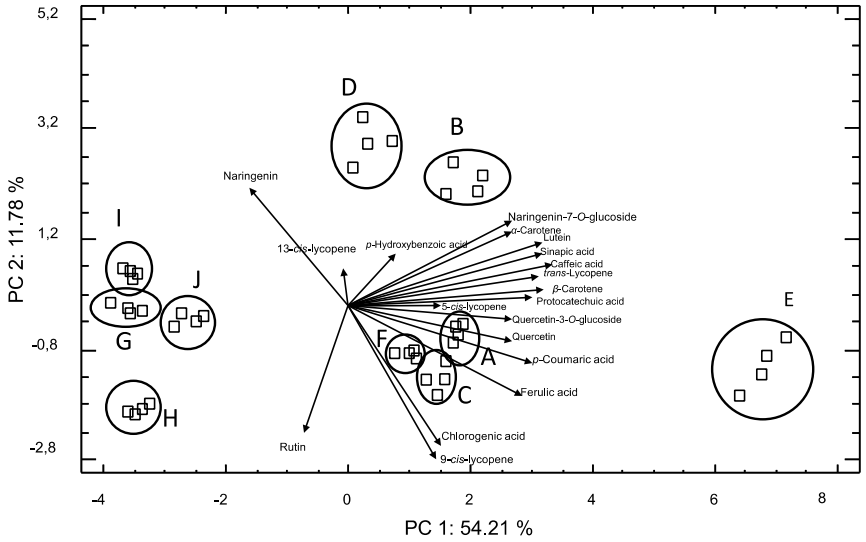

Fig. 1. Biplot of samples representing the polyphenol and carotenoid profile of Mediterranean *sofritos*.

FW, whereas levels of 9- and 13-*cis*-lycopene were lower, ranging from 0.99 to 1.88 μg/g FW and 1.72 to 2.09 μg/g FW, respectively. 15-*cis*-lycopene was not detected. Levels of *cis*-lycopene isomers were similar to those reported by Vallverdú-Queralt et al. in gazpachos (Vallverdú-Queralt, Arranz, Casals-Ribes, & Lamuela-Raventos, 2012).

3.4. Correlation between polyphenols, carotenoids and Mediterranean sofritos

A PCA was carried out to discriminate among *sofritos* (Fig. 1) according to their phenolic and carotenoid profiles. The two principal components (PC1 and PC2) obtained for each *sofrito* accounted for 65.99% of the variability of the original data. The nearer variable Y (=loading) is located to the axis origin, the lower the contribution to the class distinction among treatments. Thus, plant metabolites such as 5- and 13-*cis*-lycopene and *p*-hydroxybenzoic acid may not contribute to the discrimination between Mediterranean *sofritos* (Fig. 1). On the other hand, large loadings for variables such as chlorogenic, ferulic and *p*-coumaric acids and rutin, and also *trans*-lycopene, *β*-carotene and lutein had a high discriminating power. It can be clearly observed that certain polyphenols and carotenoids are highly correlated with a specific group of *sofritos*. *Sofrito* E, which contained tomatoes, garlic, onions and olive oil as the main ingredients, is situated on the right hand side of the plot and is highly correlated with ferulic, coumaric and protocatechuic acids, quercetin, quercetin-3-*O*-glucoside, *trans*-lycopene and *β*-carotene. In contrast, *sofritos* G–H, which include tomatoes and onions in their composition, are situated on the left hand side of the plot and are related to low levels of these metabolites. Lastly, *sofritos* A, B, C, D and F, whose main ingredients are tomatoes, onions and garlic, are situated in the middle of the plot. *Sofritos* A, B and F are highly correlated with chlorogenic, ferulic and *p*-coumaric acids, 9-*cis*-lycopene and quercetin. In contrast, *sofritos* B and D present lower levels of these metabolites and are better correlated with 13-*cis*-lycopene, *p*-hydroxybenzoic acid, naringenin and naringenin-7-*O*-glucoside. Thus, the presence of olive oil, onion and garlic influences the phenolic and carotenoid profile of Mediterranean *sofritos*.

Sofritos are interesting for their content of bioactive compounds that have beneficial effects on human health. To our knowledge, this is the first time a study has been carried out in Mediterranean *sofritos*, in which 40 phenolic compounds, including simple phenolic and hydroxycinnamoylquinic acids, and flavone, flavonol and dihydrochalcone derivatives were identified using high-resolution HPLC-ESI-LTQ-orbitrap-MS. The quantification levels of phenolic and carotenoid compounds led to the distinction of features among Mediterranean *sofritos*. Statistical analysis was performed to identify metabolites that may serve as markers for *sofritos* containing tomatoes, onions, garlic and olive oil. Due to their additional ingredients of garlic, onion and olive oil, *sofritos* presented higher levels of bioactive compounds than tomatoes. With a daily consumption of 120 g of *sofrito* added to pasta, the intake of TP would be 16–24 mg per portion and 6–10 mg of carotenoids per portion.

References

Agarwal, S., & Rao, A. V. (2000). Tomato lycopene and its role in human health and chronic diseases. *Canadian Medical Association Journal, 163*, 739–744.

Aruna, G., & Baskaran, V. (2010). Comparative study on the levels of carotenoids lutein, zeaxanthin and β-carotene in Indian spices of nutritional and medicinal importance. *Food Chemistry, 123*, 404–409.

Bakhouche, A., Lozano-Sanchez, J. S., Beltran-Debon, R. I., Joven, J., Segura-Carretero, A., & Fernandez-Gutierrez, A. (2013). Phenolic characterization and geographical classification of commercial Arbequina extra-virgin olive oils produced in Southern Catalonia. *Food Research International, 50*, 401–408.

Ballus, C. A., Meinhart, A. D., Bruns, R. E., & Godoy, H. T. (2011). Use of multivariate statistical techniques to optimize the simultaneous separation of 13 phenolic compounds from extra-virgin olive oil by capillary electrophoresis. *Talanta, 83*, 1181–1187.

Beato, V., Orgaz, F., Mansilla, F., & Montao, A. (2011). Changes in phenolic compounds in garlic (Allium sativum L.) owing to the cultivar and location of growth. *Plant Foods for Human Nutrition, 66*, 218–223.

Boileau, T. W. M., Boileau, A. C., & Erdman, J. W. Jr., (2002). Bioavailability of all-trans and cis-Isomers of Lycopene. *Experimental Biology and Medicine, 227*, 914–919.

Cassidy, A. N., Mukamal, K. J., Liu, L., Franz, M., Eliassen, A. H., & Rimm, E. B. (2013). High anthocyanin intake is associated with a reduced risk of myocardial infarction in young and middle-aged women. *Circulation, 127*, 188–196.

Dillon, S. A., Burmi, R. S., Lowe, G. M., Billington, D., & Rahman, K. (2003). Antioxidant properties of aged garlic extract: An in vitro study incorporating human low density lipoprotein. *Life Sciences, 72*, 1583–1594.

El Riachy, M., Priego-Capote, F., Leon, L., Luque de Castro, M. A. D., & Rallo, L. (2012). Virgin olive oil phenolic profile and variability in progenies from olive crosses. *Journal of the Science of Food and Agriculture, 92*, 2524–2533.

Estruch, R., Corella, D., Salas-Salvado, J., Ruiz-Gutierrez, V., Covas, M. U. I., Fiol, M., et al. (2006). Effects of a Mediterranean-style diet on cardiovascular risk factors: A randomized trial. *Annals of Internal Medicine, 145*, 1–11.

Estruch, R., Ros, E., Salas-Salvado, J., Covas, M.I., Corella, D., Arus, F., et al. (2013). Primary prevention of cardiovascular disease with a Mediterranean diet. *New England Journal of Medicine*, in press.

Failla, M., Chitchumroonchokchai, C., & Ishida, B. (2008). In vitro micellarization and intestinal cell uptake of cis isomers of lycopene exceed those of all-trans lycopene. *Journal of Nutrition, 138*, 482–486.

Fielding, J. M., Rowley, K. G., Cooper, P., & O'Dea, K. (2005). Increases in plasma lycopene concentration after consumption of tomatoes cooked with olive oil. *Asia Pacific Journal of Clinical Nutrition, 14*, 131–136.

Fraser, P. D., Enfissi, E. M. A., & Bramley, P. M. (2009). Genetic engineering of carotenoid formation in tomato fruit and the potential application of systems and synthetic biology approaches. *Archives of Biochemistry and Biophysics, 483*, 196–204.

Frohlich, K., Conrad, J., Schmid, A., Breithaupt, D. E., & Bohm, V. (2007). Isolation and structural elucidation of different geometrical isomers of lycopene. *International Journal for Vitamin and Nutrition Research, 77*, 369–375.

Gorinstein, S., Cvikrova, M., Machackova, I., Haruenkit, R., Park, Y. S., Jung, S. T., et al. (2004). Characterization of antioxidant compounds in Jaffa sweeties and white grapefruits. *Food Chemistry, 84*, 503–510.

Gorinstein, S., Drzewiecki, J., Leontowicz, H., Leontowicz, M., Najman, K., Jastrzebski, Z., et al. (2005). Comparison of the bioactive compounds and antioxidant potentials of fresh and cooked polish, Ukrainian, and Israeli garlic. *Journal of Agricultural and Food Chemistry, 53*, 2726–2732.

Gorinstein, S., Jastrzebski, Z., Namiesnik, J., Leontowicz, H., Leontowicz, M., & Trakhtenberg, S. (2007). The atherosclerotic heart disease and protecting properties of garlic: Contemporary data. *Molecular Nutrition and Food Research, 51*, 1365–1381.

Gorinstein, S., Leontowicz, H., Leontowicz, M., Namiesnik, J., Najman, K., Drzewieck, J., et al. (2008). Comparison of the main bioactive compounds and antioxidant activities in garlic and white and red onions after treatment protocols. *Journal of Agricultural and Food Chemistry, 56*, 4418–4426.

Graziani, G., Pernice, R., Lanzuise, S., Vitaglione, P., Anese, M., & Fogliano, V. (2003). Effect of peeling and heating on carotenoid content and antioxidant activity of tomato and tomato-virgin olive oil systems. *European Food Research and Technology, 216*, 116–121.

Grieb, S. M. D., Theis, R. P., Burr, D., Benardot, D., Siddiqui, T., & Asal, N. R. (2009). Food groups and renal cell carcinoma: Results from a case-control study. *Journal of the American Dietetic Association, 109*, 656–667.

Griffiths, G., Trueman, L., Crowther, T., Thomas, B., & Smith, B. (2002). Onions - A global benefit to health. *Phytotherapy Research, 16*, 603–615.

Hart, D. J., & Scott, K. J. (1995). Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. *Food Chemistry, 54*, 101–111.

Kay, C. D., Hooper, L., Kroon, P. A., Rimm, E. B., & Cassidy, A. N. (2012). Relative impact of flavonoid composition, dose and structure on vascular function: A systematic review of randomised controlled trials of flavonoid-rich food products. *Molecular Nutrition and Food Research, 56*, 1605–1616.

Lenucci, M. S., Cadinu, D., Taurino, M., Piro, G., & Dalessandro, G. (2006). Antioxidant composition in cherry and high-pigment tomato cultivars. *Journal of Agricultural and Food Chemistry, 54*, 2606–2613.

Lin, C. H., & Chen, B. H. (2005). Stability of carotenoids in tomato juice during storage. *Food Chemistry, 90*, 837–846.

Martinez-Gonzalez, M. A., Garcia-Arellano, A., Toledo, E., Salas-Salvado, J., Buil-Cosiales, P., Corella, D., et al. (2012). A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: The PREDIMED trial. *PLoS ONE, 7*, e43134.

Matea, C., Soran, M., Pintea, A., & Bele, C. (2010). Analytical determination of carotenoids in inland Ocimum basilicum L.: Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. *Agriculture, 67*, 298–302.

Medina-Remon, A., Estruch Ramon, Tresserra-Rimbau Anna, Vallverdú-Queralt Anna & Lamuela-Raventós Rosa Maria. (2012). The effect of polyphenol consumption on blood pressure. *Mini Reviews in Medicinal Chemistry* 13, 1137–1149.

Miean, K. H., & Mohamed, S. (2001). Flavonoid (Myricetin, Quercetin, Kaempferol, Luteolin, and Apigenin) content of edible tropical plants. *Journal of Agricultural and Food Chemistry, 49*, 3106–3112.

Parvu, M., Toiu, A., Vlase, L., & Alina Parvu, E. (2010). Determination of some polyphenolic compounds from Allium species by HPLC-UV-MS. *Natural Product Research, 24*, 1318–1324.

Rohn, S., Buchner, N., Driemel, G., Rauser, M., & Kroh, L. W. (2007). Thermal Degradation of Onion Quercetin Glucosides under Roasting Conditions. *Journal of Agricultural and Food Chemistry, 55*, 1568–1573.

Santas, J., Carbo, R., Gordon, M. H., & Almajano, M. P. (2008). Comparison of the antioxidant activity of two Spanish onion varieties. *Food Chemistry, 107*, 1210–1216.

Schroder, H., Fito, M., Estruch, R., Martinez-Gonzalez, M. A., Corella, D., Salas-Salvado, J., et al. (2011). A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. *The Journal of Nutrition, 141*, 1140–1145.

Silaste, M. L., Alfthan, G., Aro, A., Kesaniemi, Y. A., & Horkko, S. (2007). Tomato juice decreases LDL cholesterol levels and increases LDL resistance to oxidation. *British Journal of Nutrition, 98*, 1251–1258.

Slimestad, R., Fossen, T., & Verheul, M. J. (2008). The flavonoids of tomatoes. *Journal of Agricultural and Food Chemistry, 56*, 2436–2441.

Slimestad, R., & Verheul, M. (2009). Review of flavonoids and other phenolics from fruits of different tomato (Lycopersicon esculentum Mill.) cultivars. *Journal of the Science of Food and Agriculture, 89*, 1255–1270.

Tulipani, S., Martínez-Huélamo, M., Rotchés-Ribalta, M., Estruch, R., Ferrer, E., Andres-Lacueva, C., et al. (2011). Oil matrix effects on plasma exposure and urinary excretion of phenolic compounds from tomato sauces: Evidence from a human pilot study. *Food Chemistry, 30*, 581–590.

Vallverdú-Queralt, A., Arranz, S., Casals-Ribes, I., & Lamuela-Raventos, R. M. (2012). Stability of the Phenolic and Carotenoid Profile of Gazpachos during Storage. *Journal of Agricultural and Food Chemistry, 60*, 1981–1988.

Vallverdú-Queralt, A., Jáuregui, O., Medina-Remón, A., Andrés-Lacueva, C., & Lamuela-Raventós, R. M. (2010). Improved characterization of tomato polyphenols using liquid chromatography/electrospray ionization linear ion trap quadrupole Orbitrap mass spectrometry and liquid chromatography/electrospray ionization tandem mass spectrometry. *Rapid Communications in Mass Spectrometry, 24*, 2986–2992.

Vallverdú-Queralt, A., Jáuregui, O., Medina-Remon, A., & Lamuela-Raventos, R. M. (2012). Evaluation of a method to characterize the phenolic profile of organic and conventional tomatoes. *Journal of Agricultural and Food Chemistry, 60*, 3373–3380.

Vallverdú-Queralt, A., Martinez-Huelamo, M., Arranz-Martinez, S., Miralles, E., & Lamuela-Raventos, R. M. (2012). Differences in the carotenoid content of ketchups and gazpachos through HPLC/ESI(Li+)-MS/MS correlated with their antioxidant capacity. *Journal of the Science of Food and Agriculture, 92*, 2043–2049.

Vallverdú-Queralt, A., Medina-Remón, A., Andres-Lacueva, C., & Lamuela-Raventos, R. M. (2011). Changes in phenolic profile and antioxidant activity during production of diced tomatoes. *Food Chemistry, 126*, 1700–1707.

Vallverdú-Queralt, A., Medina-Remon, A., Casals-Ribes, I., Andres-Lacueva, C., Waterhouse, A. L., & Lamuela-Raventos, R. M. (2012). Effect of tomato industrial processing on phenolic profile and hydrophilic antioxidant capacity. *LWT - Food Science and Technology, 47*, 154–160.

Vallverdú-Queralt, A., Medina-Remon, A., Martinez-Huelamo, M., Jauregui, O., Andres-Lacueva, C., & Lamuela-Raventos, R. M. (2011). Phenolic profile and hydrophilic antioxidant capacity as chemotaxonomic markers of tomato varieties. *Journal of Agricultural and Food Chemistry, 59*, 3994–4001.

4.2.2 Publication 4. Home cooking and ingredient synergism improve lycopene isomer production in *Sofrito*

José Fernando Rinaldi de Alvarenga, Camilla Tran, Sara Hurtado-Barroso, Miriam Martinez-Huélamo, Montserrat Illan, Rosa Maria Lamuela-Raventós. *Food Research International*, 2017, 99, 851-861

There has been increasing interest in tomato products rich in carotenoids *Z*-isomers since these compounds preset higher bioavailability and antioxidant capacity compared to *E*-form. Cooking process and ingredients used in food preparation can influence the content, type and bioavailability of carotenoids. The aim of this study was to evaluate whether carotenoid content and isomerization in tomato-*sofrito* is affected by the presence of other ingredients and home cooking process. Carotenoids content was analyzed by HPLC-DAD and identification confirmed by HPLC-ESI-QqQ-MS/MS. A full factorial design (FFD) 2^4 was performed to clarify the effect of the factors EVOO (5% and 10%), onion (20% and 40%), garlic (2% and 4%) and cooking time (30 and 60 min) on carotenoid composition of tomato-*sofrito*. The FFD experiments were performed independently, randomized and in triplicate totalizing 48 experiments. Data obtained from FFD were evaluated by ANOVA and Fisher test and mathematical models for each carotenoid were constructed. During *sofrito* cooking process the content of all-*E*-lycopene decreased in all formulation compared to raw tomato and an adequate model was no possible to be constructed by the low value of r^2 and adj-r^2. In contrast with all-*E*-lycopene, the content of 5 and 9-*Z*-lycopene isomers increased in the tomato *sofrito* compared to raw tomato, as did for 13-*Z*-lycopene in certain formulations. Mathematical models were constructed for 5, 9 and 13-*Z*-lycopene isomers verifying how each factor influenced the production of these isomers. The main factor for the formation of *Z*-isomers in *sofrito* was cooking time. EVOO showed to improve carotenoids level, but when associated with longer time of cooking a decrease was observed. Onion proved to be the most interesting ingredient in the *sofrito*, combined with long cooking time, they promoted the formation of lycopene isomers. All-*E*-α-carotene content decreased during *sofrito* cooking technique compared to raw tomato. In contrast, all-*E*-β-carotene and 13-*Z*-β-carotene increased in all formulations compared to raw tomato. The models for all-*E*-α-carotene, all-E-β-carotene and 13-*Z*-β-carotene were not constructed because the values of r^2 and adj-r^2 were beyond of the established criteria. Longer time of cooking and the addition of onion in the preparation of tomato *sofrito* increased lycopene isomerization, which are more bioavailable and active compared to *E*-form. Mediterranean *sofrito* technique could be used to improve carotenoids absorption.

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Home cooking and ingredient synergism improve lycopene isomer production in *Sofrito*

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ABSTRACT

There has been increasing interest in tomato products rich in lycopene *Z*-isomers since these carotenoids present greater bioavailability and antioxidant capacity than the all-*E* lycopene form. Intrinsic food properties as well as processing and the interaction between dietary components can all influence the content, type and bioavailability of carotenoids. The aim of this study was to evaluate whether carotenoid content and isomerization in tomato-based Mediterranean *sofrito* is affected by the process of home cooking and the presence of other ingredients such as extra virgin olive oil, onion and garlic. We used a full factorial design to clarify the contribution of each ingredient to the carotenoid composition of *sofrito* and to determine whether this can be improved by the cooking time and ingredient synergism. Cooking time and onion content were associated with a higher production of 5-*Z*-lycopene, 9-*Z*-lycopene and 13-*Z*-lycopene in *sofrito*. Onion proved to be the most interesting ingredient in the *sofrito* formulation due to their enhancing effect on lycopene isomerization. The use of onion combined with an adequate processing time may improve the bioavailability of lycopene in tomato products.

1. Introduction

The PREDIMED (PREvención con Dieta MEDiterranea) study confirmed the health benefits of the vegetable-based Mediterranean diet, which are attributed to a high ingestion of phytochemicals (Estruch et al., 2013). Adherence to this diet has become popular in non-Mediterranean countries and assessment of its effects must take into account different ways of food preparation. Home cooking of typical Mediterranean dishes is interesting from the nutritional point of view, since this process can increase, decrease or change phytochemical content and bioavailability in food, thereby determining the intake and beneficial effects of bioactive compounds (Hoffman & Gerber, 2015). *Sofrito* is a typical home-made tomato-based Mediterranean sauce which also contains onion, garlic and olive oil (Vallverdú-Queralt, de Alvarenga, Estruch, & Lamuela-Raventos, 2013). Tomato is a principal dietary source of carotenoids and is associated with a regulation of the lipid profile and inflammatory biomarkers (Burton-Freeman, Talbot, Park, Krishnankutty, & Edirisinghe, 2012; Cuevas-Ramos et al., 2013; Sesso, Wang, Ridker, & Buring, 2012; Valderas-Martinez et al., 2016). Onion and garlic are widely used as gastronomic ingredients and present beneficial effects against cardiovascular diseases, hypertension, diabetes and cancer attributed to their content of organosulfur compounds and polyphenols (Corzo-Martínez, Corzo, & Villamiel, 2007).

Lycopene is the major carotenoid found in tomato and tomato products and has antioxidant capacity, affects the expression of genes and regulatory proteins, and has shown beneficial effects against cancer, cardiovascular diseases and type II diabetes (Burton-Freeman et al., 2012; Cervantes-paz, Victoria-campos, & Ornelas-paz, 2016; Cuevas-Ramos et al., 2013; Jacques, Lyass, Massaro, Vasan, & D'Agostino, 2013; Sesso et al., 2012). *Z*-lycopene isomers are currently gaining interest due to their higher bioavailability and antioxidant capacity compared to other carotenoids (Arranz et al., 2015; Bohm, Puspitasari-Nienaber, Ferruzzi, & Schwartz, 2002). Recent studies have focused on producing ingredients and products with a high content of *Z*-lycopene isomers, using organic solvents and vegetable oils as mediators (Honda et al., 2016; Lambelet, Richelle, Bortlik, Franceschi, & Giori, 2009). The presence of lipids during food preparation increases the solubility of carotenoids by favoring their incorporation in small micelles, which do not readily occur in the gastrointestinal tract (Palmero, Panozzo, Simatupang, Hendrickx, & Van Loey, 2014). Processing tomato sauce with oil increases the extractability of carotenoids from the food matrix (Colle et al., 2010a; Colle, Lemmens, Van Buggenhout, Van Loey, & Hendrickx, 2013; Vallverdú-Queralt, Regueiro, de Alvarenga, Torrado, & Lamuela-Raventos, 2015) and improves their bioavailability

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in humans (Arranz et al., 2015; Valderas-Martinez et al., 2016). The bioavailability of carotenoids is affected by intrinsic food properties, processing and the presence of other dietary components, and the complexity of these interacting factors is a challenging subject for research (Cervantes-Paz et al., 2016). The individual ingredients of *sofrito*, tomato sauce, and tomato sauce emulsified with oil have been extensively studied, but to our knowledge, a possible synergistic effect of the ingredients of home-made *sofrito* has not been studied.

The aim of this study was to evaluate how the home cooking process and the additional ingredients such as extra virgin olive oil, onion and garlic used to prepare tomato-based Mediterranean *sofrito* may interact and improve carotenoid content and isomerization.

2. Material and methods

2.1. Chemicals and standards

The standards of carotenoids all-*E*-α-carotene, all-*E*-β-carotene and all-*E*-lycopene were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol and methanol were purchased from AppliChem, Panreac Quimica SA (Barcelona, Spain). Methyl tert-butyl ether (MTBE) and hexane were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water (Milli-Q) was produced by a Millipore system (Millipore, Bedford, MA, USA).

2.2. Material

The tomatoes used (*Lycopersicon esculentum* Mill, c. v. Pera) were bought from Grupo Almería (La Cañada, Almería, Spain) and were all from the same batch and had a diameter of 57–67 mm. Extra virgin olive oil (EVOO) was provided by Manuel Heredia Halcón (Cortijo De Suerte Alta, Albedin-Baena-Córdoba). Onions and garlic were bought from Casa Ametller (Barcelona, Spain).

2.3. Factorial design

We used a full factorial design (FFD) in the present study in order to clarify the effect of individual ingredients, different cooking times and possible ingredient synergism on the carotenoid composition of *sofrito*. The factors EVOO, onion, garlic and cooking time were analyzed in a two-level FFD. Each factor had a high (+1) and low (−1) level, producing a 2^4 FFD, with 16 different experiments. The levels for each factor were: 5% (−1) and 10% (+1) for oil; 20% (−1) and 40% (+1) for onion; 2% (−1) and 4% (+1) for garlic, and 30 min (−1) and 60 min (+1) for time. The FFD experiments were performed independently in triplicate and randomized for better reproducibility, and the pure error and lack of fit were estimated in a total of 48 experiments. The level of each ingredient and cooking time were defined by consulting the literature (Colle et al., 2010a; Vallverdú-Queralt et al., 2015). Table 1 shows the variables and levels of ingredients and cooking time for each experiment. Data obtained in the FFD experiments were analyzed by analysis of variance (ANOVA) using STATISTICA 10 software (StatSoft, USA) and a regression equation of a first order model with four parameters and their interaction terms was applied for modeling (Eq. 1):

$$Y = b_0 + b_1E + b_2O + b_3G + b_4t + b_{12}EO + b_{13}EG + b_{14}Et + b_{23}OG + b_{24}Ot + b_{34}Gt + b_{123}EOG + b_{124}EOt + b_{134}EGt + b_{234}OGt + b_{1234}EOGt$$

where Y is the predicted response variable, b_0 the average value, b_1 b_2, b_3 and b_4 the linear coefficients and b_{12}, b_{13}, b_{14}, b_{23}, b_{24}, b_{34}, b_{123}, b_{124}, b_{134} and b_{234} the interaction coefficients. The independent variables are E (EVOO), O (onion), G (garlic) and t (time).

2.4. Sofrito preparation

The *sofrito* was prepared at Torribera Campus, University of Barcelona (Santa Coloma de Gramenet, Spain), following a home cooking method. The tomatoes, garlic and onions were washed, cut into small pieces, mixed with a blender (model R5 Plus, Robot Coupe®) and weighed according to the FFD. The cooking process was based on the traditional Mediterranean diet: in an uncovered pan (24 cm diameter, 15 cm height, 6.3 L volume, thickness 1.59 mm, made of inox 18/10). The EVOO was heated on an electrical cooking plate (180 mm diameter, 1500 W, model Encimera EM/30 2P, Teka®) at potency 4 (ranging from 1 to 6) for 1 min. The onion and garlic were then added and fried for 1 min, before adding the tomato, at which point the cooking process was timed, and the temperature was reduced to potency 2 to provide constant heat throughout the process (100 ± 1 °C). After preparation, the 16 *sofritos* were weighed to quantify weight loss, frozen in plastic vacuum bags, and stored at −25 °C (Table 1).

2.5. Extraction and analysis of carotenoids

The carotenoid extraction was performed under light with UV filters and ice to minimize the oxidation and isomerization of the compounds. All the samples were extracted in triplicate.

2.5.1. Extraction of carotenoids from tomato, onion, garlic and sofrito

The *sofrito* (0.5 g) was weighed and homogenized with ethanol: *n*-hexane (4:3, v/v), after which it was sonicated for 10 min and then centrifuged at 2486*g*, for 20 min at 4 °C. The apolar phase was separated into a flask and the extraction was repeated until colorless. The supernatants were combined and evaporated until dry using a vacuum concentrator (miVac DNA concentrator, Genevac LTD, England). The residue was reconstituted with 1 mL of MTBE, filtered using a 25 mm, 0.22 μm PTFE filter (Teknokroma, Spain) into an amber vial for HPLC analysis, and stored at −80 °C until analysis (Vallverdú-Queralt et al., 2013).

2.5.2. Extraction of carotenoids from EVOO

EVOO (1 g) was weighed and diluted with 1 mL of n-hexane. Thereafter, 2 mL of methanol was added, homogenized and centrifuged at 1399*g* for 3 min at 4 °C. The apolar phase and polar phase were separated and again extracted with 1 mL of *n*-hexane and 2 mL of methanol, respectively. The apolar phases were combined and evaporated until

Table 1
Experimental level of the factors used in the full factorial design (FDD).

	EVOO (%)	Onion (%)	Garlic (%)	Time (min)	Tomato (%)	Initial weight (g)	Final weight (g)		
							a	b	c
1	5	20	2	30	73	1000	497	492	515
2	10	20	2	30	68	1000	523	485	500
3	5	40	2	30	53	1000	347	390	401
4	10	40	2	30	48	1000	465	436	430
5	5	20	4	30	71	1000	486	545	491
6	10	20	4	30	66	1000	535	628	621
7	5	40	4	30	51	1000	453	454	433
8	10	40	4	30	46	1000	407	385	380
9	5	20	2	60	73	1000	446	486	423
10	10	20	2	60	68	1000	455	497	478
11	5	40	2	60	53	1000	461	466	464
12	10	40	2	60	48	1000	422	420	421
13	5	20	4	60	71	1000	398	472	513
14	10	20	4	60	66	1000	503	547	531
15	5	40	4	60	51	1000	379	500	480
16	10	40	4	60	46	1000	401	361	362

$n = 3$ (represent as a, b and c). EVOO = Extra Virgin Olive Oil. Initial weight (before cooking) and Final weight (after cooking process).

dry using a vacuum evaporator (miVac DNA concentrator, Genevac LTD, England). The residue was reconstituted up to 1 mL with MTBE and filtered using a 0.22 μm PTFE filter into a 2 mL amber vial for HPLC analysis and stored at −80 °C (Capriotti et al., 2014).

2.5.3. Analysis of carotenoids

The analysis was based on the procedure of Vallverdú-Queralt et al. (2013) with modifications and was performed using an HPLC system (HP1100 HPLC system, Hewlett-Packard, Waldbronn, Germany) equipped with a quaternary pump and autosampler and coupled with a diode array detector DAD G1315B. Separation was carried out on a C_{30} 250 × 4.6 mm, 5 μm column (YMC™, Water Co., Milford, MA, USA), with a flow rate of 0.6 mL/min at 25 °C. The mobile phase consisted of methanol (A), MTBE (B) and water (C). A gradient was used to separate the analytes under the following conditions: 0 min, 70% A; 10 min, 20% A; 20 min, 6% A; 21 min, 6% A, 23 min, 70% A, 33 min, 70% A. Water was kept constant at 4% throughout the analysis. The injection volume was 20 μL. The DAD detector was used in the range of 350 to 600 nm and the chromatograms were acquired at a wavelength of 450 nm.

To confirm the identification, a QTRAP4000 triple quadrupole mass spectrometer (Sciex, Foster City, CA, USA) equipped with an APCI ionization source in positive-ion mode was used operating in multiple reaction monitoring mode. The mass spectrometer conditions were: entrance potential (EP), 10 V; collision cell exit potential (CXP), 15 V; source temperature, 400 °C; curtain gas, 20 psi; ion source gas 1 (GS1), 45 psi; ion source gas 2 (GS2), 0 psi. The declustering potential (DP) was selected for each individual compound according to the conditions established by (Hrvolová et al., 2016).

The identification of the carotenoids was based on retention time, chromatography with standards, UV/VIS absorption spectrum: λ_{max}, spectral fine structure (%III/II) and peak cis intensity (%A_b/A_{II}), and mass spectrum (Rodriguez, 2001). Quantification was performed by HPLC-DAD, using external calibration curves for α-carotene, β-carotene and lycopene with six concentration levels. The *Z*-isomers were quantified using the same calibration curve corresponding to the all-*E*-form. The results were expressed as μg/g of tomato. The total carotenoid, total lycopene and total *Z*-lycopene content were calculated by the sum of each individual carotenoid.

3. Results and discussion

The analysis of the *sofritos* revealed the presence of 9 different carotenoids, consisting of lycopene, α-carotene, β-carotene and their isomers (Table 2) (Fig. 1). Among the *sofrito* ingredients, tomato was the principal source of carotenoids, with a high content of all-*E*-lycopene (126.31 ± 8.37 μg/g of tomato), and a low content of isomers such as 13-*Z*-lycopene (15.90 ± 1.27 μg/g of tomato) and 5-*Z*-lycopene (3.86 ± 0.60 μg/g of tomato), while 9-*Z*-lycopene was not detected, and 15-*Z*-lycopene was below the quantification limit of the calibration curve. Regarding the carotenes in tomato, the content of all-*E*-α-carotene (5.45 ± 0.25 μg/g of tomato) and all-*E*-β-carotene (2.64 ± 0.09 μg/g of tomato) was high, while the only isomer detected was 9-*Z*-β-carotene (1.94 ± 0.09 μg/g of tomato). The EVOO showed traces of all-*E*-β-carotene and all-*E*-lutein, although the latter was not found in the *sofritos*, and was therefore not considered in this study. In order to assess the influence of the ingredients and cooking time on carotenoid content and isomerization, the results are expressed in μg/g of tomato, with tomato being a constant in all samples of the FFD.

A total of 48 experiments were performed according to a four-factor two-level FFD, with 16 experiments in triplicate. Table 3 shows the different carotenoids found in the *sofrito* (response variables). The total carotenoids varied from 50.60 to 143.9 μg/g of tomato, the lowest content being in formulation 1, which had a low level of all the independent variables, while the highest was in formulation 11, which had a high onion content, a long cooking time and a low level of oil and garlic (Table 3). Vallverdú-Queralt et al. (2013) studied carotenoids in commercial Spanish *sofritos* and described between 53.10 and 81.49 μg/g of total carotenoids, similar to the results of the current study.

The highest total carotenoid content was found in the formulations cooked for 60 min, showing that cooking time was an important factor. Vallverdú-Queralt et al. (2015) also investigated the elaboration of tomato sauces with different cooking times and EVOO content, and found that total carotenoids increased with 60 min of cooking, compared with 15, 30 and 45 min, and with 10% of EVOO compared to 5%. Colle et al. (2010a) observed no significant reduction in total lycopene when the cooking temperature remained below 130 °C, and the cooking time did not influence the total lycopene content when tomato pulp was processed at different temperatures. The addition of olive oil to tomato products serves to partly dissolve the lycopene which is otherwise in an insoluble crystalline form that is unstable with heating. Thus, the presence of oil may protect lycopene from thermal oxidative degradation during cooking and enhance its susceptibility to isomerization (Colle, Lemmens, Van Buggenhout, Van Loey, & Hendrickx, 2010b; Lambelet et al., 2009).

3.1. Effect of sofrito ingredients and cooking time on lycopene and its isomers

The content of all-*E*-lycopene in the 16 different *sofritos* varied from 16.71 to 56.12 μg/g of tomato (Table 3), showing a decrease compared to raw tomato (126.31 ± 8.37). The all-*E*-lycopene content in commercial *sofritos* has been reported to range between 29.86 and 40.50 μg/g of fresh weight (FW) of *sofrito* (Vallverdú-Queralt et al., 2013), which is similar to that of the home-cooked samples. The decrease in all-*E*-lycopene during thermal processing, with or without the presence of lipids, by degradation and isomerization has been previously reported (Colle et al., 2010a, 2010b, 2013; Colle, Van Buggenhout, Lemmens, Van Loey, & Hendrickx, 2012; Honda et al., 2016; Vallverdú-Queralt et al., 2015).

Table 2
Identification of carotenoid compounds present in Mediterranean *sofrito*.

	Compound	rt	λ_{max} (nm)	%III/II	%A_b/A_{II}	$[M + H]^+$ (*m*/*z*)	MS^2 product ions (*m*/*z*)
1	13-*Z*-β-carotene	15.80	340,425,445,475	28	40	537	413
2	all-*E*-α-carotene	16.24	425,445,475	68		536	444
3	all-*E*-β-carotene	16.65	450,480	28		537	413
4	15-*Z*-lycopene	17.67	348,363,440,460,490	55	68	n.d	n.d
5	not identified	18.28	n.d.				
6	13-*Z*-lycopene	18.72	348,363,440,468,500	50	49	537	413
7	9-*Z*-lycopene	20.30	365,445,470,500	65	14	537	413
8	all-*E*-lycopene	22.94	445,473,505	73		537	413
9	5-*Z*-lycopene	22.98	365,445,473,505	71	10	537	413

rt (retention time); λ_{max} (wavelength); (%III/II) spectral fine structure; (%Ab/II) peak cis intensity. n.d.(not detected).

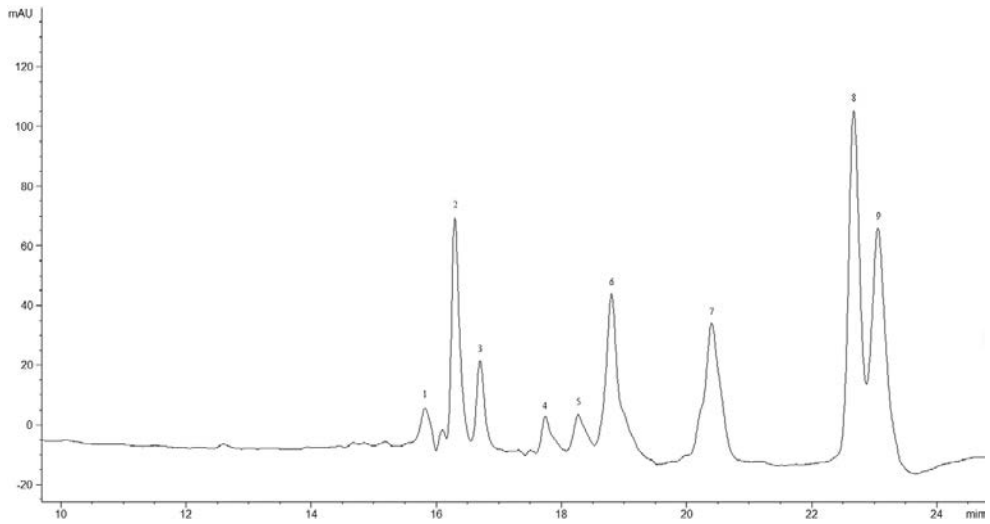

Fig. 1. Carotenoid HPLC-DAD chromatogram of a sofrito sample. Peak identification according to Table 2.

ANOVA analysis was used to determine the factors that significantly affect all-*E*-lycopene content in *sofrito*, using a *p*-value to determine the statistical significance of the factor in the model and F-value to determine the importance of the factor for the model compared with Fisher's F-value in the confidence level ($\alpha = 0.05$) (Seyed Shahabadi & Reyhani, 2014). Factors E, O and t and the interaction factors EG, Ot, Gt and EGt presented a significant F-value and a statistically significant *p*-value ($p \leq 0.001$). The interaction factors EO, Et, OG EOt presented a *p*-value of $p \leq 0.01$ and factor G had a *p*-value of $p \leq 0.05$ (Table 4). In order to determine the suitability of the model, the coefficient of determination (r^2) and the adjusted coefficient of determination (adj-r^2) were evaluated. According to Seyed Shahabadi and Reyhani (2014), the model must have an r^2 close to 1 and should be at least 0.80 for a good fit. It is also preferable to analyze the adj-r^2, because it adjusts for the number of terms in the model, and a value higher than 0.90 indicates that the process is well explained by the regression model. The all-*E*-lycopene presented an r^2 value of 0.86031, adj-r^2 of 0.80105 and a significant lack of fit ($p > 0.05$), indicating that the model did not satisfactorily describe how the ingredients and cooking time affected the all-*E*-lycopene content; therefore a regression equation was not designed.

Since the degradation and isomerization of carotenoids involve multiple mechanisms of action, it is difficult to find a model for the influence of ingredients and cooking time (Boon, McClements, Weiss, & Decker, 2010). The decrease in all-*E*-lycopene content could be related to many factors: its degradation can be accelerated with increased oxygen, temperature and processing time. It is also influenced by the ingredients in the formulation, such as oils sensitive to oxidation, which form radicals that can isomerize or degrade carotenoids (Colle, Lemmens, Knockaert, Van Loey, & Hendrickx, 2015; Colle et al., 2013; Murador, da Cunha, & de Rosso, 2014). Furthermore, the structure of all-*E*-lycopene is able to isomerize in 72 different structurally favorable *Z*-isomers, predominantly the 5-*Z*-, 9-*Z*- and 13-*Z*-lycopene forms, thereby making

Table 3
Experimental response values of the carotenoid content on different formulations of *sofrito* according to the full factorial design, expressed as μg/g of tomato.

	13-*Z*-β-carotene	all-*E*-α-carotene	all-*E*-β-carotene	13-*Z*-lycopene	9-*Z*-lycopene	all-*E*-lycopene	5-*Z*-lycopene	Total *Z*-lycopene[a]	Total lycopene	Total carotenoid
1	2.15 ± 0.02	2.6 ± 0.1	3.3 ± 0.2	7.7 ± 0.3	5.97 ± 0.02	17 ± 2	12.1 ± 0.4	25.79 (60.68)	42.49	50.60
2	2.4 ± 0.1	3.14 ± 0.01	5.3 ± 0.3	12.5 ± 0.4	13 ± 1	28 ± 2	15.5 ± 0.5	41.08 (59.48)	69.07	79.96
3	2.42 ± 0.06	2.9 ± 0.2	3.9 ± 0.3	12 ± 1	10.0 ± 0.9	26 ± 0.7	15.0 ± 0.4	37.43 (59.24)	63.19	72.41
4	3.1 ± 0.2	3.2 ± 0.3	5.6 ± 0.6	17.9 ± 0.1	17 ± 1	15 ± 2	20.0 ± 0.5	54.62 (78.52)	69.56	81.41
5	2.6 ± 0.2	2.60 ± 0.09	3.6 ± 0.2	7.5 ± 0.3	5.7 ± 0.1	23 ± 2	13.6 ± 0.9	26.70 (53.27)	50.12	58.93
6	3.1 ± 0.2	3.8 ± 0.1	4.7 ± 0.3	12.8 ± 0.3	13 ± 1	40.8 ± 0.9	17.0 ± 0.7	42.75 (51.16)	83.55	95.11
7	2.6 ± 0.1	3.1 ± 0.2	4.53 ± 0.08	7.9 ± 0.2	8.69 ± 0.05	17 ± 2	15.3 ± 0.2	31.96 (65.89)	48.50	59.02
8	2.9 ± 0.2	3.1 ± 0.2	6.2 ± 0.6	12.1 ± 0.5	21 ± 2	38.9 ± 0.7	35.0 ± 0.7	68.50 (63.76)	107.4	119.6
9	2.29 ± 0.08	2.8 ± 0.2	3.6 ± 0.3	20.7 ± 0.9	17.9 ± 0.6	55 ± 4	29 ± 1	67.33 (54.93)	122.6	131.3
10	2.29 ± 0.08	3.07 ± 0.06	5.2 ± 0.2	11 ± 1	11.9 ± 0.1	50.4 ± 0.3	24.4 ± 0.9	47.77 (48.66)	98.16	108.7
11	3.1 ± 0.2	3.2 ± 0.2	4.1 ± 0.2	25 ± 2	28.7 ± 3	33 ± 3	48 ± 1	100.8 (75.53)	133.4	143.9
12	3.0 ± 0.2	3.1 ± 0.1	4.4 ± 0.3	13.5 ± 0.8	16 ± 1	53 ± 2	30 ± 2	59.17 (52.73)	112.2	122.6
13	2.418 ± 0.003	2.8 ± 0.2	3.7 ± 0.3	14.6 ± 0.8	18 ± 1	43 ± 2	22.3 ± 0.5	54.47 (55.92)	97.40	106.3
14	2.63 ± 0.07	3.20 ± 0.05	4.9 ± 0.6	10.7 ± 0.7	9.5 ± 0.5	56 ± 4	18.9 ± 0.4	39.09 (41.06)	95.21	106.0
15	3.2 ± 0.3	3.45 ± 0.02	4.6 ± 0.4	20 ± 1	22.7 ± 0.6	40 ± 3	30.31 ± 0.09	72.72 (64.44)	112.9	124.1
16	2.6 ± 0.2	2.8 ± 0.1	4.7 ± 0.1	12.7 ± 0.7	14.3 ± 0.4	29 ± 1	24 ± 2	50.86 (63.52)	80.07	90.16

Mean ± SD ($n = 3$).
[a] (%) values of isomers of lycopene related of total lycopene.

Table 4
ANOVA (*F*-value of regression coefficients), lack of fit, coefficient of variation of the first order model for the carotenoid content of processed *sofrito* according to the full factorial design.

	13-*Z*-β-carotene	all-*E*-α-carotene	all-*E*-β-carotene	13-*Z*-lycopene	9-*Z*-lycopene	all-*E*-lycopene	5-*Z*-lycopene
E	5.08*	25.04***	141.82***	26.70***	0.39^{ns}	127.94***	0.02^{ns}
O	74.64***	6.13*	18.93***	108.34***	311.04***	147.93***	796.11***
G	17.26***	6.32*	2.89^{ns}	104.44***	9.10**	4.19*	54.03***
t	0.00^{ns}	0.00^{ns}	6.42*	277.23***	317.52***	908.12***	1303.21***
E O	8.69**	61.04***	6.91*	6.25*	0.62^{ns}	9.65**	0.36^{ns}
E G	2.95^{ns}	0.16^{ns}	2.70^{ns}	15.23***	11.47**	25.73***	140.53***
E t	30.07***	32.37***	14.57***	522.73***	774.15***	19.11**	783.01***
O G	17.44***	4.25*	10.85**	17.05***	0.19^{ns}	8.02**	0.31^{ns}
O t	10.75**	2.51^{ns}	13.04**	2.10^{ns}	5.05*	51.73***	18.82***
G t	10.38**	2.74^{ns}	0.18^{ns}	1.03^{ns}	28.50***	124.63***	550.61***
E O G	17.35***	17.53***	1.93^{ns}	1.90^{ns}	24.89***	18.81***	119.53***
E O t	6.73*	0.00^{ns}	10.56*	4.39*	22.84***	12.59**	228.62***
E G t	0.01^{ns}	3.40^{ns}	0.35^{ns}	20.72***	2.46^{ns}	105.12***	1.10^{ns}
O G t	0.00^{ns}	0.39^{ns}	0.29^{ns}	27.74***	12.45**	0.95^{ns}	107.81***
Lack of Fit	0.05^{ns}	2.00^{ns}	0.80^{ns}	0.06^{ns}	0.08^{ns}	222.11***	3.53^{ns}
CV%	4.94	4.28	6.37	5.75	6.02	5.14	3.49
r^2	0.8627	0.8265	0.8759	0.9726	0.9793	0.8603	0.9914
r^2-Adj	0.8044	0.7528	0.8232	0.9609	0.9706	0.8011	0.9878

E (Extra Virgin Olive Oil); O (Onion); G (Garlic); t (time).
* $p \le 0.05$.
** $p \le 0.01$.
*** $p \le 0.001$.
^{ns} = not significative.

it difficult to predict how it will isomerize or degrade (Lambelet et al., 2009).

In contrast with all-*E*-lycopene, the content of 5-*Z*-lycopene and 9-*Z*-lycopene isomers increased in the *sofrito* compared to raw tomato, as did the 13-*Z*-lycopene isomer in certain formulations. The content of 5-*Z*-lycopene ranged from 12.10 to 47.52 μg/g of tomato, being higher than in raw tomato (3.86 ± 0.60 μg/g of tomato) and highest in formulation 11 (5% E, 40% O, 2% G and 60 t) and lowest in formulation 1 (5% E, 20% O, 2% G and 30 t) (Table 3). The linear model significantly fit the experimental data, indicated by an r^2 of 0.9142 and adj-r^2 of 0.98778. The model presented a statistically insignificant lack of fit ($p \ge 0.05$) and a low coefficient of variation (3.49%). The linear factor E and the interaction factors EO, OG and EGt presented a statistically insignificant F-value compared to Fisher's F-value ($p \le 0.05$) and a *p*-value of up to $p \le 0.05$, and were therefore not included in the model (Table 4). The graphs of predicted and observed values (Fig. 2) showed good agreement with the experimental data. These statistical parameters confirm the consistency of the model, indicating its suitability to predict 5-*Z*-lycopene content and analyze the role of each factor in isomerization. Excluding the insignificant terms and performing a new ANOVA, the regression coefficient was used to develop the following equation for the model:

$$5\text{—}Z\text{—lycopene}(\mu g/g\ \text{of tomato}) = +21.24393 - 1.34894O - 0.60043t - 0.97890EG + 0.06312Et + 0.07299Ot + 0.11531Gt + 0.06956EOG - 0.00468EOt - 0.01126OGt$$

Fig. 3 depicts the main effects of the different factors and their interactions on the content of 5-*Z*-lycopene in a three-dimensional surface plot. It can be seen that cooking time was the main factor affecting the isomerization of 5-*Z*-lycopene (Fig. 2d, e and f). Lambelet et al. (2009) studied the isomerization of lycopene in an enriched tomato extract and found that unlike other isomers, 5-*Z*-lycopene was originally present in a small amount and increased over time. Vallverdú-Queralt et al. (2015) described the same pattern: unlike 9-*Z* and 13-*Z*, the content of 5-*Z* increased over time. Moreover, 5-*Z*-lycopene required significantly less activation energy, indicating that its isomerization from the all-*E*-lycopene occurred more readily. However, a different behavior has also been observed with the 5-*Z* isomer presenting a considerably larger rotational barrier for this conversion and a slower isomerization (Colle et al., 2010b).

In addition to time, another relevant factor in the isomerization of 5-*Z*-lycopene was the use of onion (Fig. 3b, c and f). The influence of onion in the isomerization of lycopene could be related to electron transfer due to the presence of sulfur compounds. Arita et al. (2005) found that sulfides, which are major compounds in onion and garlic, accelerated the photodegradation of carotenoids: that is, the more sulfur atoms, the greater the degradation. Unfortunately, in that study the effect of the sulfur compounds on isolation or a possible isomerization of the carotenoids was not evaluated.

The oil factor caused a small increase in the content of 5-*Z*-lycopene in formulations with greater EVOO content, but the combination of a long cooking time and high oil content had a negative effect on5-*Z*-lycopene levels (Fig. 3e).

9-*Z*-lycopene was only found in the processed samples, ranging from 5.97 to 28.67 μg/g of tomato. Formulation 1 (5%E, 20% O, 2% G and 30 min t) had the lowest content and formulation 11 (5% E, 40% O, 2% G and 60 min t) the highest (Table 3). All the linear and interaction factors presented an F-value and *p*-value ≤ 0.05, except for factor E and interaction factors EO, OG and EGt. Model adequacy was good (Fig. 1); high r^2 and adj-r^2values were calculated (0.97934 and 0.97057, respectively), and the lack of fit was insignificant (Table 4). The regression coefficient of the statistically significant factors was used to design the following equation for the model:

$$9\text{—}Z\text{—lycopene}(\mu g/g\ \text{of tomato}) = 13.23581 - 0.43356O - 4.89285G + 0.03013Ot + 0.08034Gt + 0.02677EOG - 0.00190EOt - 0.00452OGt$$

The analysis of the surface plots showed a behavior similar to 5-*Z*-lycopene (Fig. 4). The isomerization of 9-*Z*-lycopene was particularly interesting, since it only appeared in the processed samples. The factors of time (Fig. 4d, e and f) and onion (Fig. 4b, c and f) had a greater influence on its isomerization, than on 5-*Z*-lycopene. The content of 9-*Z*-lycopene was increased by EVOO in correlation with onion and time, although 9-*Z*-lycopene showed a greater reduction with the combination of olive oil and a long cooking time than the 5-*Z* isomer. Colle et al. (2010b) found that in comparison with 5-*Z*-lycopene, 9-*Z*-lycopene isomerization needed more activation energy and presented a lower rotational barrier. In addition, it showed a constant reaction rate similar to 5-*Z*-lycopene in tomato/oil emulsions, which would explain the same behavior related to the time factor found in the current study.

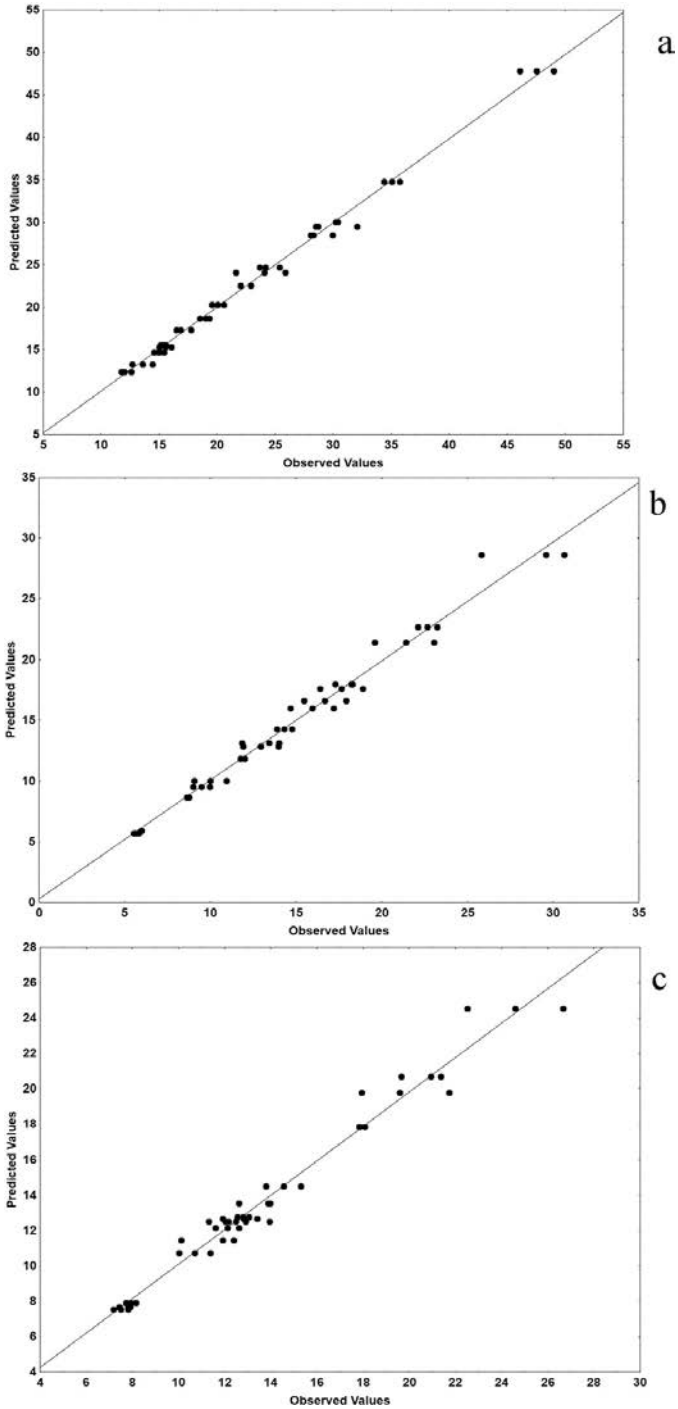

Fig. 2. Comparison of predicted values *vs* observed values of 5-*Z*-lycopene (a), 9-*Z*-lycopene (b) and 13-*Z*-lycopene (c).

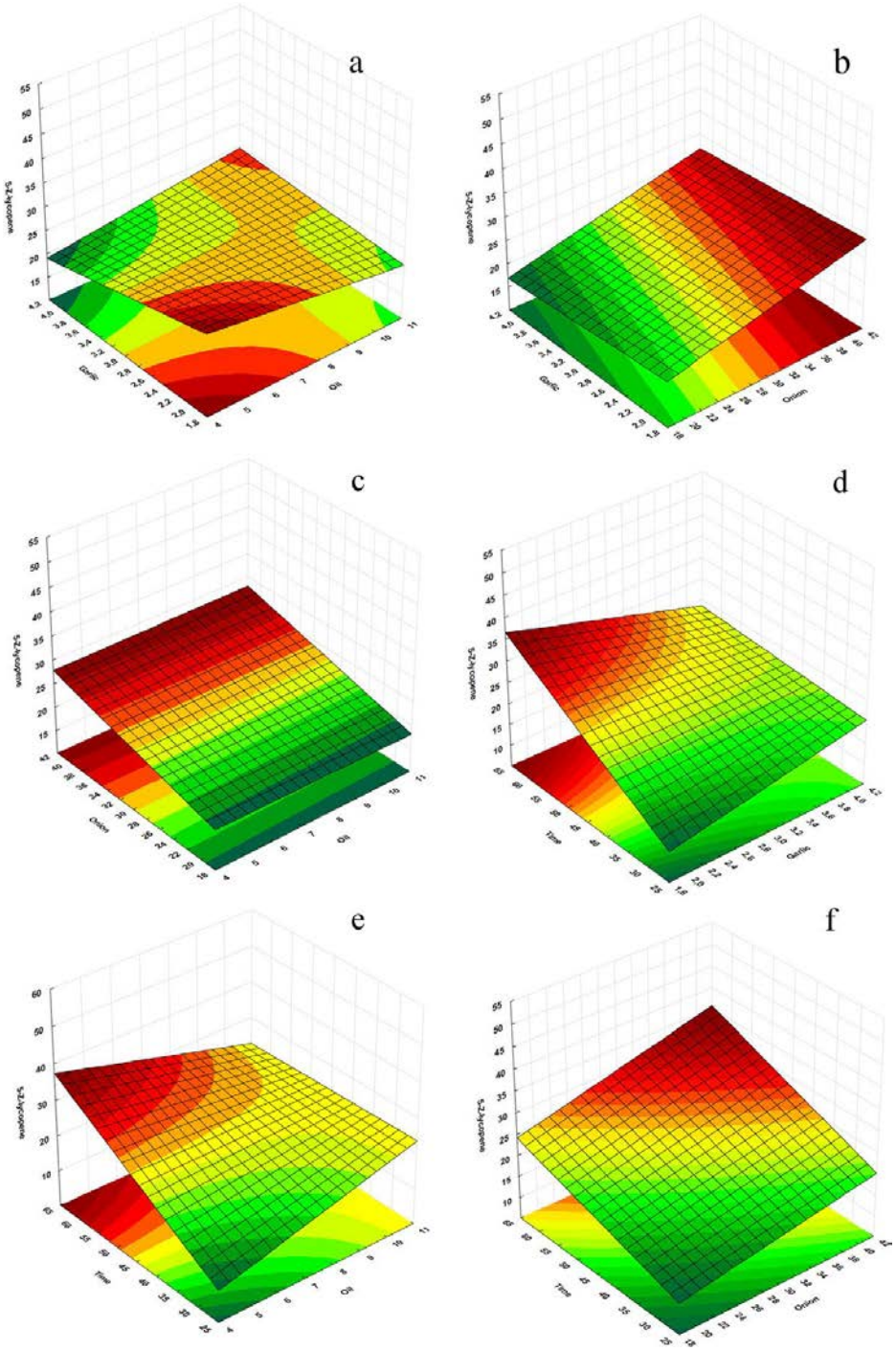

Fig. 3. Response surface plot of the combined effects of: garlic and olive oil at time/onion on level 0 (a); garlic and onion at olive oil/time on 0 level (b); onion and olive oil at garlic/time on level 0 (c); time and garlic at onion/olive oil on level 0 (d); time and olive oil at onion/garlic on level 0 (e); time and onion at garlic/olive oil on level 0 (f) on the content of 5-Z-lycopene of home cooked Mediterranean *sofrito*. (level 0: garlic = 3%; olive oil = 7.5%; onion = 30% and time = 45 min).

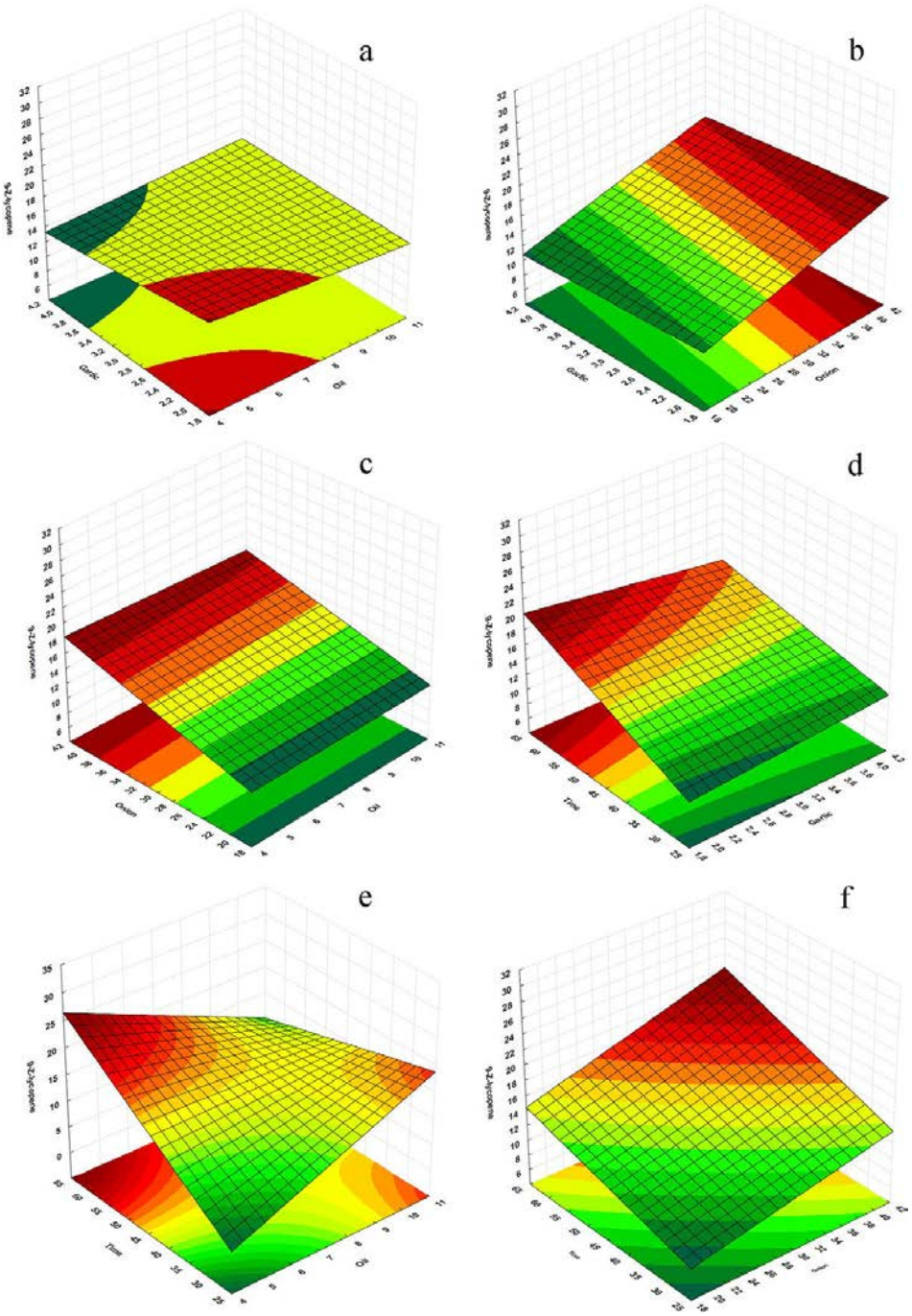

Fig. 4. Response surface plot of the combined effects of: garlic and olive oil at time/olive onion on level 0 (a); garlic and onion at olive oil/time on 0 level (b); onion and olive oil at garlic/time on level 0 (c); time and garlic at onion/olive oil on level 0 (d); time and olive oil at onion/garlic on level 0 (e); time and onion at garlic/olive oil on level 0 (f) on the content of 9-*Z*-lycopene of home cooked Mediterranean *sofrito*. (level 0: garlic = 3%; olive oil = 7.5%; onion = 30% and time = 45 min).

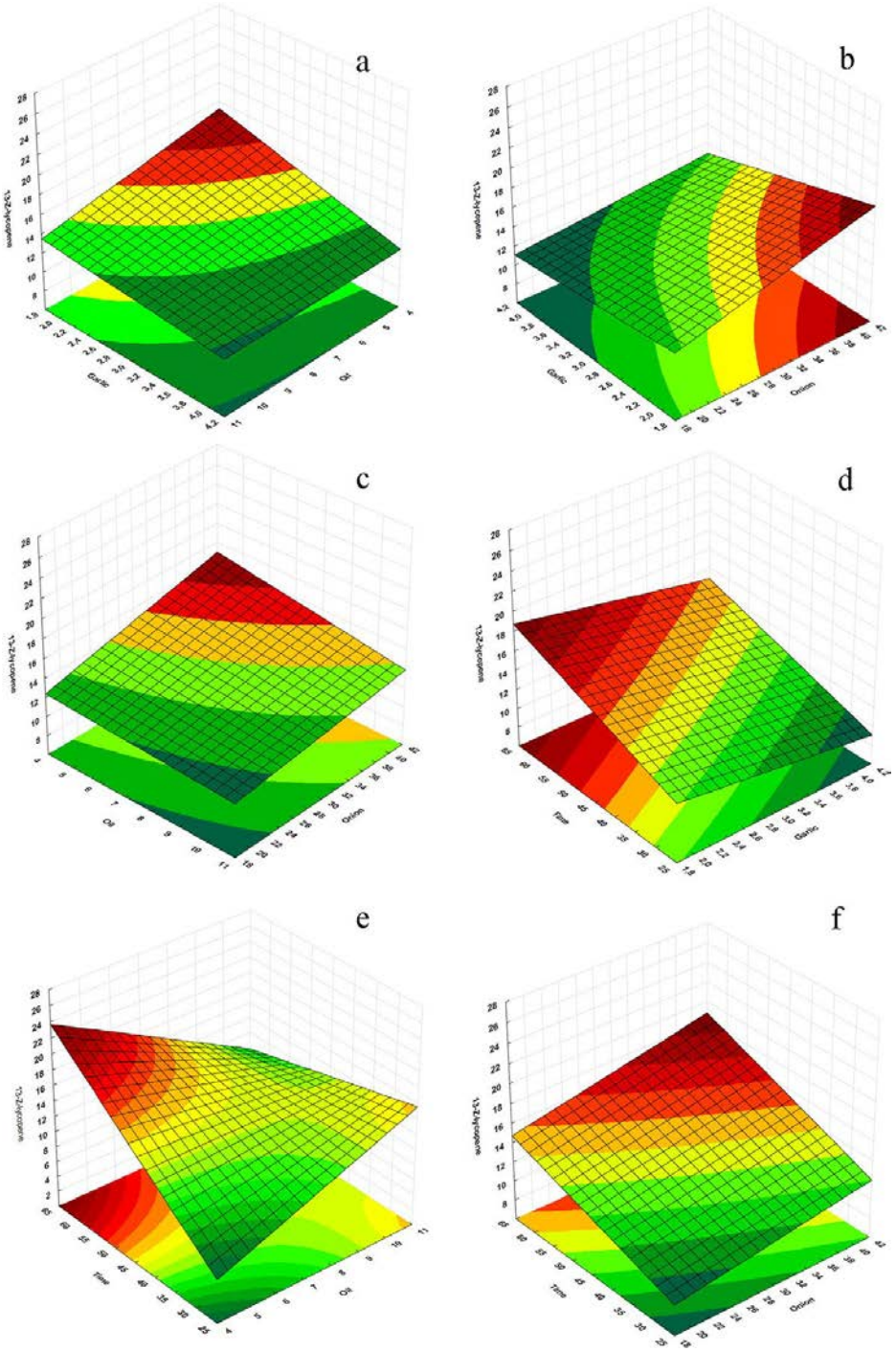

Fig. 5. Response surface plot of the combined effects of: garlic and oil at time/onion on level 0 (a); garlic and onion at olive oil/time on 0 level (b); onion and olive oil at garlic/time on level 0 (c); time and garlic at onion/olive oil on level 0 (d); time and olive oil at onion/garlic on level 0 (e); time and onion at garlic/olive oil on level 0 (f) on the content of 13-*Z*-lycopene of home cooked Mediterranean *sofrito*. (level 0: garlic = 3%; olive oil = 7.5%; onion = 30% and time = 45 min).

The content of 13-*Z*-lycopene was highest in formulation 11 (5% E, 40% O, 2% G and 60 min t) at 24.59 ± 2.07 μg/g of tomato, and lowest in formulation 5 (5%E, 20% O, 4% G and 30 min t) at 7.49 ± 0.31 μg/g of tomato (Table 3). The statistical parameters confirmed the model adequacy with high r^2 (0.97255) and adj-r^2 values (0.9609), a low coefficient of variation (5.75%), insignificant lack of fit and the graph of predicted *versus* observed values (Table 4 and Fig. 2). The linear factors presented a statistically significant F-value and *p*-value of ≤0.001. The significant interaction factors were EG, Et, OG, EGt and OGt with a significant *p*-value of $p \leq 0.001$ and EO and EOt with a significance of $p \leq 0.05$. The statistically significant regression coefficients were used to design the following equation for the model:

$$13{-}Z{-}\text{lycopene}(\mu g/g \text{ of tomato}) = -18.9291 + 2.4553E + 0.4211O - 1.3256G + 0.6949t + 0.3626EG - 0.0661Et - 0.1049OG$$

The surface plots of 13-*Z*-lycopene content showed cooking time to be the main factor for its isomerization (Fig. 5), since it was the source of energy for the system. Onion was another enhancing factor, albeit to a lesser extent than in the case of 5-*Z* and 9-*Z*, which could be attributed to a process of retro-isomerization to the all-*E*-lycopene, due to the lower stability of 13-*Z*-lycopene (Lambelet et al., 2009). A high garlic content had a slightly negative effect on the isomerization of 13-*Z*-lycopene (Fig. 5a, b and d). The content of EVOO had no effect when combined with garlic or onion. The effect of EVOO in combination with time showed the same pattern as in 5-*Z*, and 9-*Z*:13-*Z* formation was strongly reduced with high levels of oil and a long cooking time, although separately these two factors increased the isomer content. Lambelet et al. (2009) described the isomerization of 13-*Z*-lycopene as unstable, decreasing over time due to a retro-isomerization to all-*E*-lycopene. Vallverdú-Queralt et al. (2015) found an increase in the isomerization of 13-*Z*-lycopene during the first 45 min of cooking, with the content remaining the same thereafter until 60 min, suggesting that its content increases with the length of cooking.

Overall, the main factor for the formation of *Z*-lycopene isomers in *sofrito* was cooking time, probably because this variable is correlated with the quantity of energy required for the isomerization reaction (Colle et al., 2010a, 2010b, 2013; Honda et al., 2016; Lambelet et al., 2009; Vallverdú-Queralt et al., 2015). Time is essential in the thermal process because it helps to release the carotenoids by softening cell walls and denaturing the protein-carotenoid complex, which determines possible isomer formation and the carotenoid content of the final product (Murador et al., 2014).

Carotenoids are of value as functional ingredients in foods due to their health benefits. However, the instability, degradation and isomerization of carotenoids in foods require extensive knowledge of their behavior and careful selection of each ingredient in the formulation to obtain the greatest possible nutritional value (Boon et al., 2010). The bioavailability of carotenoids in tomato products, especially lycopene, can be improved by the addition of EVOO (Arranz et al., 2015), since it enhances carotenoid solubility and provides the necessary environment for isomerization (Colle et al., 2013; Honda et al., 2016). Nevertheless, a longer cooking time combined with a high level of EVOO was found to decrease the content of *Z*-isomers, possibly due to exposure to oxygen in the home cooking process, among other factors (Colle et al., 2010a, 2010b, 2015, 2013; Honda et al., 2016). Garlic had no noticeable effect on lycopene isomerization, probably due to its low content in the formulations. Onions proved to be the most interesting ingredient in the *sofritos* in that, combined with a long cooking time, they promoted the formation of lycopene isomers, and are therefore a potential ingredient to improve the bioavailability of lycopene in food. Further research is required to explore the influence of sulfur compounds in the production of lycopene isomers, and clarify a possible catalytic function.

3.2. Effect of ingredients and cooking time on α-carotene, β-carotene and their isomers

Table 3 shows the content of all-*E*-α-carotene, all-*E*-β-carotene and 13-*Z*-β-carotene in the different formulations. All-*E*-α-carotene content decreased during the cooking of *sofrito*; while its content in raw tomato was 5.45 ± 0.25 μg/g of tomato, in the formulations it ranged from 2.604 to 3.783 μg/g of tomato. In contrast, all-*E*-β-carotene and 13-*Z*-β-carotene increased in all the formulations compared to raw tomato, ranging from 3.33 to 6.19 μg/g of tomato and 2.15 to 3.22 μg/g of tomato, respectively. The models describing the effect of cooking time and *sofrito* ingredients on the content of all-*E*-α-carotene, all-*E*-β-carotene and 13-*Z*-β-carotene presented an insignificant lack of fit and a good coefficient of variation, but the r^2 and adj-r^2were beyond the criteria established in this study, and the models were not constructed.

4. Conclusions

In the present study, we analyzed the effect of the ingredients and cooking time on the content and isomerization of carotenoids in *sofrito*. We found that longer cooking times and the addition of onion in the preparation of *sofrito* increased lycopene isomerization, which is of interest because the *Z*-forms are more bioavailable and active compared to all-*E*-lycopene. Thus, the use of onion in tomato sauce recipes should be promoted to improve lycopene *Z*-isomer formation.

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References

Arita, S., Ando, S., Hosoda, H., Sakaue, K., Nagata, T., Murata, Y., ... Tada, M. (2005). Acceleration effect of sulfides on photodegradation of carotenoids by UVA irradiation. *Bioscience, Biotechnology, and Biochemistry*, *69*(9), 1786–1789. http://dx.doi.org/10.1271/bbb.69.1786.

Arranz, S., Martínez-Huélamo, M., Vallverdu-Queralt, A., Valderas-Martinez, P., Illán, M., Sacanella, E., ... Lamuela-Raventos, R. M. A. (2015). Influence of olive oil on carotenoid absorption from tomato juice and effects on postprandial lipemia. *Food Chemistry*, *168*, 203–210. http://dx.doi.org/10.1016/j.foodchem.2014.07.053.

Bohm, V., Puspitasari-Nienaber, N. L., Ferruzzi, M. G., & Schwartz, S. J. (2002). Trolox equivalent antioxidant capacity of different geometrical isomers of α-carotene, β-carotene, lycopene, and zeaxanthin. *Journal of Agricultural and Food Chemistry*, *50*(1), 221–226. http://dx.doi.org/10.1021/jf010888q.

Boon, C. S., McClements, D. J., Weiss, J., & Decker, E. a. (2010). Factors influencing the chemical stability of carotenoids in foods. *Critical Reviews in Food Science and Nutrition*, *50*(6), 515–532. http://dx.doi.org/10.1080/10408390802565889.

Burton-Freeman, B., Talbot, J., Park, E., Krishnankutty, S., & Edirisinghe, I. (2012). Protective activity of processed tomato products on postprandial oxidation and inflammation: A clinical trial in healthy weight men and women. *Molecular Nutrition & Food Research*, *56*(4), 622–631. http://dx.doi.org/10.1002/mnfr.201100649.

Capriotti, A. L., Cavaliere, C., Crescenzi, C., Foglia, P., Nescatelli, R., Samperi, R., & Laganà, A. (2014). Comparison of extraction methods for the identification and quantification of polyphenols in virgin olive oil by ultra-HPLC-QToF mass spectrometry. *Food Chemistry*, *158*, 392–400. http://dx.doi.org/10.1016/j.foodchem.2014.02.130.

Cervantes-paz, B., Victoria-campos, C. I., & Ornelas-paz, J. D. J. (2016). *Carotenoids in natureVol. 79. http://dx.doi.org/10.1007/978-3-319-39126-7*.

Colle, I. J. P., Lemmens, L., Tolesa, G. N., Van Buggenhout, S., De Vleeschouwer, K., Van Loey, A. M., & Hendrickx, M. E. (2010a). Lycopene degradation and isomerization kinetics during thermal processing of an olive oil/tomato emulsion. *Journal of Agricultural and Food Chemistry*, *58*(24), 12784–12789. http://dx.doi.org/10.1021/jf102934u.

Colle, I., Lemmens, L., Van Buggenhout, S., Van Loey, A., & Hendrickx, M. (2010b). Effect of thermal processing on the degradation, isomerization, and bioaccessibility of lycopene in tomato pulp. *Journal of Food Science*, *75*(9), 753–759. http://dx.doi.org/10.1111/j.1750-3841.2010.01862.x.

Colle, I. J. P., Van Buggenhout, S., Lemmens, L., Van Loey, A. M., & Hendrickx, M. E. (2012). The type and quantity of lipids present during digestion influence the in vitro

bioaccessibility of lycopene from raw tomato pulp. *Food Research International*, *45*(1), 250–255. http://dx.doi.org/10.1016/j.foodres.2011.10.041.

Colle, I. J. P., Lemmens, L., Van Buggenhout, S., Van Loey, A. M., & Hendrickx, M. E. (2013). Modeling lycopene degradation and isomerization in the presence of lipids. *Food and Bioprocess Technology*, *6*(4), 909–918. http://dx.doi.org/10.1007/s11947-011-0714-4.

Colle, I. J. P., Lemmens, L., Knockaert, G., Van Loey, A., & Hendrickx, M. (2015). Carotene degradation and isomerization during thermal processing: A review on the kinetic aspects. *Critical Reviews in Food Science and Nutrition*, 0–48. http://dx.doi.org/10.1080/10408398.2013.790779 (8398 December).

Corzo-Martínez, M., Corzo, N., & Villamiel, M. (2007). Biological properties of onions and garlic. *Trends in Food Science and Technology*, *18*(12), 609–625. http://dx.doi.org/10.1016/j.tifs.2007.07.011.

Cuevas-Ramos, D., Almeda-Valdés, P., Chávez-Manzanera, E., Meza-Arana, C. E., Brito-Córdova, G., Mehta, R., ... Gómez-Pérez, F. J. (2013). Effect of tomato consumption on high-density lipoprotein cholesterol level: A randomized, single-blinded, controlled clinical trial. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *6*, 263–273. http://dx.doi.org/10.2147/DMSO.S48858.

Estruch, R., Ros, E., Salas-Salvado, J., Covas, M. -I., Coreila, D., Ards, F., ... Martinez-Gonzalez, M. A. (2013). Primary prevention of cardiovascular disease with a Mediterranean diet. *New England Journal of Medicine*, *368*(14), 1279. http://dx.doi.org/10.1056/NEJMoa1200303.

Hoffman, R., & Gerber, M. (2015). Food processing and the Mediterranean diet. *Nutrients*, *7*(9), 7925–7964. http://dx.doi.org/10.3390/nu7095371.

Honda, M., Horiuchi, I., Hiramatsu, H., Inoue, Y., Kitamura, C., Fukaya, T., & Takehara, M. (2016). Vegetable oil-mediated thermal isomerization of (all - *E*)-lycopene: facile and efficient production of *Z*-isomers. *European Journal of Lipid Science and Technology*. http://dx.doi.org/10.1002/ejlt.201500446 (n/a–n/a).

Hrvolová, B., Martínez-Huélamo, M., Colmán-Martínez, M., Hurtado-Barroso, S., Lamuela-Raventós, R., & Kalina, J. (2016). Development of an advanced HPLC–MS/MS method for the determination of carotenoids and fat-soluble vitamins in human plasma. *International Journal of Molecular Sciences*, *17*(10), 1719. http://dx.doi.org/10.3390/ijms17101719.

Jacques, P. F., Lyass, A., Massaro, J. M., Vasan, R. S., & D'Agostino, R. B. (2013). Relationship of lycopene intake and consumption of tomato products to incident CVD. *The British Journal of Nutrition*, *110*(3), 545–551. http://dx.doi.org/10.1017/S0007114512005417.

Lambelet, P., Richelle, M., Bortlik, K., Franceschi, F., & Giori, A. M. (2009). Improving the stability of lycopene *Z*-isomers in isomerised tomato extracts. *Food Chemistry*, *112*(1), 156–161. http://dx.doi.org/10.1016/j.foodchem.2008.05.053.

Murador, D. C., da Cunha, D. T., & de Rosso, V. V. (2014). Effects of cooking techniques on vegetable pigments: A meta-analytic approach to carotenoid and anthocyanin levels. *Food Research International*, *65 (PB)*, 177–183. http://dx.doi.org/10.1016/j.foodres.2014.06.015.

Palmero, P., Panozzo, A., Simatupang, D., Hendrickx, M., & Van Loey, A. (2014). Lycopene and ??-carotene transfer to oil and micellar phases during in vitro digestion of tomato and red carrot based-fractions. *Food Research International*, *64*, 831–838. http://dx.doi.org/10.1016/j.foodres.2014.08.022.

Rodriguez, D. (2001). *A guide to carotenoid analysis in foods. Life Sciences.* Washington, D. C: ILSI PRESS International Life Sciences Institute One Thomas Circle, N.W.

Sesso, H. D., Wang, L., Ridker, P. M., & Buring, J. E. (2012). Tomato-based food products are related to clinically modest improvements in selected coronary biomarkers in women. *The Journal of Nutrition*, *142*(2), 326–333. http://dx.doi.org/10.3945/jn.111.150631.

Seyed Shahabadi, S. M., & Reyhani, A. (2014). Optimization of operating conditions in ultrafiltration process for produced water treatment via the full factorial design methodology. *Separation and Purification Technology*, *132*, 50–61. http://dx.doi.org/10.1016/j.seppur.2014.04.051.

Valderas-Martinez, P., Chiva-Blanch, G., Casas, R., Arranz, S., Martínez-Huélamo, M., Urpi-Sarda, M., ... Estruch, R. (2016). Tomato sauce enriched with olive oil exerts greater effects on cardiovascular disease risk factors than raw tomato and tomato sauce: A randomized trial. *Nutrients*, *8*(3), 1–14. http://dx.doi.org/10.3390/nu8030170.

Vallverdú-Queralt, A., de Alvarenga, J. F. R., Estruch, R., & Lamuela-Raventos, R. M. (2013). Bioactive compounds present in the Mediterranean sofrito. *Food Chemistry*, *141*(4), 3366–3372. http://dx.doi.org/10.1016/j.foodchem.2013.06.032.

Vallverdú-Queralt, A., Regueiro, J., de Alvarenga, J. F. R., Torrado, X., & Lamuela-Raventos, R. M. (2015). Carotenoid profile of tomato sauces: Effect of cooking time and content of extra virgin olive oil. *International Journal of Molecular Sciences*, *16*(5), 9588–9599. http://dx.doi.org/10.3390/ijms16059588.

4.2.3 Publication 5. Mediterranean *sofrito* home-cooking technique enhances polyphenol content in tomato sauce

José Fernando Rinaldi de Alvarenga, Paola Quifer-Rada, Victoria Westrin, Sara Hurtado-Barroso, Xavier Torrado-Prat, Rosa Maria Lamuela-Raventós. Sent to *Journal of Science of Food and Agriculture*.

Tomatoes are highly daily consumed in Mediterranean countries compared to other vegetables and epidemiological studies suggested that processed tomato-based products have a greater correlation with a low incidence of chronic-degenerative diseases. *Sofrito*, a basic culinary technique widely used in the Mediterranean, may preserve polyphenols and help to increase their intake in the Mediterranean population. The aim of this study was to evaluate how the *sofrito* cooking technique could improve tomato polyphenols content in *sofrito* sauce. Phenolic compounds content was analyzed by HPLC-ESI-QqQ-MS/MS. A full factorial design (FFD) 2^4 was performed to clarify the effect of the factors EVOO (5% and 10%), onion (20% and 40%), garlic (2% and 4%) and cooking time (30 and 60 min) on polyphenols content of tomato-*sofrito*. The FFD experiments were performed independently, randomized and in triplicate totalizing 48 experiments. Data obtained from FFD were evaluated by ANOVA and Fisher test and mathematical models for each phenolic compound were constructed. Among of 16 polyphenols typically found in tomato, ferulic acid, caffeic acid, caffeic acid hexoside I and II were excluded of from the statistical analysis for being part of the composition of the other ingredients. When compared to raw tomato, the content of chlorogenic acid, ferulic acid hexoside and narigenin increased after cooking process, criptochlorogenic acid remained stable and dicaffeoylquinic acid and coumaric acid hexoside I and II decreased independently of the cooking time and ingredients content used in the *sofrito* preparation. Mathematical models were constructed for chlorogenic acid, ferulic acid hexoside and naringenin to understand how cooking and ingredients synergism improve phenolic compounds content. Cooking time decreased the content of phenolic compounds in tomato *sofrito.* The use of EVOO helps to extract polyphenols from the food matrix that contribute to the increase its content. The presence of a high content of onion can protect some phenolic compounds from oxidation during the *sofrito* cooking process. Mediterranean *sofrito* cuisine technique could contributes to polyphenol consumption in the Mediterranean population.

MEDITERRANEAN SOFRITO HOME-COOKING TECHNIQUE ENHANCES POLYPHENOL CONTENT IN TOMATO SAUCE

Running title: *Sofrito* cooking process enhances tomato polyphenol content

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Abstract

BACKGROUND: *Sofrito*, a basic culinary technique widely used in the Mediterranean, may preserve dietary polyphenols and enhance their intake in the Mediterranean population. The aim of this study was to investigate if the *sofrito* technique improves the polyphenol content in a tomato-based *sofrito* sauce. **RESULTS:** A full factorial design was applied using mathematical models. The content of chlorogenic acid, ferulic acid hexoside and naringenin was higher in the *sofrito* sauce than in raw tomato. The bioaccessibility of some tomato polyphenols was enhanced by the presence of olive oil and they were protected from oxidation during the cooking process by the use of onion. **CONCLUSION:** The use of olive oil and onion in Mediterranean cooking as a base for sauces and dishes, with an appropriate cooking time, enhances the polyphenol content of food. Thus, Mediterranean cuisine contributes to the health effects of the Mediterranean diet.

Keywords: phenolic compounds, matrix effect, extra virgin olive oil, onion, cooking

1. Introduction

The health benefits of the Mediterranean diet are associated with a high intake of phytochemicals from vegetables and legumes [1,2], yet have proved difficult to reproduce in non-Mediterranean populations; cultural differences between countries, including culinary techniques, may be among the factors responsible for this lack of success [1]. The impact of cooking on bioactive compounds has been analyzed [2,3,] but only a few studies have focused on the effect of Mediterranean culinary techniques on polyphenol intake in the Mediterranean population [4,5.]

Tomatoes are highly prevalent in the Mediterranean diet because of their versatility, being consumed directly or as an ingredient in many food recipes [6]. Due to their year-round availability

and accessible price, the daily consumption of tomatoes in Mediterranean countries is higher than that of other vegetables and fruits [7,8]. The tomato *sofrito* sauce, the basis of many Mediterranean recipes, is prepared by frying onion and garlic in olive oil. This sauce has been reported to contain 40 different phenolic compounds and a high content of carotenoids [9]. Epidemiological studies have shown that the consumption of processed tomato-based products has a greater correlation with a low incidence of chronic-degenerative diseases compared to raw tomato [10]. Moreover, tomato *sofrito* consumption is associated with improved cardiovascular risk parameters and insulin sensitivity, and activates thermogenesis by browning fat tissue [11,12]. This key component of the Mediterranean diet has also recently been correlated with the manifestation of less aggressive prostate cancer [13].

The aim of this study was to evaluate how the *sofrito* cooking technique and ingredients used to prepare tomato *sofrito* sauce could improve the content of tomato phenolic compounds.

2. Material and methods

2.1 Material

Tomatoes (Licopersicon esculentum Mill, c. v. Pera) of equal diameter from the same batch were bought from Grupo Almeria (La Cañada, Almeria, Spain). Onions and garlic were bought from Casa Ametller (Barcelona, Spain). Manuel Heredia Halcón (Cortijo De Suerte Alta, Albedin-Baena-Cordoba) supplied the extra virgin olive oil (EVOO).

2.2 *Sofrito* cooking process

The *sofritos* were prepared based on a traditional Mediterranean recipe in an industrial kitchen at Torribera Campus, University of Barcelona (Santa Coloma de Gramenet, Spain). The ingredients were washed, crushed with a mixer (model R5 Plus, Robot Coupe®), and weighed according to a previously published full factorial design (FFD) 5. The EVOO was heated for 1 min in a pan (24 cm diameter, 15 cm height, 6.3 L volume, 1.59 mm thickness, made of inox 18/10) without a lid, followed by the addition of onion and garlic. After frying for 3 minutes, the tomato was added and the cooking time was monitored. The temperature of the electrical cooking plate (180 mm diameter, 1500W, model Encimera EM/30 2P, Teka®) was adjusted to remain constant throughout the cooking process (100 ± 1 ºC). Once prepared, all the *sofritos* were weighed to evaluate water loss (Table 1), and frozen in plastic vacuum bags for storage at -25º C.

2.3 Full factorial design and statistical analysis

In order to understand the effect of each ingredient, the cooking time, and any ingredient synergism on the tomato phenolic compounds, a full factorial design (FFD) was used with the factors EVOO (E), onion (O) and garlic (G) content, and cooking time (t). The four factors had a high (+1) and low (-1) level, providing a 2^4 FFD for a total of 16 formulations. These were independently analyzed in triplicate and randomized for better reproducibility and error

estimation, resulting in a total of 48 experiments. The levels for the different factors are described in Table 1. Data obtained from the FFD were evaluated by analysis of variance (ANOVA) and the

Table 1. Experimental level of the factors used in the Full Factorial Design (FDD).

	EVOO (%)	Onion (%)	Garlic (%)	Time (min)	Tomato (%)	Initial weight (g)	Final weight (g)		
							a	b	c
1	5	20	2	30	73	1000	497	492	515
2	10	20	2	30	68	1000	523	485	500
3	5	40	2	30	53	1000	347	390	401
4	10	40	2	30	48	1000	465	436	430
5	5	20	4	30	71	1000	486	545	491
6	10	20	4	30	66	1000	535	628	621
7	5	40	4	30	51	1000	453	454	433
8	10	40	4	30	46	1000	407	385	380
9	5	20	2	60	73	1000	446	486	423
10	10	20	2	60	68	1000	455	497	478
11	5	40	2	60	53	1000	461	466	464
12	10	40	2	60	48	1000	422	420	421
13	5	20	4	60	71	1000	398	472	513
14	10	20	4	60	66	1000	503	547	531
15	5	40	4	60	51	1000	379	500	480
16	10	40	4	60	46	1000	401	361	362

n = 3 (represent as a, b and c)
EVOO = Extra Virgen Olive Oil. Initial weight (before cooking) and Final weight (after cooking process).

Fisher test using STATISTICA 10 software (StatSoft, USA). A possible first-order regression equation with the factors and the interaction between them was applied for modeling as follows (Equation 1):

$$Y = b0+b1E+b2O+b3G+b4t +b12EO+b13EG+b14Et+b23OG+b24Ot+b34Gt+b123EOG+ b124EOt+b134EGt+b234OGt+b1234EOGt$$

In order to construct an adequate model, the following criteria were established: a coefficient of regression (r^2) and adjusted coefficient of regression (r^2-adj) greater than 80% (<0.80), a non-significant lack of fit ($p>0.05$) and a coefficient of variation less than 10% (<10%). The normality of the data was verified and a linear correlation plot of observed versus predicted values was obtained.

2.4 Extraction and Analysis of Polyphenols

Polyphenol extraction was performed under lights with UV filters and ice to minimize compound degradation. All samples were extracted in triplicate.

2.4.1 Extraction of polyphenols from tomato, onion, garlic and *sofrito*

The *sofrito* and each ingredient were weighed (0.5g) and homogenized with 5 mL of methanol:ultra-pure water (80:20, v:v) for 1 min, sonicated for 10 min in an ice bath and centrifuged at 4000 rpm for 15 min at 4º C. The supernatant was transferred into a 10 mL glass tube and the extraction was repeated. After that, the supernatants were combined and evaporated using a vacuum evaporator (miVac DNA concentrator, Genevac LTD, England). The residue was

suspended in up to 2 mL of ultra-pure water with 0.1% of formic acid, filtered through a 0.22 µm PTFE filter into a 2 mL amber vial and stored at -80 ºC [9].

2.4.2 Extraction of polyphenols from EVOO

EVOO (1g) was weighed and diluted with 1 mL of n-hexane. After the addition of 2 mL of methanol, it was homogenized and centrifuged at 3000 rpm for 3 min at 4ºC. The nonpolar and polar phases were separated and extracted again with 1 mL of n-hexane and 2 mL of methanol, respectively. The polar phases were combined, and 50 mg of C18 was added to remove the polar fatty acids, followed by homogenization for 1 min and centrifugation. After the extract was maintained at -80 ºC for 15 min, a visual inspection was made to ensure that no lipids were present. The clean liquid was evaporated until dryness using a vacuum evaporator. The residue was reconstituted using 2 mL of ultra-pure water with 0.2% of acetic acid, filtered with a 0.22 µm PTFE filter into a 2 mL amber vial and stored at -80 ºC [14.]

2.4.3 Targeted polyphenol analysis by UHPLC-ESI-QqQ-MS/MS

The quantification of phenolic compounds from tomato was carried out in an UHPLC Acquity system equipped with a binary pump and an autosampler from Waters (Milford, MA, United States) coupled to an API 3000 triple quadrupole mass spectrometer (ABSciex, Framingham, MA, USA) equipped with a Turbo Ion spray source in negative-ion mode for the MS/MS data analysis. Quantification of polyphenols was performed using multiple reaction monitoring (MRM) mode. The separation was performed in a BEH C18 column (50 mm x 2.1 mm) i.d., 1.7 µm from Waters. Chromatography conditions and mass spectrometer settings were according to a method previously validated by our group [15].

The quantification was carried out using ethylgallate as an internal standard. The polyphenols were quantified using the corresponding standard or a compound of the same class. In order to compare the content of each phenolic compound in the different *sofrito* sauces, the samples of the FFD were expressed per g of fresh tomato.

3. Results and discussion

3.1 Polyphenols in the *sofrito* sauce and its ingredients

We investigated the effect of the *sofrito* ingredients and cooking time on 16 polyphenols typically found in tomatoes[15]. Analysis of raw tomato revealed a high content of caffeic acid (CfA) and its hexoside forms (I and II), chlorogenic acid (CA), criptochlorogenic acid, coumaric acid hexosides (CoAH), ferulic acid hexoside (FAH), naringenin and rutin (Table 2). The phenolic compounds found in onion were CfA hexoside I and II, ferulic acid (FA), and quercetin. Garlic contained FA and CfA and its hexoside forms I and II, and EVOO only presented FA.

The polyphenols found in ingredients other than tomato (FA, CfA, CfA hexoside I and II) were excluded from the statistical analysis in order to construct a mathematical model describing

Table 2. Experimental response values of phenolic compounds content (µg/g of tomato.) on processed *sofrito* according to the full factorial design and ingredients.

chlorogenic acid	criptochlorogenic acid	coumaric hexoside I	coumaric hexoside II	dicaffeoylquinic	ferulic hexoside	naringenin	Naringenin -7- glucoside	rutin	caffeic acid	caffeic acid hexoside I	caffeic acid hexoside II	ferulic acid	querc
5.4 ± 0.2	2.5 ± 0.2	1.31 ± 0.01	1.37 ± 0.08	0.41 ± 0.04	6.3 ± 0.5	3.47 ± 0.04	0.028 ± 0.003	3.15 ± 0.06	0.9 ± 0.1	2.9 ± 02	1.02 ± 0.07	0.17 ± 0.02	1.4 ±
5.6 ± 0.2	2.60 ± 0.04	1.36 ± 0.1	1.4 ± 0.1	0.35 ± 0.02	6.4 ± 0.3	3.5 ± 0.1	0.024 ± 0.002	3.13 ± 0.03	1.52 ± 0.07	3.1 ± 0.2	1.05 ± 0.03	0.3 ± 0.2	1.5 ±
6.7 ± 0.3	2.8 ± 0.2	0.983 ± 0.005	1.09 ± 0.03	0.34 ± 0.02	7.06 ± 0.05	3.4 ± 0.4	0.0185 ± 0.0008	2.9 ± 0.2	1.07 ± 0.09	3.04 ± 0.05	0.96 ± 0.08	0.4 ± 0.1	3.3 ±
6.0 ± 0.3	2.4 ± 0.2	0.99 ± 0.03	0.95 ± 0.04	0.34 ± 0.04	7.2 ± 0.7	2.7 ± 0.2	0.016 ± 0.001	2.35 ± 0.09	1.29 ± 0.06	3.2 ± 0.5	1.09 ± 0.09	0.5 ± 0.1	5.8 ±
3.8 ± 0.2	2.0 ± 0.1	0.92 ± 0.01	0.88 ± 0.01	0.264 ± 0.005	4.4 ± 0.2	2.74 ± 0.07	0.0192 ± 0.0001	2.23 ± 0.03	0.92 ± 0.02	2.3 ± 0.3	0.88 ± 0.09	0.3 ± 0.1	4.7 ±
5.4 ± 0.1	2.89 ±0.08	0.79 ± 0.03	0.87 ± 0.09	0.370 ± 0.007	6.5 ± 0.4	2.5 ± 0.5	0.027 ± 0.001	2.31 ± 0.05	1.27 ± 0.03	3.0 ± 0.4	1.10 ± 0.08	0.4 ± 0.1	1.59 ±
3.8 ± 0.1	2.34 ± 0.07	0.58 ± 0.07	0.79 ± 0.04	0.30 ± 0.02	5.2 ± 0.6	2.16 ± 0.09	0.0161 ± 0.0004	2.2 ± 0.1	1.00 ± 0.04	2.3 ± 0.1	0.88 ± 0.06	0.5 ± 0.1	6.7 ±
4.5 ± 0.3	2.4 ± 0.2	0.57 ± 0.08	0.84 ± 0.08	0.295 ± 0.007	4.3 ± 0.4	2.34 ± 0.08	0.021 ± 0.002	2.17 ± 0.07	1.3 ± 0.2	2.2 ± 0.2	0.9 ± 0.1	0.55 ± 0.05	5 ±
4.0 ± 0.5	2.7 ± 0.3	1.08 ± 0.02	1.4 ± 0.2	0.35 ± 0.04	4.6 ± 0.7	2.5 ± 0.2	0.037 ± 0.002	2.70 ±0.02	0.84 ± 0.09	2.8 ± 0.4	1.0 ± 0.2	0.5 ± 0.2	1.9 ±
3.7 ± 0.2	2.5 ± 0.4	0.9 ± 0.1	1.2 ± 0.1	0.34 ± 0.06	4.4 ± 0.2	2.4 ± 0.2	0.024 ± 0.002	1.85 ± 0.08	1.5 ± 0.2	2.7 ± 0.6	1.0 ± 0.2	0.13 ± 0.01	2.0 ±
5.8 ± 0.1	2.75 ±0.03	0.907 ± 0.006	1.1 ± 0.1	0.360 ± 0.003	6.6 ± 0.1	2.5 ± 0.2	0.024 ± 0.001	2.44 ± 0.05	1.5 ± 0.4	2.5 ± 0.5	1.0 ± 0.2	0.39 ± 0.04	6.1 ±
5.4 ± 0.4	2.7 ± 0.2	0.81 ± 0.06	1.04 ± 0.05	0.38 ± 0.03	6.3 ± 0.5	1.92 ± 0.02	0.014 ± 0.002	2.26 ± 0.09	1.6 ± 0.4	2.57 ± 0.06	1.11 ± 0.06	0.5 ± 0.1	6.0 ±
3.1 ± 0.1	2.1 ± 0.1	0.84 ± 0.02	1.03 ± 0.02	0.248 ± 0.003	3.2 ± 0.1	1.87 ± 0.02	0.0175 ± 0.0001	2.39 ± 0.09	1.08 ± 0.07	2.21 ± 0.04	0.95 ± 0.02	0.4 ± 0.2	1.4 ±
3.2 ± 0.3	2.2 ± 0.1	0.86 ± 0.03	1.1 ± 0.1	0.26 ± 0.03	3.63 ± 0.02	1.5 ± 0.1	0.0131 ± 0.0002	2.5 ± 0.3	1.1 ± 0.1	2.6 ± 0.2	1.03 ± 0.04	0.26 ± 0.04	1.0 ±
3.4 ± 0.3	2.6 ± 0.2	0.78 ± 0.08	1.1 ± 0.1	0.23 ± 0.03	4.2 ± 0.6	1.67 ± 0.01	0.0316 ± 0.0009	2.7 ± 0.3	1.32 ± 0.2	2.9 ± 0.4	1.2 ± 0.2	0.65 ± 0.05	4.1 ±
3.2 ± 0.2.	2.37 ± 0.07	0.75 ± 0.06	1.00 ± 0.04	0.23 ± 0.03	3.8 ± 0.5	1.3 ± 0.1	0.0224 ± 0.0002	2.3 ± 0.2	1.15 ± 0.09	2.5 ± 0.1	1.13 ± 0.08	0.6 ± 0.2	2.6 ±
n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.12 ± 0.02	n.(
n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.30 ± 0.03	0.112 ± 0.002	0.73 ± 0.07	34 ±
n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.8 ± 0.1	0.36 ± 0.02	0.36 ± 0.04	4.4 ± 0.4	n.(
4.4 ± 0.4	2.6 ± 0.2	1.58 ± 0.09	2.5 ± 0.1	0.61 ± 0.02	4.5 ± 0.4	1.9 ± 0.2	0.076 ± 0.009	2.94 ± 0.05	1.36 ± 0.03	4.7 ± 0.2	1.53 ± 0.06	0.10 ± 0.01	0.236 ±

Mean ± SD (n =3)
E (Extra Virgen Olive Oil); O (Onion), G (Garlic); T (Tomato)

the behavior of tomato polyphenols during the cooking process and to determine the effect of the sofrito technique on these bioactive compounds.

Table 2 shows the tomato polyphenols quantified in the *sofrito* formulations. The content of CA, FAH and naringenin increased after the cooking process, reaching maximum values of 6.7 μg/g, 7.2 μg/g and 3.5 μg/g, respectively, compared to the respective values of 4.4 μg/g, 4.5 μg/g and 1.9 μg/g in raw tomato. Criptochlorogenic acid remained stable during the cooking process, whereas rutin presented a slight increase in formulations 1 and 2 after 30 min of cooking compared to raw tomato, decreasing in the other formulations. Dicaffeoylquinic acid and CoAH I and II levels in the *sofritos* decreased, independently of the cooking time or amount of ingredients used in the formulations.

All the phenolic compounds in Table 2 were included in the statistical analysis and selected according to the criteria described in Section 2.3. CA, CoAH I, FAH, naringenin and naringenin-7-O-glucoside fulfilled the criteria, and a model was generated for each of these compounds to describe the effect of the *sofrito* technique on the polyphenol content of the tomato sauce. CA, FAH and naringenin were chosen to investigate how cooking and ingredient synergism may affect the different classes of phenolic compounds.

3.2 Effect of the *sofrito* technique on CA

The content of CA in the 16 different tomato *sofrito* sauce formulations ranged from 3.1 to 6.7 μg/g of tomato (Table 2). ANOVA was performed to determine the factors that affect the CA content in the *sofrito*, using a p-value and F-value in the confidence level (α = 0.05) to determine the statistical significance and importance of the factors for the model, respectively. Factors O, G, and t, and the interaction factors EG, EO, Et, OG, Ot and EGt all presented a statistically significant p-value ($p \leq 0.05$) and significant F-values (Table 3). The CA model presented an r^2 of 0.9510 and an adj-r^2 of 0.9303; the lack of fit was statistically insignificant ($p \geq 0.05$) and the CV% was low (5.64%). The graphs of predicted and observed values (Figure 1) agree with the experimental data. These statistical parameters confirm the consistency of the linear model, indicating its suitability for describing how the ingredients and cooking time influenced the CA content. To improve the model, the insignificant terms were excluded, a new ANOVA was performed, and the regression coefficients were used to design the equation for the model (Table 3):

$$\text{CA(μg/g of tomato)} = 5.722652 + 0.117481O - 0.384765G$$

$$-0.048519t - 0.005029EO + 0.115502EG - 0.035970OG + 0.001259Ot$$

Figure 2 depicts the effect of the factors and their interactions on the CA content in a surface plot. Cooking time was the variable that most affected the CA levels (Figure 2c, 2e and 2f), which decreased along the cooking process. On the other hand, the presence of onions

Table 3. ANOVA (*F*-value), lack of fit, coefficient of variation and significant regression coefficients of the first order model for the polyphenol content of processed *sofrito* according to the full factorial design.

	chlorogenic acid		criptochlorogenic acid	coumaric hexoside I		coumaric hexoside II	dicaffeoylquinic	ferulic hexoside		naringenin		naringenin-7-glucoside	
	F-value	RC	*F*-value	*F*-value	RC	*F*-value	*F*-value	*F*-value	RC	*F*-value	RC	*F*-value	RC
Mean		5.722652			2.989461				8.367237		5.884412		
E	2.35		0.42	4.25*		1.67	0.46	0.63		14.85***	-0.057117	56.40***	
O	39.17***	0.117481	3.06	97.23***	-0.025638	24.94***	2.00	18.04***		20.95***	-0.016713	40.53***	
G	278.12***	-0.384765	12.80**	166.47***	-0.382392	54.81***	76.41***	130.44***		134.80***	-0.403614	18.10***	
t	165.34***	-0.048519	0.00	9.75**	-0.028226	7.48*	11.74**	78.13***	-0.105396	187.29***	-0.030680	11.39**	0.001482
E O	9.44**	-0.005029	9.00**	0.20		0.30	0.14	9.73**		2.35		1.21	
E G	23.51***	0.115502	6.80*	0.09		1.57	4.16*	1.64		0.89		52.34***	0.001653
E t	11.67**		4.27*	1.30		0.40	0.11	2.96		1.33		118.30***	
O G	62.43***	-0.035970	0.02	1.99		15.68***	0.74	23.24***	-0.037746	0.00		177.07***	-0.000346
O t	17.20***	0.001259	3.47	20.30***	0.000329	2.51	2.26	16.60***	0.002039	1.44		47.26***	-0.000037
G t	0.00		1.50	53.61***	0.005353	14.18***	10.20**	0.20		0.65		8.69**	-0.000409
E O G	0.29		2.09	0.08		0.05	7.43*	9.96**		8.18**		9.44**	
E O t	2.90		3.89	0.07		0.05	0.79	2.64		0.13		0.13	
E G t	9.59**		5.59*	6.29*		0.01	4.75*	0.21		2.35		6.13*	-0.000016
O G t	0.66		1.10	0.01		0.47	2.61	0.03		0.00		93.94***	0.000016
Lack of Fit	0.06^{ns}		0.16^{ns}	2.46^{ns}		3.52^{ns}	2.11^{ns}	3.74^{ns}		1.51^{ns}		0.14^{ns}	
CV%	5.64		7.24	6.87		8.42	8.83	8.06		7.92		6.37	
r^2	0.95104		0.62675	0.91301		0.77752	0.78397	0.89174		0.91801		0.95225	
r^2-Adj	0.93027		0.4684	0.8761		0.68313	0.69232	0.84581		0.88322		0.93199	

*p≤0.05; **p≤0.01; ***p≤0.001; ns = not significative
E (Extra Virgen Olive Oil); O (Onion); G (Garlic); t (time); RC (regression coeficient)

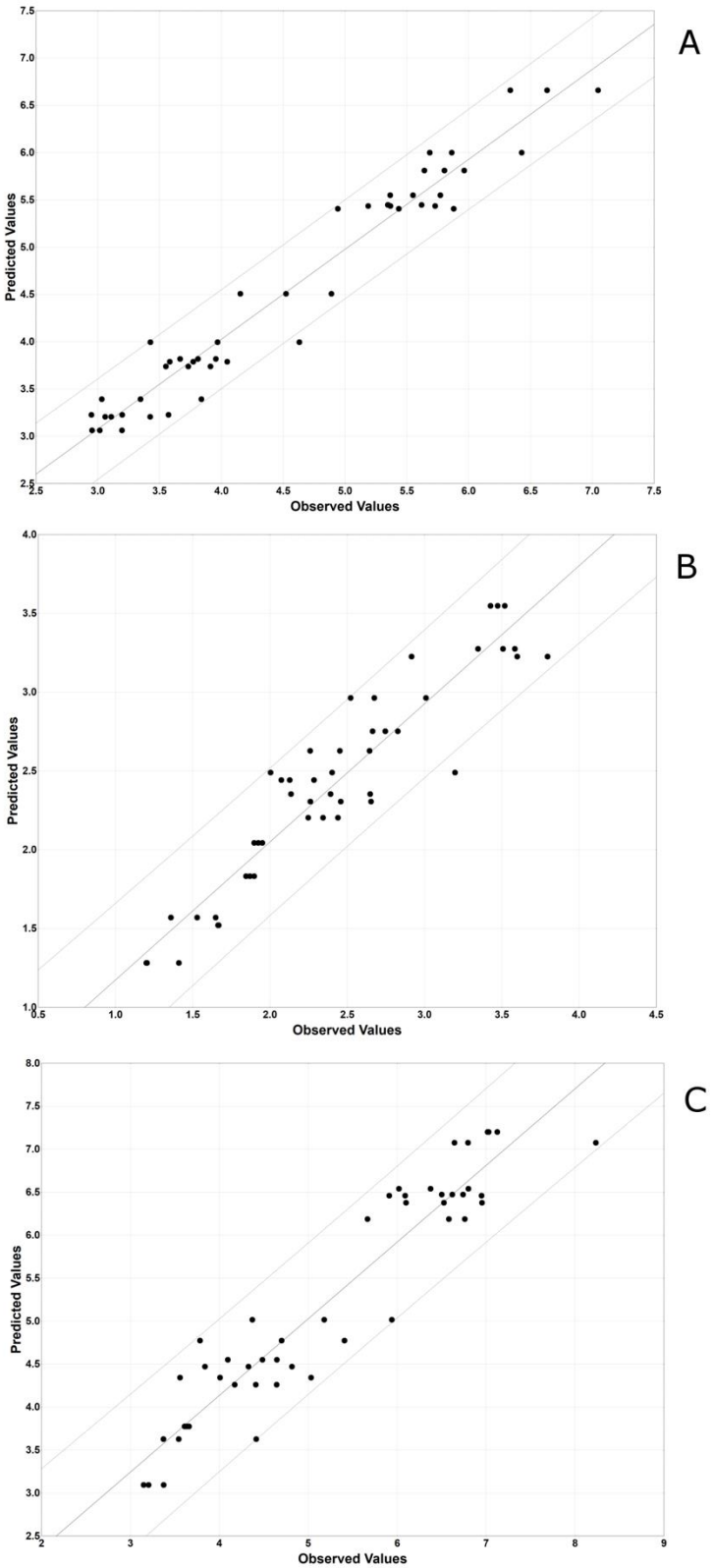

Figure 1. Comparison of predicted vs. observed values of chlorogenic acid (a), naringenin (b) and ferulic acid hexoside (c).

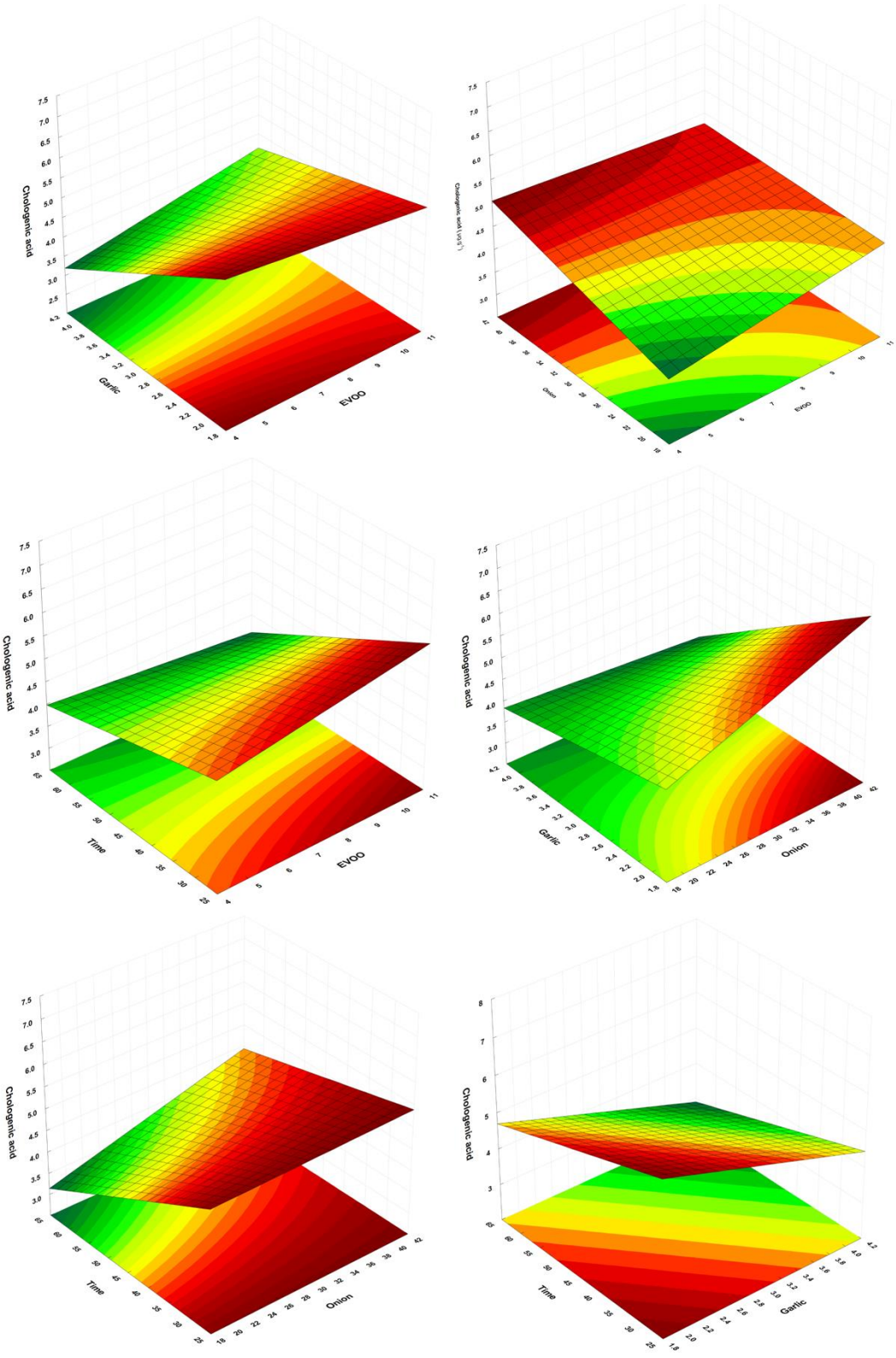

Figure 2. Response surface plot of the combined effects of: garlic and oil at time/onion on level 0 (a); oil and onion at garlic/time on level 0 (b); time and oil at garlic/garlic on level 0 (c); onion and garlic at time/oil on level 0 (d); time and onion at oil/garlic on level 0 (e); time and garlic at oil/onion on level 0 (f) the content of chlorogenic acid of home-cooked Mediterranean sofrito. (level 0: garlic = 3%; olive oil = 7.5%; onion = 30% and time = 45 min).

contributed to preserving CA in the sauce, showing a positive main factor (O) and interaction factor with time (Ot) (Figure 2b, 2d and 2e). A high content (10%) of EVOO was associated with an increase in CA (Figure 1c), albeit not statistically significant, and the main factor did not affect the CA content.

In a study of industrial Spanish *sofritos*, Vallverdú-Queralt et al (2013) reported a CA content between 4.43 and 5.50 µg/g, which is in accordance with our results. The observed increase in CA could be attributed to the hydrolysis of dicaffeoylquinic acid present in tomato, as free hydroxyl phenol groups may be enhanced by the hydrolysis of quinic acid[16]. The positive effect of onion on CA in the sofrito could be related with its content of other antioxidants, such as quercetin, which is an effective scavenger of free radicals in an emulsion system[17]. In a study of polyphenols in tomato sauces, Vallverdú-Queralt et al. (2014) found that EVOO improved CA levels, which also increased after 15 to 30 min of cooking, decreasing thereafter. Ramírez-Anaya et al. (2015) studied the effect of different domestic cooking techniques using olive oil on Mediterranean vegetables and found a higher CA content in sautéed than in raw tomatoes. Tomas et al. (2017), comparing industrial- and home-processed tomato sauces, found that CA levels in the latter were lower than in raw tomato (17.9 to 13.3 mg/100g of dry weight). However, the domestic process was based on a long cooking time (60 min at 100 ºC) and without the addition of other ingredients, which supports the preserving effect of the *sofrito* cooking technique on polyphenols.

3.3 Effect of the *sofrito* technique on naringenin

The highest naringenin content was observed in formulation 2 at 3.5 ± 0.1 µg/g of tomato, being lowest in formulation 16 at 1.3 ± 0.1 µg/g of tomato (Table 2). Statistical analysis confirmed the suitability of the model, with an r^2 of 0.9180 and adj-r^2 of 0.8832, an acceptable CV% (7.92) and a non-statistically significant lack of fit. All the linear factors presented a significant F-value and p-value and the only interaction factor with statistical significance was EOG. After running a new ANOVA with the significant terms, the regression coefficients were used to design the model equation (Table 3):

$$\text{naringenin(µg/g of tomato)} = 5.884412 - 0.057117E$$

$$-0.016713O - 0.403614G - 0.030680t$$

Analysis of the surface plots revealed that a high amount of garlic (4%) and a long processing time (60 min) significantly reduced the naringenin content in the *sofrito* (Figure 3f). Although onion and EVOO had a slight influence, the surface graph was flat (Figure 3b), indicating that these factors had little effect on naringenin and probably helped to preserve its content during cooking. Naringenin is trapped in the cuticle of ripe tomato, interacting with insoluble polyesters, and can be released by the presence of oil and mechanical and thermal processes [6].

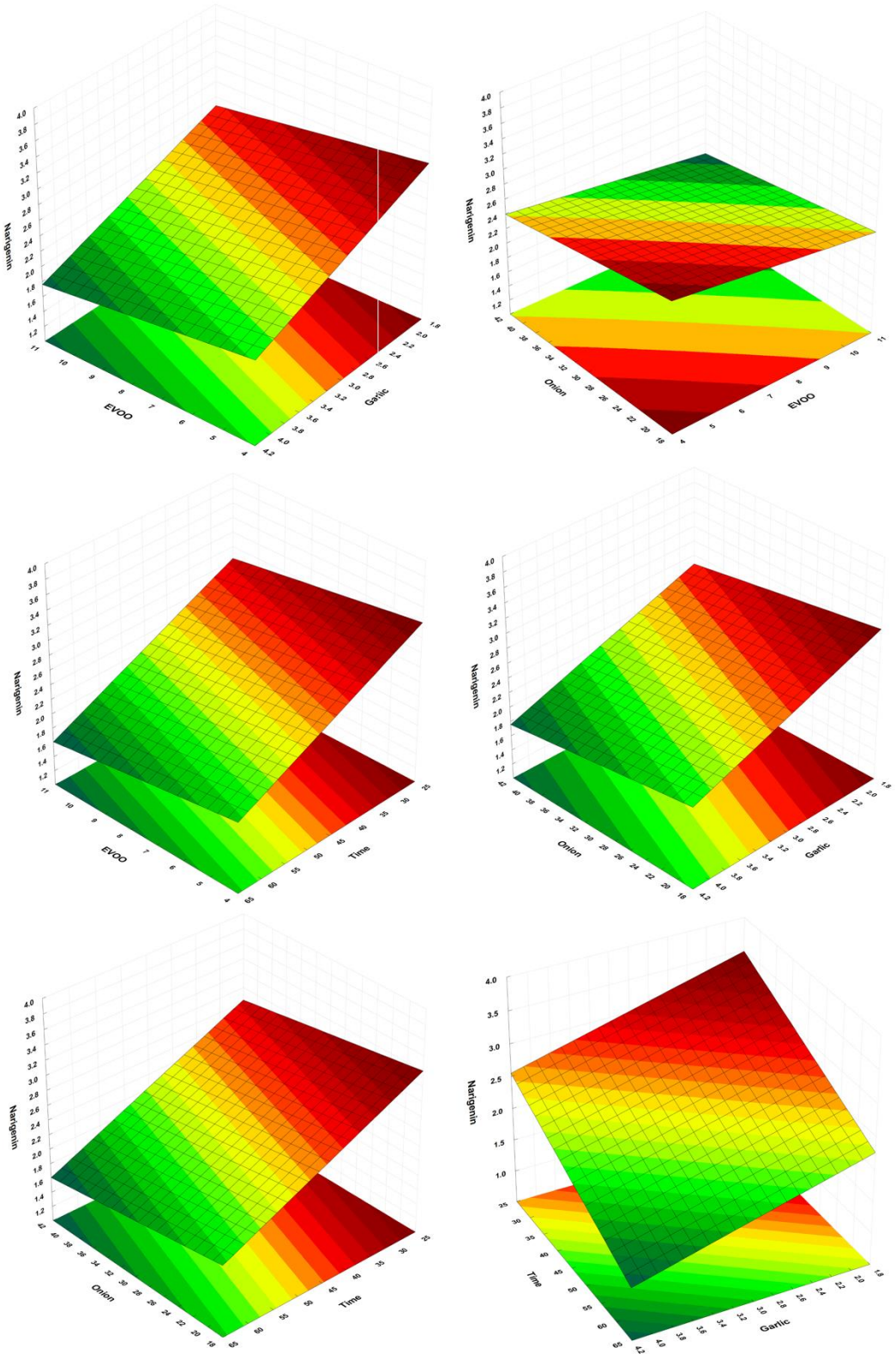

Figure 3. Response surface plot of the combined effects of: garlic and oil at time/onion on level 0 (a); oil and onion at garlic/time on level 0 (b); time and oil at garlic/garlic on level 0 (c); onion and garlic at time/oil on level 0 (d); time and onion at oil/garlic on level 0 (e); time and garlic at oil/onion on level 0 (f) the content of naringenin of home-cooked Mediterranean sofrito. (level 0: garlic = 3%; olive oil = 7.5%; onion = 30% and time = 45 min).

Zhang et al. (2015) describe a high solubility of naringenin in ethyl acetate compared to methanol, indicating a less polar structure compared to other polyphenols. In addition, temperature improves naringenin solubility in non-polar solvents such as petroleum ether and hexane, corroborating the extraction effect of oil during the cooking process. Vallverdú-Queralt et al (2014) found that the naringenin content in tomato sauces increased with 15 to 45 min of cooking, but decreased thereafter. In the same study, naringenin levels were higher with 10% of EVOO than 5%, probably because of enhanced solubility, whereas the oil content did not affect the degradation pattern. Martínez-Huélamo et al. (2016) found that processing increased the naringenin concentrations in tomato sauce, compared to the raw fruit, even more so if oil was used. Tomas et al. (2017) reported a statistically significant increase of naringenin in home-made versus industrial tomato sauce and fresh fruit. Thus, the home-cooking process improves the content of naringenin in tomato sauces, and the use of olive oil in the *sofrito* technique enhances its solubilization and bioaccessibility.

3.4 Effect of the *sofrito* technique on FAH

A high amount of FAH was found in the *sofrito* formulations, above all in formulation 4 (7.2 ± 0.7 µg/g of tomato) followed by formulation 13 (3.2 ± 0.1 µg/g of tomato) (Table 2). Statistical analysis showed that the linear factors O, G and t and the interaction factors OG, EO, Ot and EOG presented a significant F-value and p-value. The model was suitable, with an r^2 value of 0.8917 and adj-r^2 value of 0.8458, a CV% of 8.06 and a non-significant lack of fit. The regression coefficients of the significant factors were used to design a mathematical equation model (Table 3):

$$\text{FAH(µg/g of tomato)} = 8.367237 - 0.105396t - 0.037746OG + 0.002039Ot$$

The surface plots show that the cooking time reduced the FAH in the *sofrito* samples (Figure 4c, 4e and 4f), whereas high amounts of EVOO (10%) had a protective effect (Figure 4c), resulting in higher FAH levels at both 30 and 60 min than when using less oil. A high onion content (40%) also had a protective effect on FAH, since after a cooking period of 60 min, levels were greater than when using less onion (Figure 4e). Martínez-Huélamo et al. (2016) reported that, compared with raw tomatoes, the FAH content of tomato sauces was not affected by processing but decreased when olive oil was added. In contrast, in the current work, the presence of oil did not negatively affect FAH levels. A synergistic effect with onion promoted by the *sofrito* technique probably avoided its degradation. Kelebek et al. (2017) found that industrial tomato paste had higher FAH concentrations than the raw tomatoes used for its elaboration, independently of the inclusion of a hot or cold break process. The low impact of processing on FAH could be because

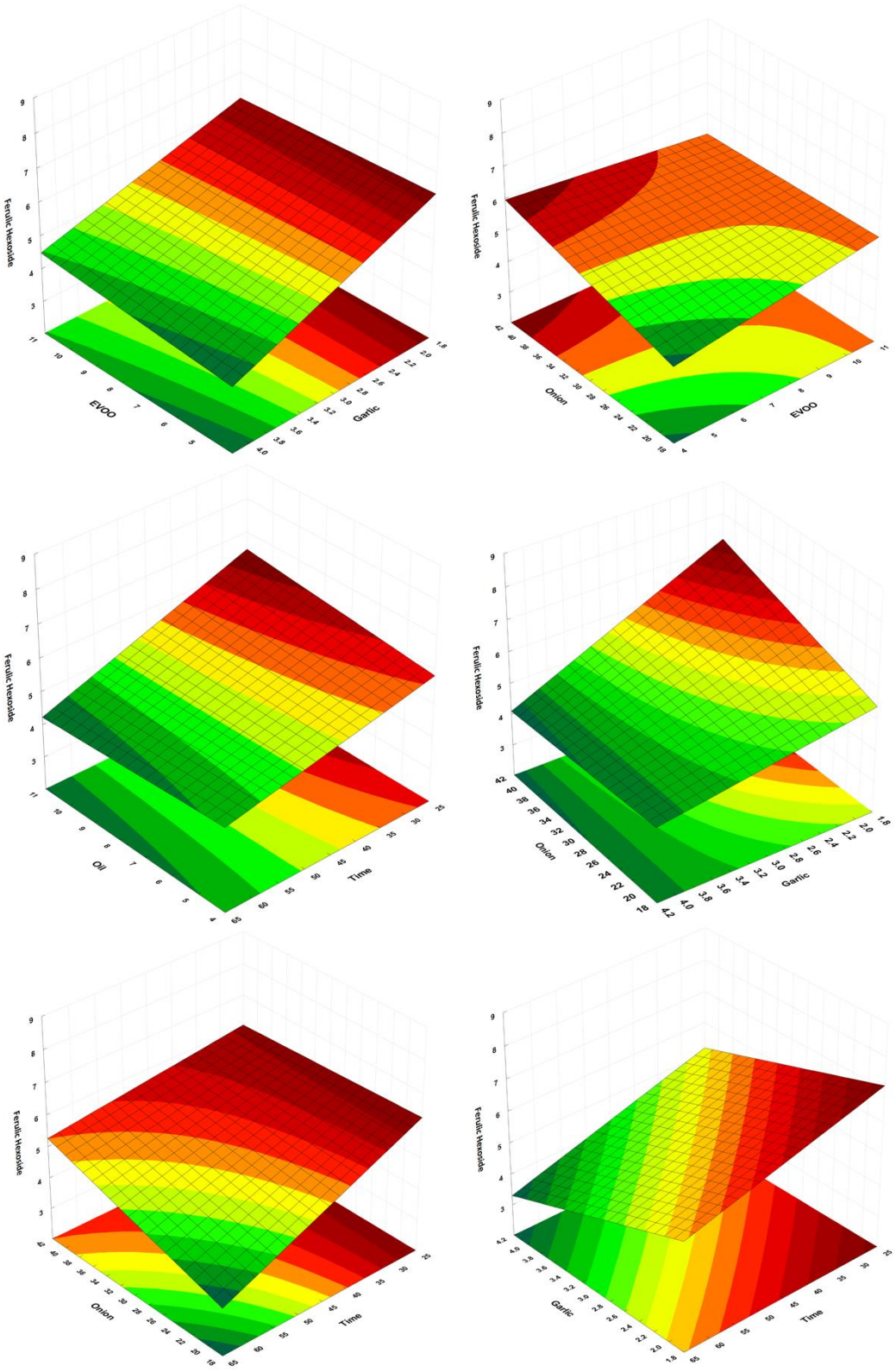

Figure 4. Response surface plot of the combined effects of: garlic and oil at time/onion on level 0 (a); oil and onion at garlic/time on level 0 (b); time and oil at garlic/garlic on level 0 (c); onion and garlic at time/oil on level 0 (d); time and onion at oil/garlic on level 0 (e); time and garlic at oil/onion on level 0 (f) the content of ferulic acid hexoside of home-cooked Mediterranean sofrito. (level 0: garlic = 3%; olive oil = 7.5%; onion = 30% and time = 45 min.

phenolics in their hexoside forms are less susceptible to oxidative degradation compared to free forms in which the sugar moiety diminishes antioxidant scavenging[16].

3.5 Synergistic effect of the *sofrito* ingredients and cooking technique on tomato polyphenols

The cooking process can increase polyphenols in food by physical processes such as cellular disruption, which releases compounds from the food matrix, and by chemical processes that change molecular structures and promote hydrolysis. Cooking can also reduce phenolic compounds by exposing them to oxidation [2]. In the *sofrito*, the cooking time was the main factor affecting the polyphenol content. A short cooking period can help to extract phenolics from the food matrix [4,6,16,19,21], and resulted in a higher content of CA, naringenin and FAH in the *sofrito* compared to raw tomato. However, after a medium-long thermal process, the amounts of these compounds started to decrease due to oxidation.

The presence of EVOO is known to improve the bioaccessibility of carotenoids as well as polyphenols, promoting their release from the food matrix. Vallverdú-Queralt et al. (2014) reported a higher content of CA, p-coumaric acid, protocathechuic acid, naringenin, rutin and quercetin in tomato sauces prepared with a higher amount of EVOO. The literature attributes the polyphenol increase in the presence of oil to different mechanisms, including the concentration effect after evaporation of moisture [22] and the diffusion of polyphenols in the oil when heated [23]. In our study, the evaporation effect can be excluded, as all the samples were expressed per gram of fresh tomato added in the formulation, but the diffusion mechanism may explain the impact of the *sofrito* technique. The structure/solubility relation of phenolic compounds is important to determine their solubility in oil, which improves their bioaccessibility. Polyphenols such as CA and naringenin are solubilized in the oil phase during cooking, whereas FAH is conjugated with a sugar that hampers solubility.

In a previous study, onion was found to significantly impact carotenoid isomerization and preservation during the tomato *sofrito* sauce preparation [5], and a similar protective effect was observed here for CA and to a lesser extent naringenin. The *sofrito* cooking technique could involve the mechanism proposed by Becker, Ntouma, and Skibsted (2007) in which quercetin, the major polyphenol in onion, has a synergistic effect with other chain-breaking antioxidants, such as carotenoids, in oil/water systems. Quercetin is able to inhibit lipid oxidation in multiphasic systems, especially in oil/water emulsions. Its good solubility allows quercetin to be localized at the oil-water interface, where oxidation takes place, and to scavenge free radicals in both phases, protecting other compounds from oxidation during the cooking process. The onion protective effect was notable in CA and FAH and less so in naringenin, as this polyphenol also presents a good solubility in oil/water phases and could help quercetin in the scavenging process. Yin, Hwang, & Chan (2002) attribute an antioxidant activity to organosulfur compounds, typically present in onion and garlic, this being another possible mechanism for the protective effect of

onion on tomato polyphenols during cooking. The process of frying onion in olive oil to prepare *sofrito* could liberate quercetin into the oil phase, thus improving the radical scavenging process in the system and avoiding oxidation of other bioactive compounds.

There is still no consensus in the literature on the best way to cook vegetables to preserve their bioactive compounds [2]. Furthermore, each food has a unique composition, containing compounds with different thermabilities, which the same cooking technique affects differently[25]. Tomas et al. (2017) reported that the content of specific phenolic compounds such as naringenin increased in home-cooked tomato sauce, thus generating interest in how the cooking process influences polyphenol profiles. In our study, the use of the *sofrito* technique contributed to preserving tomato polyphenols and improved their bioaccessibility.

4. Conclusion

In this study, we analyzed the effect of the sofrito technique on the tomato polyphenol content, which was reduced by a longer cooking time. However, the use of EVOO helps to extract polyphenols from the food matrix and may also improve their bioaccessibility. The use of onion in the sofrito process protected some phenolic compounds from oxidation during the cooking process and could have application as a functional ingredient to avoid bioactive compound oxidation in tomato sauce products. More studies on how bioactive compounds can be influenced by home-cooking techniques, ingredients, and the synergism between these factors should be performed to understand how Mediterranean cuisine contributes to polyphenol consumption in the Mediterranean population.

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6. Conflict of Interest

Dr. Lamuela-Raventos reports having received lecture fees from Adventia and Cerveceros de España. The other authors declare that they have no conflict of interest.

7. References

1 Hoffman R and Gerber M. Food Processing and the Mediterranean Diet. Nutrients 7:7925–7964 (2015).

2 Murador D, Braqa AR, Cunha D Da, and Rosso V de. Alterations in phenolic compound levels and antioxidant activity in response to cooking technique effects: A meta-analytic investigation. Crit Rev Food Sci Nutr 8398:00–00 (2016).

3 Murador DC, Cunha DT da, and Rosso VV de. Effects of cooking techniques on vegetable pigments: A meta-analytic approach to carotenoid and anthocyanin levels. Food Res Int Elsevier Ltd; 65:177–183 (2014).

4 Ramírez-Anaya JDP, Samaniego-Sánchez C, Castañeda-Saucedo MC, Villalón-Mir M, and La Serrana HLG De. Phenols and the antioxidant capacity of Mediterranean vegetables prepared with extra virgin olive oil using different domestic cooking techniques. Food Chem 188:430–438 (2015).

5 Alvarenga JFR de, Tran C, Hurtado-Barroso S, Martinez-Huélamo M, Illan M, and Lamuela-Raventos RM. Home cooking and ingredient synergism improve lycopene isomer production in Sofrito. Food Res Int Elsevier Ltd; :1–11 (2017).

6 Tomas M, Beekwilder J, Hall RD, Sagdic O, Boyacioglu D, and Capanoglu E. Industrial processing versus home processing of tomato sauce: Effects on phenolics, flavonoids and in vitro bioaccessibility of antioxidants. Food Chem Elsevier Ltd; 220:51–58 (2017).

7 Gahler S, Otto K, and Böhm V. Alterations of Vitamin C, Total Phenolics, and Antioxidant Capacity as Affected by Processing Tomatoes to Different Products. J Agric Food Chem 51:7962–7968 (2003).

8 Gómez-Romero M, Arráez-Román D, Segura-Carretero A, and Fernández-Gutiérrez A. Analytical determination of antioxidants in tomato: Typical components of the Mediterranean diet. J Sep Sci 30:452–461 (2007).

9 Vallverdú-Queralt A, Alvarenga JFR De, Estruch R, and Lamuela-Raventos RM. Bioactive compounds present in the Mediterranean sofrito. Food Chem 141:3366–3372 (2013).

10 Trumbo PR. Promises and Perils of Lycopene / Tomato Supplementation and Cancer Prevention Are there Adverse Effects of Lycopene Exposure? 1. J Nutr 135:2060–2061 (2005).

11 Rodriguez-Rodriguez R, Jiménez-Altayó F, Alsina L, Onetti Y, Rinaldi de Alvarenga JF, Claro C, Ogalla E, Casals N, and Lamuela-Raventos RM. Mediterranean tomato-based sofrito protects against vascular alterations in obese Zucker rats by preserving NO bioavailability. Mol Nutr Food Res 61:1601010 (2017).

12 Sandoval V, Rodríguez-Rodríguez R, Martínez-Garza Ú, Rosell-Cardona C, Lamuela-Raventós RM, Marrero PF, Haro D, and Relat J. Mediterranean Tomato-Based Sofrito Sauce Improves Fibroblast Growth Factor 21 (FGF21) Signaling in White Adipose Tissue of Obese ZUCKER Rats. Mol Nutr Food Res 21:1700606 (2018).

13 Stojanovic J, Giraldi L, Arzani D, Pastorino R, Biondi A, Persiani R, Boccia S, and Leoncini E. Adherence to Mediterranean diet and risk of gastric cancer. Eur J Cancer Prev Informa UK Ltd.; 0:1 (2017).

14 Capriotti AL, Cavaliere C, Crescenzi C, Foglia P, Nescatelli R, Samperi R, and Laganà A. Comparison of extraction methods for the identification and quantification of polyphenols in virgin olive oil by ultra-HPLC-QToF mass spectrometry. Food Chem Elsevier Ltd; 158:392–400 (2014).

15 Lecce G Di, Martínez-Huélamo M, Tulipani S, Vallverdú-Queralt A, and Lamuela-Raventós RM. Setup of a UHPLC-QqQ-MS method for the analysis of phenolic compounds in cherry tomatoes, tomato sauce, and tomato juice. J Agric Food Chem 61:8373–8380 (2013).

16 Vallverdú-Queralt A, Regueiro J, Rinaldi de Alvarenga JF, Torrado X, and Lamuela-Raventos RM. Home Cooking and Phenolics: Effect of Thermal Treatment and Addition of Extra

Virgin Olive Oil on the Phenolic Profile of Tomato Sauces. J Agric Food Chem 62:3314–3320 (2014).

17 Becker EM, Ntouma G, and Skibsted LH. Synergism and antagonism between quercetin and other chain-breaking antioxidants in lipid systems of increasing structural organisation. Food Chem 103:1288–1296 (2007).

18 Zhang L, Song L, Zhang P, Liu T, Zhou L, Yang G, Lin R, and Zhang J. Solubilities of naringin and naringenin in different solvents and dissociation constants of naringenin. J Chem Eng Data 60:932–940 (2015).

19 Martínez-Huélamo M, Vallverdú-Queralt A, Lecce G Di, Valderas-Martínez P, Tulipani S, Jáuregui O, Escribano-Ferrer E, Estruch R, Illan M, and Lamuela-Raventós RM. Bioavailability of tomato polyphenols is enhanced by processing and fat addition: Evidence from a randomized feeding trial. Mol Nutr Food Res 60:1578–1589 (2016).

20 Kelebek H, Selli S, Kadiroglu P, Kola O, Kesen S, Uçar B, and Çetiner B. Bioactive compounds and antioxidant potential in tomato pastes as affected by hot and cold break process. Food Chem 220:31–41 (2017).

21 Capanoglu E, Beekwilder J, Boyacioglu D, Hall R, and Vos R de. Changes in Antioxidant and Metabolites Profiles during Production of Tomato Paste. :964–973 (2008).

22 Provesi JG, Dias CO, and Amante ER. Changes in carotenoids during processing and storage of pumpkin puree. Food Chem 128:195–202 (2011).

23 Fillion L and Henry CJ. Nutrient losses and gains during frying: a review. Int J Food Sci Nutr 49:157–168 (1998).

24 Yin MC, Hwang SW, and Chan KC. Nonenzymatic antioxidant activity of four organosulfur compounds derived from garlic. J Agric Food Chem 50:6143–6147 (2002).

25 Bernhardt S and Schlich E. Impact of different cooking methods on food quality: Retention of lipophilic vitamins in fresh and frozen vegetables. J Food Eng 77:327–333 (2006).

4.2.4 Publication 6. Using extra virgin olive oil to cook vegetables enhances polyphenol and carotenoid extractability: a study applying the *sofrito* technique

José Fernando Rinaldi de Alvarenga, Paola Quifer-Rada, Fernanda Francetto Juliano, Sara Hurtado-Barroso, Montserrat Illan, Xavier Torrado-Prat, Rosa Maria Lamuela-Raventós. *Molecules*, 2019, 24 (8), 1555.

Mediterranean diet is characterized by a high intake of phytochemicals that are correlated with improvements in cardiovascular and metabolic health. However, Mediterranean diet reported health effects are difficult to reproduce in non-Mediterranean populations, possibly by different cooking practices. Olive oil is the main source of fat in the Mediterranean diet and the most frequently used ingredient in Mediterranean cuisine. Cooking with olive oil has been calling attention by its capacity to act as a food excipient that could increase bioaccessibility and bioavailability of phytochemicals. The aim of this study was understanding the enhancing effect of olive oil on the extraction of bioactive compounds from other ingredients in the cooking process, using *sofrito* as a representative model of the Mediterranean diet. *Sofrito* samples were fractionated in insoluble, water and oil fraction in order to understand a possible incorporation of phytochemicals in the oil fraction. The different fractions were analyzed by their content of phenolic compounds using a HPLC-ESI-QqQ-MS/MS and their content of carotenoids using a HPLC-DAD and HPLC-APCI-QqQ-MS/MS. Among polyphenols, the insoluble fraction showed the highest content of phenolic compounds, follow by oil and water fraction. The oil fraction was found to contain phenolic compounds not present in the EVOO before cooking such as naringenin, ferulic acid and quercetin. Among carotenoids, the insoluble fraction showed the higher total carotenoids content (53.89 μg/g of *sofrito*) compared to oil fraction (23.83 μg/g of *sofrito*), however, before cooking process no quantifiable carotenoids were detected in the EVOO. Moreover, oil was the only fraction containing carotenoids such as all-*E*-lutein, apo-8-β-carotenal, 9,13-*Z*-lycopene and 9,5-*Z*-lycopene and higher content of lycopene isomers. The use of olive oil in Mediterranean cuisine may improve the extractability of bioactive compounds such as polyphenols and carotenoids from the food matrix and could contribute to its bioaccessibility, bioavailability and health effects.

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Using Extra Virgin Olive Oil to Cook Vegetables Enhances Polyphenol and Carotenoid Extractability: A Study Applying the *sofrito* Technique

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Abstract: Olive oil is the main source of fat in the Mediterranean diet and the most frequently used ingredient in Mediterranean cuisine. Cooking with olive oil has been attracting attention because it can act as a food excipient, thereby increasing the bioaccessibility and bioavailability of ingested bioactive compounds. The aim of this study was to understand the effect of cooking with olive oil on the bioactive components in other ingredients (tomato, onion, and garlic) of *sofrito* sauce, a representative model of Mediterranean cuisine. After the cooking process, polyphenols from tomato, onion, and garlic were detected in the olive oil, especially naringenin, ferulic acid, and quercetin, as well as a high content of carotenoid *Z*-isomers, which are more bioavailable than the *E*-isomers. Therefore, traditional Mediterranean cuisine could play an important role in the health-improving effects of the Mediterranean diet.

Keywords: phenolic compounds; matrix effect; carotenoid isomerization; tomato; onion; garlic; naringenin; lycopene

1. Introduction

The Mediterranean diet, characterized by a high intake of phytochemicals from vegetables and legumes, has been correlated with improvements in cardiovascular and metabolic health [1,2]. This association is supported by evidence from the intervention trial PREDIMED, a Mediterranean diet reference study with more than 7000 participants, in which a Mediterranean diet supplemented with extra virgin olive oil (EVOO) reduce the risk of cardiovascular events among high-risk population [2]. Similar results have been observed in other Mediterranean diet clinical trials [3]. However, the health outcomes of a Mediterranean diet are reportedly difficult to reproduce in non-Mediterranean populations, possibly because of different cooking practices [1]. Cooking can negatively affect the

phytochemical content of food through oxidation, degradation, or the formation of pro-oxidant compounds. In contrast, it may improve the bioavailability of phytochemicals by altering chemical structures and releasing bioactive compounds from the food matrix [1,4,5].

Traditional Mediterranean cuisine has unique characteristics that could affect the content of bioactive compounds in cooked food [6]. The *sofrito* is a typical technique of lightly frying onion and garlic in EVOO. This sauce is an ingredient used to prepare many Mediterranean dishes and recipes [6,7]. The tomato *sofrito* sauce has been reported to contain 40 different phenolic compounds and a high content of carotenoids [7] and its consumption is associated with improved cardiovascular risk parameters and insulin sensitivity, and activates thermogenesis by browning fat tissue [8,9]. Moreover, tomato *sofrito* consumption is one of a 14-item validated questionnaire used to evaluate the adherence of traditional Mediterranean diet, in which was correlated with low incidence of abdominal fat and obesity and manifestation of less aggressive prostate cancer [10–12]. For this reason, we consider *sofrito* a key component of the Mediterranean diet. Olive oil, the predominant source of fat in the Mediterranean diet, displays a singular fatty acid composition, with a higher content of phenolic compounds than other edible oils [13,14]. One of the beneficial effects of olive oil is that it improves the bioavailability of lipophilic compounds such as carotenoids by acting as a food excipient, and enhances nutrient extraction and bioaccessibility [15–18]. Moreover, cooking with olive oil increases levels of carotenoid *Z*-isomers, which are currently gaining interest due to their higher bioavailability and antioxidant capacity by their geometrical structure compared to carotenoid *E*-isomer form [19]. The polar phenolic compounds, on the other hand, are less soluble in olive oil than carotenoids. Polyphenol bioavailability is low in comparison with other macro- and micronutrients. Only a small percentage of dietary polyphenols (5–10% of total intake) can be directly absorbed in the small intestine, after deconjugation reactions during digestion. The rest (90–95%) may be metabolized by enzymes in the gut microbiome, producing metabolites more easily absorbed in the large intestine, or eliminated through the feces [20]. The absorption of polyphenols could be improved if they are incorporated into olive oil, which would facilitate their entry into epithelial cells by passive diffusion [21]. Recent reports indicate that cooking with olive oil can improve the bioaccessibility and bioavailability of polyphenols [22–24], although the underlying mechanisms remain unclear.

The aim of this study is to understand the enhancing effect of olive oil on the extraction of bioactive compounds from other ingredients (tomato, onion, and garlic) in the cooking process, using *sofrito* as a representative model of the Mediterranean diet.

2. Results

2.1. Identification and Quantification of Phenolic Compounds in the Ingredients

The quantification of phenolic compounds in each ingredient used to prepare the *sofrito* is shown in Table 1. The content of each polyphenol typically found in tomato was quantified by the validated Tomato method, which was also used to quantify polyphenols in onion and garlic. The major polyphenol found in onion was quercetin (34 ± 1 μg/g) [25] followed by p-coumaric (5.9 ± 0.1 μg/g) and ferulic acid (0.62 ± 0.08 μg/g) (Table 1). Polyphenol levels in garlic were much lower, with only two compounds detected, caffeic acid (0.8 ± 0.1 μg/g) and caffeic acid-*O*-hexoside I (0.36 ± 0.02 μg/g) (Table 1).

Table 1. Polyphenols monitored and quantified in the ingredients of the tomato-based *sofrito* sauce expressed in μg/g of ingredient.

Compound	rt	MRM (*m*/*z*)	DP (V)	FP (V)	EP (V)	CE (V)	T	O	G	E
Tomato Method [A]										
caffeic acid-*O*-hexoside I	0.33	341→179	−40	−170	−10	−20	4.7 ± 0.2	0.30 ± 0.03	0.36 ± 0.02	n.d.
caffeic acid-*O*-hexoside II	0.51	341→179	−40	−170	−10	−20	n.d.	n.d.	n.d.	n.d.
chlorogenic acid *	0.53	353→191	−50	−180	−10	−20	4.4 ± 0.4	n.d.	n.d.	n.d.
protocatechuic *	0.54	153→109	−40	−150	−10	−20	0.0014 ± 0.0001	n.d.	n.d.	n.d.
coumaric acic-*O*-hexoside	0.55	325→163	−40	−150	−10	−25	1.58 ± 0.09	n.d.	n.d.	n.d.
ferulic acid-*O*-hexoside	0.59	355→193	−40	−170	−10	−25	4.5 ± 0.4	n.d.	n.d.	n.d.
caffeic acid *	0.69	179→135	−40	−170	−10	−20	1.36 ± 0.03	n.d.	0.8 ± 0.1	n.d.
Rutin *	0.74	609→300	−60	−230	−10	−50	2.94 ± 0.05	n.d.	n.d.	n.d.
ethyl gallate * (IS)	1.01	197→169	−60	−200	−10	−25	-	-	-	-
naringenin-7-*O*-glucoside *	1.39	433→271	−50	−280	−10	−30	0.076 ± 0.009	n.d.	n.d.	n.d.
dicaffeoylquinic acid	1.41	515→353	−50	−180	−10	−20	0.61 ± 0.02	n.d.	n.d.	n.d.
Quercetin *	2.08	301→151	−60	−210	−10	−30	0.236 ± 0.002	34 ± 1	n.d.	n.d.
naringenin *	2.65	271→151	−50	−190	−10	−30	1.9 ± 0.2	n.d.	n.d.	n.d.
Olive Oil Method [B]										
1-acetoxypinoresinol	-	451→325	−60	−180	−8	−25	n.d.	n.d.	n.d.	n.d.
dihydroxyoleuropein aglycone (DHOA)	-	409→180	−30	−140	−10	−30	n.d.	n.d.	n.d.	n.d.
dihydroxyphenylacetic acid	-	167→123	−40	−170	−10	−10	n.d.	n.d.	n.d.	n.d.
hydroxytyrsol acetate. (3,4-DHPEA-AC II)	-	195→153	−30	−140	−10	−30	n.d.	n.d.	n.d.	n.d.
p-coumaroyl-6-oleoside	-	535→427	v30	−140	−10	−30	n.d.	n.d.	n.d.	n.d.
tyrosol	-	137→106	−25	−90	−10	−20	n.d.	n.d.	n.d.	n.d.
hydroxytyrosol-*O*-glucoside	1.01	315→153	−40	−250	−10	−20	n.d.	n.d.	n.d.	0.038 ± 0.004
Hydroxytyrosol *	1.13	153→123	−40	−250	−10	−20	n.d.	n.d.	n.d.	14.3 ± 0.8
p-coumaric acid *	1.57	163→119	−40	−150	−10	−25	0.108 ± 0.007	5.9 ± 0.1	0.057 ± 0.008	0.200 ± 0.006
hydroxycarboxymethyl elenolic acid I (HCM-EA I)	1.65	199→155	−30	−140	−10	−30	n.d.	n.d.	n.d.	n.d.
vanillic acid *	2.56	167→123	−30	−200	−10	−30	n.d.	n.d.	n.d.	n.d.
m-coumaric acid *	3.26	163→119	−40	−150	−10	−25	0.71 ± 0.02	0.014 ± 0.001	n.d.	n.d.
ethyl gallate * (IS)	4.62	197→169	−60	−200	−10	−25	-	-	-	-
o-coumaric acid *	4.75	163→119	−40	−150	−10	−25	1.9 ± 0.1	0.012 ± 0.002	n.d	n.d
ferulic acid *	5.15	193→134	−40	−170	−10	−20	1.85 ± 0.08	0.62 ± 0.08	7.2 ± 0.3	0.0358 ± 0.0004

Table 1. *Cont.*

Compound	rt	MRM (*m*/*z*)	DP (V)	FP (V)	EP (V)	CE (V)	T	O	G	E
Tomato Method [A]										
hydroxytyrsol acetate. (3,4-DHPEA-AC)	5.69	195→180	−30	−140	−10	−30	n.d.	n.d.	n.d.	n.d.
hydroxyelenolic acid	6.02	257→137	−30	−140	−10	−30	n.d.	n.d.	n.d.	1.17 ± 0.2
elenolic acid *	6.31	241→127	−30	−140	−10	−30	n.d.	n.d.	n.d.	7.1 ± 0.5
verbascoside *	6.4	623→161	−90	−210	−10	−50	n.d.	n.d.	n.d.	n.d.
isolariciresinol *	6.44	359→344	−60	−100	−13	−30	n.d.	n.d.	n.d.	n.d.
lactone	7.16	321→185	−40	−250	−10	−20	n.d.	n.d.	n.d.	0.68 ± 0.06
secoisolariciresinol	7.24	361→165	−60	−50	−6	−35	n.d.	n.d.	n.d.	
hydroxycarboxymethyl elenolic acid II (HCM-EA II)	7.3	199→155	−30	−140	−10	−30	n.d.	n.d.	n.d.	n.d.
hydroxydecarboxymethyl oleuropein aglycone (HDCM-OA)	7.32	335→199	−30	−140	−10	−30	n.d.	n.d.	n.d.	6.6 ± 0.6
lariciresinol *	7.45	359→329	−40	−100	v4	−15	n.d.	n.d.	n.d.	n.d.
hydroxy oleuropein aglycone I (HOA I)	7.6	393→257	−30	−140	−10	−30	n.d.	n.d.	n.d.	3.41 ± 0.04
oleuropein *	7.68	539→275	−30	−140	−10	−30	n.d.	n.d.	n.d.	5.46 ± 0.08
oleuropein aglycone (3,4-DHPEA-EA) II	7.91	377→307	−30	−140	−10	−30	n.d.	n.d.	n.d.	n.d.
luteolin *	8.15	285→133	−100	−340	−10	−50	n.d.	n.d.	n.d.	0.89 ± 0.06
decarboxylmethyl oleuropein aglycone (3,4-DHPEA-EDA)	8.4	319→181	−30	−140	−10	−30	n.d.	n.d.	n.d.	0.114 ± 0.005
pinoresinol *	8.58	357→151	−60	−180	−8	−25	n.d.	n.d.	n.d.	0.089 ± 0.001
oleuropein derivative II	8.64	377→307	−30	−140	−10	−30	n.d.	n.d.	n.d.	7.8 ± 0.2
hydroxy oleuropein aglycone II (HOA II)	8.8	393→257	−30	−140	−10	−30	n.d.	n.d.	n.d.	n.d.
oleuropein derivative III	8.92	377→307	−30	−140	−10	−30	n.d.	n.d.	n.d.	n.d.
oleocanthal (4-HPEA-EDA)	9.0	303→165	−25	−90	−7	−15	n.d.	n.d.	n.d.	n.d.
ligstroside aglycon I	9.01	361→291	−30	−140	−10	−30	n.d.	n.d.	n.d.	39 ± 1
apigenin *	9.11	269→117	−70	−200	−10	−50	n.d.	n.d.	n.d.	0.36 ± 0.03
oleuropein derivative I	9.14	377→241	−30	−140	−10	−30	n.d.	n.d.	n.d.	5.46 ± 0.08

Table 1. *Cont.*

Compound	rt	MRM (*m*/*z*)	DP (V)	FP (V)	EP (V)	CE (V)	T	O	G	E
					Tomato Method [A]					
ligstroside aglycon II	10.0	361→291	−30	−140	−10	−30	n.d.	n.d.	n.d.	30.9 ± 0.6
oleuropein aglycone (3,4-DHPEA-EA)	10.0	377→307	−30	−140	−10	−30	n.d.	n.d.	n.d.	n.d.
oleuropein derivative IV	10.3	377→307	−30	−140	−10	−30	n.d.	n.d.	n.d.	n.d.
ligstroside aglycon III	11.2	361→291	−30	−140	−10	−30	n.d.	n.d.	n.d.	17.3 ± 0.6
methyl oleuropein aglycone (methyl 3,4-DHPEA-EA)	11.2	391→255	−30	−140	−10	−30	n.d.	n.d.	n.d.	0.045 ± 0.001

[A] Tomato method was used to quantify phenolic compounds typically from tomato, onion and garlic. [B] Olive oil method was used to quantify phenolic compounds typically from olive oil. * Compounds confirmed by standards. Results were expressed in µg/g of ingredient by mean ± SD, n = 3. rt (retention time); MRM (multiple reaction monitoring); DP (declustering potential); FP (focusing potential); EP (entrance potential); CE (collision energy); T (tomato); O (onion); G(garlic); E (extra virgin olive oil); n.d. (not detected).

The content of polyphenols typical from olive oil was quantified in the EVOO as well as in the other ingredients of the tomato-based *sofrito* sauce by Olive oil method. All ingredients were found to contain *p*-coumaric acid and ferulic acid. Additionally, *m*-coumaric and *o*-coumaric acid were detected in tomato and onion, but not in garlic and EVOO. The major polyphenols in EVOO were hydroxytyrosol (14.3 ± 0.8 μg/g), hydroxytyrosol derivatives (oleuropein (5.46 ± 0.08 μg/g), oleuropein derivate I (7.8 ± 0.2 μg/g), hydroxy oleuropein aglycone (3.41 ± 0.04 μg/g) and hydroxydecarboxymethyl oleuropein aglycone (6.6 ± 0.6 μg/g), ligstroside aglycone I (39 ± 1 μg/g), and elenolic acid (7.1 ± 0.5 μg/g) (Table 1). The two methods were used to ensure a suitable selection of candidates for investigating polyphenol migration to the oil fraction in the *sofrito* preparation. To evaluate the migration process, the candidate polyphenols should not be part of the original composition of the EVOO.

2.2. Identification and Quantification of Carotenoids in the Ingredients

The analysis of carotenoids in the different *sofrito* fractions revealed the presence of 27 different compounds, only 21 of which were identified, namely lutein, α-carotene, β-carotene, ζ-carotene, lycopene, phytofluene, and apo-β-carotenoids and their isomers (Table 2). The oil fraction had the most diverse carotenoid profile, and all-*E*-lutein, apo-8-β-carotenal, 9,5-*Z*-lycopene, and 9,13-*Z*-lycopene were only quantified in this *sofrito* fraction (Table 3). Tomato was the only ingredient with carotenoids in its composition, showing a high content of all-*E*-lycopene (126 ± 8 μg/g) and some lycopene isomers such as 5-*Z*-lycopene (3.9 ± 0.6 μg/g) and 13-*Z*-lycopene (16 ± 1 μg/g). Also, β-carotene (3 ± 1 μg/g) and α-carotene (1.94 ± 0.09 μg/g) were found.

Table 2. Carotenoid identification in the *sofrito* oil fraction.

Peak	Compound	rt (min)	λ_{max} (nm)	%III/II	%A_b/II	$[M + H]^+$ (*m*/*z*)	MS^2 ion products (*m*/*z*)
1	all-*E*-lutein *	10.51	(420), 445, 476	40	14	551 [M + H-18]	429 [M − 122]
2	n.i.	11.12	445, 472			-	-
3	n.i.	11.93	(330), (347), (416), 440, 470	41	21	-	-
4	n.i.	12.69	(330), (345), (422), 440, 468	30	15	-	-
5	n.i.	14.31	434, 456			-	-
6	n.i.	15.20	430, 452, 485	38	4	-	-
7	Apo-10-β-carotenal	15.58	448			-	n.d.
8	Apo-8-β-carotenal *	15.99	460			417	325 [M − 92]
9	all-*E*-α-carotene *	16.39	425, 445,475			537	444 [M − 92]
10	*Z*-phytofluene	16.43	332, 349, 368	72		543	406 [M + H − 137]
11	phytofluene	16.79	332, 349, 368	90		543	406 [M + H − 137]
12	all-*E*-ζ-carotene	17.31	385, 401, 420	104		541	472 [M + H − 69]
13	*Z*-β-carotene	17.05	418, 437, 461	13			444 [M − 92]; 413 [M − 123]
14	13-*Z*-β-carotene	17.91	(335), (347), 448, 470	28	32	537	444 [M − 92]; 413 [M − 123]
15	all-*E*-β-carotene *	18.95	(425), 453, 478	28		537	444 [M − 92]; 413 [M − 123]
16	9-*Z*-β-carotene *	19.71	(350), 426, 453, 480	28	28	537	444 [M − 92]; 413 [M − 123]
17	n.i.	20.37	(350), 426, 453, 480		20	537	444 [M − 92]; 413 [M − 123]
18	n.i.	21.34	(350), 455, 485				n.d.
19	9,13-*Z*-lycopene	22.17	(348), (362), 437, 460, 488	47	28	537	444 [M − 92]; 413 [M − 123]
20	15-*Z*-lycopene	23.11	(348), (362), 438, 461, 490	48	69	537	444 [M − 92]; 413 [M − 123]
21	all-*E*-γ-carotene	23.41	(364), 438, 461, 491	58		537	444 [M − 92]
22	13-*Z*-lycopene	23.89	(348), (362), 440, 466, 495	49	40	537	444 [M − 92]; 413 [M − 123]
23	9,5-di-*Z*-lycopene	24.57	(348), (362), 438, 460, 489	54	22	537	444 [M − 92]; 413 [M − 123]

Table 2. *Cont.*

Peak	Compound	rt (min)	λ_{max} (nm)	%III/II	$\%A_b$/II	$[M + H]^+$ (*m*/*z*)	MS^2 ion products (*m*/*z*)
24	9-Z-lycopene	26.88	(348), (362), 441, 467, 497	70	17	537	444 [M − 92]; 413 [M − 123]
25	7-Z-lycopene	27.23	(348), (363), 441, 467, 497	70	17	537	444 [M − 92]; 413 [M − 123]
26	all-*E*-lycopene *	31.29	447, 472, 503	74	0	537	444 [M − 92]; 413 [M − 123]
27	5-Z-lycopene	31.89	(365), 446, 472, 503	74	5	537	444 [M − 92]; 413 [M − 123]

λ_{max} (UV/VIS absorption spectra); %III/II (spectral fine structure); $\%A_b$/AII (peak cis intensity); n.i. (not identified); n.d. (not detected); * Compounds confirmed by standards.

2.3. Identification and Quantification of Phenolic Compounds in the Sofrito Water, Oil and Insoluble Fractions

Using a targeted approach, among all phenolic compounds analyzed only 21 were possible to quantify in the different *sofrito* fractions (Table 3). With the exception of pinoresinol, which remained stable, the level of EVOO polyphenols was considerably reduced in the oil-fraction, possibly because of migration to the food matrix [26] or degradation [27] (Table 3). Carrasco-Pancorbo and co-workers reported an oxidation of phenolic compounds during thermal treatment of EVOO, however pinoresinol was stable during the first hour of processing [27]. In contrast, the oil fraction was enriched with polyphenols from other ingredients, namely caffeic acid, caffeic acid hexoside, chlorogenic acid, naringenin, protocatechuic acid, and quercetin, which were not detected in the EVOO before the cooking process (Table 1). The phenolic compound with the highest transfer efficiency was naringenin (1.7 ± 0.03 μg/g), found at levels close to those in raw tomato (1.9 ± 0.2 μg/g) (Tables 1 and 3). Ferulic acid was also efficiently transferred to the oil fraction (0.55 ± 0.06 μg/g), where its content was higher than in the initial composition of EVOO (0.0358 μg/g), and its major source were the ingredients garlic (7.2 μg/g) and tomato (1.85 μg/g). Quercetin was not initially detected in EVOO but after cooking showed a concentration of 0.04 ± 0.02 μg/g, onion being the major source of this compound (Table 3).

Analysis of the phenolic content in each fraction expressed in *sofrito* equivalents revealed that the insoluble fraction was the major source of phenolic compounds, with levels very close to the phenolic content of the entire *sofrito*. The second richest fraction in polyphenols was oil, followed by water. The highest content of EVOO phenolics was found in the insoluble fraction, probably the result of frying onions to prepare the *sofrito*. Expressing the results in *sofrito* equivalents, the predominant polyphenols in the oil fraction were naringenin and ferulic acid (0.29 ± 0.06 and 0.10 ± 0.02 μg/g of *sofrito*, respectively). These results would explain the biphasic kinetic curve in plasma described by Martínez-Huélamo and co-workers [24], who reported that the presence of a lipid matrix increased the absorption of ferulic acid and naringenin in volunteers who consumed tomato sauce with olive oil versus tomato sauce without olive oil.

Table 3. Quantification of polyphenols in water, oil and insoluble fractions of *sofrito* expressed in μg/g of *sofrito* or ingredients.

Compound	μg/g of Ingredient or Fraction [A]		μg/g of *sofrito* [B]			
	EVOO	Oil Fraction	*Sofrito*	Water	Oil	Insoluble
Polyphenols						
apigenin	0.36 ± 0.03	0.16 ± 0.02 ***	1.64 ^{a} ±0.007	0.008 ^{c} ± 0.002	0.028 ^{b} ± 0.006	1.5 ^{a} ± 0.1
elenolic acid	22 ± 2	0.083 ± 0.06 ***	0.110 ^{b} ± 0.004	0.010 ^{c} ± 0.002	0.014 ^{c} ± 0.002	1.49 ^{a} ± 0.08
ferulic acid	0.0358 ± 0.00004	0.55 ± 0.06 ***	0.64 ^{b} ± 0.6	0.007 ^{c} ± 0.002	0.10 ^{c} ± 0.02	0.75 ^{a} ± 0.09
ligstroside I	39 ± 1	1.8 ± 0.3 ***	0.109 ^{c} ± 0.004	0.007 ^{c} ± 0.001	0.31 ^{b} ± 0.07	0.6 ^{a} ± 0.1
luteolin	0.89 ± 0.06	0.13 ± 0.01 **	0.169 ^{b} ± 0.007	0.013 ^{c} ± 0.004	0.022 ^{c} ± 0.002	2.9 ^{a} ± 0.4
m-coumaric	n.d.	<LoQ	<LoQ	0.018 *** ± 0.004	<LoQ	0.40 *** ± 0.02
o-coumaric	n.d.	0.08 ± 0.01	<LoQ	0.020 ^{b} ± 0.004	0.015 ^{b} ± 0.003	0.54 ^{a} ± 0.06
p-coumaric	0.200 ± 0.006	0.08 ± 0.01 **	0.12 ^{a} ± 0.02	0.0019 ^{b} ± 0.0004	0.015 ^{b} ± 0.003	0.16 ^{a} ± 0.02
oleuropein	5.46 ± 0.08	1.14 ± 0.08 ***	0.111 ^{b} ± 0.004	0.026 ^{b} ± 0.008	0.20 ^{b} ± 0.03	2.5 ^{a} ± 0.2
pinoresinol	0.089 ± 0.001	0.09 ± 0.01	0.35 *** ± 0.20	<LoQ	0.016 *** ± 0.003	<LoQ
caffeic acid	n.d.	0.032 ± 0.001	1.8 ^{a} ± 0.2	0.006 ^{c} ± 0.002	0.0055 ^{c} ± 0.0003	0.21 ^{b} ± 0.01
caffeic acid-*O*-hexoside	n.d.	0.0183 ± 0.0007	2.6 ^{a} ± 0.3	0.0023 ^{c} ± 0.0005	0.0032 ^{c} ± 0.0002	0.16 ^{b} ± 0.02
chlorogenic acid	n.d.	0.0012 ± 0.0003	5.4 ^{a} ± 0.6	0.0025 ^{b} ± 0.0009	<LoQ	0.064 ^{b} ± 0.008
dicalfeoylquinic acid	n.d.	n.d.	0.35 ^{a} ± 0.01	0.0018 ^{c} ± 0.0004	n.d.	0.077 ^{b} ± 0.002
ferulic acid-*O*-hexoside	n.d.	n.d.	6 ^{a} ± 2	0.05 ^{c} ± 0.02	n.d.	1.2 ^{b} ± 0.2
naringenin	n.d.	1.7 ± 0.03	2.8 ^{b} ± 0.1	0.021 ^{c} ± 0.006	0.29 ^{c} ± 0.06	3.4 ^{a} ± 0.6
naringenin-7-*O*-glucoside	n.d.	n.d.	0.025 ^{b} ± 0.003	0.010 ^{b} ± 0.003	n.d.	0.60 ^{a} ± 0.06
protocatechuic acid	n.d.	0.009 ± 0.001	n.d.	0.003 ^{b} ± 0.001	0.0016 ^{b} ± 0.0002	0.06 ^{a} ± 0.02
quercetin	n.d.	0.04 ± 0.02	5 ^{b} ± 1	0.03 ^{c} ± 0.01	0.007 ^{c} ± 0.003	10 ^{a} ± 3
rutin	n.d.	n.d.	2.6 ^{a} ± 0.1	0.023 ^{c} ± 0.006	n.d.	0.62 ^{b} ± 0.08
Carotenoids						
all-*E*-lutein	<LoQ	4.2 ± 0.2	n.d.	n.d.	0.71 ± 0.2	n.d.
Apo-8-β-carotenal	n.d.	2.34 ± 0.01	n.d.	n.d.	0.40 ± 0.03	n.d.
all-*E*-α-carotene	n.d.	2.14 ± 0.03	3.6 ^{a} ± 0.1	0.020 ^{d} ± 0.005	0.37 ^{c} ± 0.03	1.61 ^{b} ± 0.06
13-*Z*-β-carotene	n.d.	3.1 ± 0.2	<LoQ	0.022 ^{c} ± 0.005	0.53 ^{b} ± 0.08	1.98 ^{a} ± 0.08
all-*E*-β-carotene	<LoQ	8.0 ± 0.8	7.3 ^{a} ± 0.5	0.029 ^{c} ± 0.007	1.4 ^{b} ± 0.2	6.2 ^{a} ± 0.6
9-*Z*-β-carotene	n.d.	6.5 ± 0.4	3.4 ^{b} ± 0.2	0.023 ^{d} ± 0.06	1.1 ^{c} ± 0.2	6.4 ^{a} ± 0.6
9,13-*Z*-lycopene	n.d.	2.15 ± 0.06	n.d.	n.d.	0.37 ± 0.04	n.d.
15-*Z*-lycopene	n.d.	2.6 ± 0.3	<LoQ	n.d.	0.45 *** ± 0.08	2.9 *** ± 0.2
13-*Z*-lycopene	n.d.	8 ± 1	14.6 ^{a} ± 0.7	0.035 ^{d} ± 0.008	1.4 ^{c} ± 0.4	7.1 ^{b} ± 0.6
9,5-*Z*-lycopene	n.d.	4.1 ± 0.5	n.d.	n.d.	0.7 ± 0.2	n.d.
9-*Z*-lycopene	n.d.	40 ± 4	25 ^{a} ± 2	0.040 ^{c} ± 0.009	7 ^{b} ± 1	9.1 ^{b} ± 0.7
all-*E*-lycopene	n.d.	20 ± 2	46.8 ^{a} ± 0.7	0.038 ^{d} ± 0.009	3.4 ^{c} ± 0.7	9.6 ^{b} ± 0.6
5-*Z*-lycopene	n.d.	36 ± 3	42 ^{a} ± 2	0.004 ^{d} ± 0.01	6 ^{c} ± 1	9 ^{b} ± 1
Total carotenoids	-	139.13	142.7	0.221	23.83	53.89

EVOO (extra virgin olive oil); <LoQ (below the limit of quantification); n.d. (not detected). Results were expressed in mean ± SD; n = 3. [A] Statistical analysis between EVOO and the oil fraction (expressed in g of oil) applying t-test. Values in a row with symbols show significant differences from * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$, [B] Statistical analysis between Sofrito and fractions (expressed in g of sofrito) applying Dunn's Kruskal–Wallis multiple comparison. Values in a row with different letter show significant differences ($p \leq 0.05$).

2.4. Identification and Quantification of Carotenoids in the Sofrito Water, Oil and Insoluble Fractions

The insoluble fraction had the highest total carotenoid content (53.89 μg/g of *sofrito*) (Table 3), with the all-E-lycopene form (9.6 ± 0.6 μg/g) and the isomers 9-*Z*-lycopene and 5-*Z*-lycopene (9.1 ± 0.7 and 9 ± 1 μg/g, respectively) being predominant. The total carotenoid content in the oil (23.83 μg/g of *sofrito*) (Table 3) was much lower than in the insoluble fraction, but before the cooking process no quantifiable carotenoids were detected in the EVOO. Moreover, oil was the only fraction containing the carotenoids all-*E*-lutein (0.71 ± 0.2 μg/g of *sofrito*), apo-8-β-carotenal (0.40 ± 0.03 μg/g of *sofrito*), 9,13-*Z*-lycopene (0.37 ±0.04 μg/g of *sofrito*), and 9,5-*Z*-lycopene (0.7 ± 02 μg/g of *sofrito*) in quantifiable amounts, which indicates that olive oil can incorporate and solubilize these bioactive compounds. The oil fraction showed almost the same amount of total carotenoids as raw tomato (139.13 versus 150.48 μg/g, respectively), which indicates an efficient migration of carotenoids from the ingredients,

given that they were not found in the EVOO before cooking. Another notable result was the presence of carotenoid isomers in the oil fraction that were absent in raw tomato, indicating that they underwent isomerization and incorporation in the oil fraction during the *sofrito* process.

3. Discussion

3.1. Incorporation of Phenolic Compounds in Extra Virgin Olive Oil (EVOO) during the sofrito Process

The health benefits of dietary phenolic compounds depend on their absorption and bioavailability, which can be affected by differences in cell wall structure, glycoside location in cells, and especially by binding with the food matrix [21]. The cooking process helps to improve bioavailability by promoting chemical structural changes and the release of phytochemicals attached to the food matrix [4]. However, the results of this study show that the major source of polyphenols in the *sofrito* was the insoluble fraction, where they are still bound to the food matrix. Thus, their absorption requires release by the digestion process or the intestinal microbiota. The composition of the microbiota and human intervariabilities can influence this absorption [20]. The enrichment of the insoluble fraction with polyphenols from EVOO could be due to the frying of onions, part of the *sofrito* preparation [26]. Ramírez-Anaya and co-workers [26] studied polyphenols in Mediterranean vegetables prepared with different domestic cooking techniques and identified EVOO polyphenols in sautéed vegetables, indicating a migration from the oil fraction.

In a reverse migration, the *sofrito* oil fraction was found to contain phenolic compounds not present in the EVOO before cooking. Vallverdú-Queralt and co-workers [18], in a study of the effect of oil addition and processing time (15 to 60 min) on the phenolic profile of tomato sauce, found an increase in naringenin when 10% of EVOO was used compared to 5%, with the highest content found at 45 min. Naringenin is trapped in the cuticle of ripe tomato fruit, where it interacts with the insoluble polyesters that constitute tomato peel fiber [28,29]. Thus, mechanical and thermal processing plus the addition of oil could release this compound from the food matrix [23,24,29]. Among the different *sofrito* fractions, approximately 8% of naringenin was found in the oil. The affinity of naringenin for the lipophilic constituents of olive oil may improve its extraction and could contribute to absorption. Once incorporated in the oil fraction, it can enter the epithelial cells by passive diffusion [21,24]. Ban and colleagues [30], in an attempt to improve the bioaccessibility of phenolic compounds, incorporated naringenin in edible oil-producing nanoparticles. Thus protected, the polyphenol showed a good stability and resistance in the harsh conditions of gastrointestinal digestion, demonstrating its affinity for lipophilic environments. Martínez-Huélamo and co-workers [24], comparing the bioavailability of phenolic compounds in raw tomato, tomato sauce, and tomato sauce supplemented with refined olive oil, reported that naringenin content and bioaccessibility were higher after processing than in raw tomato. Additionally, the addition of oil to the tomato sauce enhanced the absorption of naringenin, which supports the possibility that the migration of tomato polyphenols to the EVOO in the *sofrito* preparation may improve their bioavailability [23].

During the cooking process, the hydrolysis of flavonoid glycosides produces free hydroxyl phenol groups, which enhances the lipophilicity of the molecule [13,14]. The molecular structure and the presence of a sugar are important factors for the incorporation of phenolic compounds in the oil matrix. Naringenin, ferulic acid, and quercetin showed good solubility in oil, reflected in the increase in their content in the oil fraction after the *sofrito* preparation.

3.2. Incorporation of Carotenoids in Extra Virgin Olive Oil (EVOO) during the sofrito Process

The insoluble fraction of the *sofrito* sauce had the highest carotenoid content, probably due to the presence of chromoplasts in tomato fruit, which remain intact after the cooking process [17] and maintain the chemically stable all-*E*-isoform that predominates in nature [31]. The presence of lycopene and β-carotene isomers in the insoluble fraction confirm that the isomerization reaction starts in the chromoplast cells during the cooking process, which supplies the thermal energy required [32–36].

Carotenoid degradation is avoided if temperatures are maintained between 105 °C and 120 °C [17], which are usual conditions in traditional Mediterranean cuisine. Moreover, thermal treatment helps to release carotenoids by softening cell walls and denaturing the protein-carotenoid complex, allowing the formation of carotenoid isomers and improving their bioaccessibility in the final product [5].

The addition of oil to tomato products helps to partly dissolve lycopene, which is otherwise in an insoluble crystalline form, and may protect lycopene from thermal oxidation during the cooking process and improve its isomerization susceptibility [18,32,35,37]. Moreover, the presence of fat increase the absorption of carotenoids [38,39]. Mutsokoti and co-workers [17] reported that several factors influence the efficiency of carotenoid transfer to the oil phase, including the natural barriers of the food matrix and carotenoid hydrophobicity and structure, the latter being the most important. In the current study, all-*E*-lycopene showed a low solubility in oil, representing 18% of the total lycopene in the oil fraction, whereas *Z*-lycopene forms constituted 82%. Mutsokoti and co-workers [17] reported a less efficient transfer to the oil fraction for lycopene compared to β-carotene. Palmero and colleagues [40] described the same pattern, suggesting that lycopene was entrapped by the matrix, with low transfer to the oil phase and consequently poor micellarization after in vitro digestion. However, neither of the authors discriminated between all-*E*-lycopene and its isomers. The properties and functions of carotenoids are strongly related to the size and shape of the compounds, the flexibility of the *Z*-forms favoring their absorption and transport in comparison with the *E*-form [31].

The presence of multi-*Z*-lycopene isomers in the oil fraction could indicate that this was a stable environment for carotenoid isomers. Multi-*Z*-isomers have been synthesized using a Wittig reaction, obtained by a catalytic reaction with iodine, and isolated from tomatoes, but there is only one report of multi-*Z*-lycopene isomers being obtained by thermal isomerization [41]. In the *sofrito* oil fraction, two di-*Z*-lycopene isomers were characterized, 9,5-*Z*-lycopene and 9,13-*Z*-lycopene. Li and co-workers [42] found di-*Z*-isomers in different tomato cultivars, although the contents were not quantifiable. Lin and Chen [43] found 9,13-*Z*-lycopene and 9,13′-*Z*-lycopene in tomato juice, but at much lower levels (0.17 to 1.03 μg/g) than in the *sofrito* oil fraction. Similarly, Kelebek and co-workers [44], studying the effect of hot and cold breaks in tomato paste processing, reported di-*Z*-lycopene, also at lower levels (0.49 to 0.87 mg/100 g dry matter). The current study differs in that EVOO was employed and phenolic compounds from other ingredients like tomato, onion, and garlic could migrate to the oil fraction. The favorable environment for the production of multi-*Z*-lycopene isomers can be attributed to polyphenols and other chain-braking antioxidants present in different lipid systems. These polyphenols can protect against oxidation in multiphasic systems, like oil/water emulsions, acting together with lipophilic antioxidants such as α-tocopherol [37,45]. Quercetin and naringenin, found in the tomato-based *sofrito*, show good solubility in oil and water, and especially at the oil-water interface, where oxidation takes place [45]. The action of these free radical scavengers could explain the enhanced content of carotenoid *Z*-isomers and di-*Z*-isomers in the oil fraction of the *sofrtio*.

4. Materials and Methods

4.1. Chemicals and Standards

Acetonitrile, ethanol, methanol, formic acid, and acetic acid were purchased from AppliChem, Panreac Quimica SA (Barcelona, Spain). Hexane, methyl tert-butyl ether (MTBE), all-*E*-α-carotene, all-*E*-β-carotene, all-*E*-lutein, all-*E*-lycopene caffeic acid, *p*-coumaric acid, chlorogenic acid, ferulic acid, isolariciresinol, larisiresinol, luteolin, naringenin, oleuropein, pinoresinol, protocatchuic acid, quercetin, rutin, and secoisolariciresinol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Narigenin-7-*O*-glucoside, hydroxytyrosol, and ethyl gallate were acquired from Extrasynthese (Genay, France), vanillic acid and apigenin from Fluka (St. Louis, MO, USA), and verbascoside from HWI Analytic. C18 ODS SPE bulk sorbent was purchased from Agilent Technologies (Santa Clara, CA, USA). Ultrapure water was obtained using a Milli-Q purification system (Millipore, Bedford, MA, USA).

4.2. Material

To prepare the *sofrito* sauce, we used the tomato variety "pera" (*Lycopersicon esculentum* Mill, c. v. Pera) because it is the traditional variety for making tomato sauce and *sofrito*, and moreover it has high content of polyphenols compared to other varieties [46]. They were bought from Grupo Almería (La Cañada, Almería, Spain) from the same batch and had similar diameter (57–67 mm) to avoid composition and climate variability. Olive oil certificated as Extra Virgin category (EVOO) was kindly provided by Manuel Heredia Halcón (Cortijo De Suerte Alta, Albendin-Baena-Córdoba, Córdoba, Spain). Onions and garlic were bought from Ametller Origen to use the products from the same producer to avoid variability, since they provide high quality local products (Barcelona, Spain).

4.3. Home-Cooking sofrito Process

In order to study the incorporation of bioactive compounds in EVOO during the cooking process, *sofrito* tomato sauce was chosen as a representative model of traditional Mediterranean cuisine. The *sofrito* recipe was based on previous results of a study applying a full factorial design to evaluate the effect of EVOO, onion, and garlic contents on the carotenoids in a tomato-based *sofrito* sauce (18). The tomato *sofrito* sauce that was cooked in triplicate using 100g of EVOO, 400g of onion, 40g of garlic, and 460g of tomato is the formulation that showed higher content of carotenoids (18).

The tomato-based *sofrito* was prepared at the Food and Nutrition Torribera Campus, University of Barcelona (Santa Coloma de Gramenet, Spain). Tomatoes, garlic, and onion were washed, tomato and onions were cut into small pieces (approximately 5mm), garlic were mixed in a blender (R5 Plus, Robot Coupe®, Montceau, Bourgogne, France) and weighed. The bioactive compounds of the ingredients were analyzed after the cutting process to avoid the effect of the process. The *sofrito* cooking process was according to traditional Mediterranean cuisine: in an uncovered pan (24 cm diameter, 15 cm height, 6.3 L volume, 1.59 mm thicknesss, and made of inox 18/10, Paderno, Orfengo, Italy), the EVOO was heated on an electrical cooking plate (180 mm diameter, 1500 W, model Encimera EM/30 2P, Teka®, Madrid, Spain) using a potency of 4 (of a range from 1 to 6) for 1 min. After that, the onion and garlic were added and fried for 1 min, and then the tomato was added. From this point the cooking process was timed and the potency was reduced to 2 for low heat cooking following the traditional Mediterranean cuisine and continued for 30 min. The cooking heat was monitored and kept constant throughout the process (100 ± 1 °C) for reproducibility of the samples. After the cooking process, the *sofrito* samples were weighed to quantify the water loss, packaged in plastic vacuum bags, and stored at −25 °C (18).

4.4. Isolation of Oil, Water and Insoluble Fractions

In order to isolate the oil, water, and insoluble fractions from the *sofrito* sauce, 20 g of sample was weighed in 50 mL falcon tubes and centrifuged at 20,000 rpm for 30 min at 15 °C, according to the protocol of Palmero et al. (2013) [47]. After the separation of the oil fraction, which consisted of the EVOO added to the formulation, the water fraction, and the insoluble fraction, the different parts were weighed to verify how much each one contributed to the *sofrito* composition.

4.5. Extraction and Analysis of Polyphenols

4.5.1. Polyphenol Extraction

The solid fraction and ingredients used before cooking (0.5 g) were extracted with 5 mL of methanol:ultra-pure water (80:20, *v*/*v*) with 0.1% of formic acid for 1 min, sonicated for 10 min in an ice bath, and centrifuged at 4000 rpm for 15 min at 4 °C (Sigma 1-16KL, Sigma, Osterode am Harz, Germany). The supernatant was transferred into a tube and the extraction was repeated. After that, the supernatants were combined and evaporated using a vacuum evaporator (miVac DNA concentrator, Genevac LTD, Warminster, England). The residue was suspended in up to 2 mL of ultra-pure water with 0.1% of formic acid [7].

The oil fraction (1 g) was diluted with 1 mL of *n*-hexane and after the addition of 2 mL of methanol, it was homogenized and centrifuged at 3000 rpm for 3 min at 4 °C. The nonpolar and polar phases were separated and extracted again with 1 mL of *n*-hexane and 2 mL of methanol, respectively. The polar phases were combined, cleaned up with 50 mg of C18 to remove the polar fatty acids, homogenized for 1 min and centrifuged. In order to check for the presence of residual lipids, the extract was placed in an ultra-freezer for 15 min and a visual inspection was performed. The clean liquid was evaporated until dryness using a vacuum evaporator and the residue was suspended in up to 2 mL of ultra-pure water with 0.1% of formic acid [48].

The water fraction (1 g) was weighed and centrifuged at 4000 rpm for 15 min at 4 °C. The supernatant was transferred to a 2 mL volumetric flask to which ultra-pure water with 0.1% of formic acid was added. All the extracts were filtered thought a 0.22 µm polytetrafluoroethylene (PTFE) filter into a 2 mL amber vial for HPLC and stored at −80 °C until analysis.

4.5.2. Polyphenol Analysis by UPLC-ESI-QqQ-MS/MS

The polyphenols typically found in tomato, onion and garlic were identified and quantified by a UHPLC-MS/MS method validated for tomato polyphenols (Tomato method) [49] and EVOO polyphenols were analyzed using a another method more appropriate for them (Olive oil method) [50]. A UPLC Acquity system equipped with a binary pump, autosampler, and oven from Waters (Milford, MA, United States) with a BEH C18 column (50 mm × 2.1 mm) i.d., 1.7 µm (Waters, Milford, MA, United States) was used. The injection volume was 10 µL, the samples were maintained at 4 °C and the column at 30 °C.

The separation of tomato polyphenols (Tomato method) was carried out with a phase A consisting of acetonitrile (0.1% formic acid) and phase B of water (0.1% formic acid). A gradient elution was applied as follows: 0 min, 10%A; 0.5 min, 10%A, 1.5 min, 15% A; 2.0 min, 20% A; 2.5 min, 50%A; 3.0 min, 100% A; 3.5 min 100% A, and 4.5 min, 10% A. For the EVOO polyphenols (Olive oil method), the separation was performed with a phase A consisting of acetonitrile (0.2% acetic acid) and phase B of water (0.2% acetic acid). The gradient elution was: 0 min, 5%A; 2.5 min, 5%A; 12.5 min, 40%A; 12.6 min, 100%A; 13.5 min, 100%A; 13.6 min, 5%A, and 15.0 min, 5%A. A flow rate of 400 µL/min was applied for both methods.

An API 3000 triple quadrupole mass spectrometer (ABSciex, Framingham, MA, USA), coupled with a Turbo Ionspray source in negative ion mode was used for the MS/MS analysis. The settings of the Turbo Ionspray were: capillary voltage, -3500 V; nebulizer gas (N2), 10 (arbitrary units); curtain gas (N_2), 12 (arbitrary units); collision gas (N_2), 4 (arbitrary units); and drying gas (N_2) heated at 400 °C introduced at a flow rate of 8000 cm^3/min. To improve the detection, the declustering potential, focusing potential, and collision energy were optimized for each compound by direct infusion experiments: 10 ppm individual standard solutions, dissolved in 1:1 (*v*/*v*) mobile phase, were infused at a flow rate of 5 µL/min with a model syringe pump (Harvard Apparatus, Holliston, MA, USA). A full-scan data acquisition in profile mode, scanning from 100 to 800 *m*/*z*, was used in cycles of 2 s with a step size of 0.1 u and a pause between each scan of 0.002 s (Table 1).

The polyphenols were quantified using multiple reaction monitoring mode (MRM), tracking the transition of parent ion and product ions specific for each compound. The quantification was performed using the internal standard method, applying ethyl gallate as the internal standard, and quantified with calibration curves related to the corresponding standard. The limit of quantification (LoQ) was: 1.13 ng/mL for chlorogenic acid; 9.75 ng/mL for caffeic acid, protocatechuic acid, ferulic acid and verbascoside; 27.0 ng/mL for naringenin-7-O-glucoside, *p*-coumaric, isolariciresinol, lariciresinol; 75.0 ng/mL naringenin, quercetin, rutin, apigenin, luteolin, hydroxytyrosol, and oleuropein. When commercial standards were not available, the compound was quantified using a standard of the same class of polyphenol. The results were expressed as µg/g of fraction.

4.6. Extraction and Analysis of Carotenoids

4.6.1. Carotenoid Extraction

The solid fraction and ingredients (0.5 g) and water fractions (1 g) were weighed and homogenized with 5 mL of ethanol *n*-hexane (4:3, *v*/*v*), and then sonicated for 10 min in an ice bath and centrifuged at 4000 rpm for 20 min at 4 °C. The apolar phase was separated in a different flask and the extraction was repeated until it was colorless. All the supernatants were combined and evaporated to dryness using a vacuum concentrator (miVac DNA concentrator, Genevac LTD, Warminster, England). The residue was suspended in 1 mL of MTBE, filtered using a 0.22 µm PTFE filter, and stored in a 2 mL amber vial at −80 °C [36].

The oil fraction (1 g) was extracted using 3 mL cold acetone (8 °C), then sonicated for 10 min in an ice bath and centrifuged in the same conditions. The extract was kept at freezer temperature (−25 °C) for 30 min and the separation of acetone from residual fat was visually checked. After that, the extraction was repeated until it was colorless. The carotenoids were transferred to 2 mL of hexane and washed 4 times with 5 mL of ultrapure water to remove the acetone. The hexane was evaporated until dryness using a vacuum concentrator and the residue was suspended in 1 mL of MTBE, filtered with a 0.22 µm PTFE filter in a 2 mL amber vial, and stored at −80 °C.

4.6.2. Carotenoid Analysis by HPLC-DAD and HPLC-APCI-QqQ-MS/MS

The carotenoid analysis was based on the procedure of Vallverdú-Queralt et al. (2015) [36] with modifications. An HPLC system (HP1100 HPLC system, Hewlett-Packard, Waldbronn, Germany) equipped with a quaternary pump and autosampler and coupled with a diode array detector (DAD G1315B) was used. Separation was carried out using a C30 250 × 4.6 mm, 5 µm column (YMCTM, Water Co., Milford, MA, USA), with a flow rate of 600 µL/min at 25 °C. The injection volume was 20 µL. The mobile phase consisted of methanol (A), MTBE (B) and water (C). A gradient was used to separate the carotenoid compounds under the following conditions: 0 min, 70% A; 15 min, 20% A; 30 min, 6% A; 31 min, 6% A; 33 min, 70% A; and 43 min, 70% A. Water was kept constant at 4% throughout the analysis. The DAD detector was applied in the range of 350 to 600 nm and the chromatograms were acquired at a 450 nm wavelength.

A QTRAP4000 triple quadrupole mass spectrometer (Sciex, Foster City, CA, USA) equipped with an APCI ionization source operating in positive-ion and multiple reaction monitoring mode was used to confirm the identification. The carotenoids were separated using the same column and a different mobile phase was applied: methanol (A) and MTBE:methanol (80:20, *v*/*v*) (B), both fortified with 0.7 g/L of ammonium acetate and 0.1% acetic acid. The linear gradient of A was: 0 min, 90%; 10 min, 75%; 20 min, 50%; 25 min, 30%; 35 min, 10%; 37 min, 6%; 39 min, 90%; and 50 min, 90%, with a flow rate of 600 µL/min. The mass spectrometer conditions were: entrance potential (EP), 10 V; collision cell exit potential (CXP), 15 V; temperature source, 400 °C, curtain gas, 20 psi, ion source gas 1 (GS1), 45 psi; ion source gas 2 (GS2), 0 psi. Declustering potential (DP) was selected for the carotenoids according to previously established conditions [51].

The identification was based on retention time, chromatography with standards, mass spectra, and UV/VIS absorption spectra: λ_{max}, spectral fine structure (%III/II) and peak cis intensity (%A_b/A_{II}). The carotenoids were quantified by HPLC-DAD with external calibration curves of α-carotene, β-carotene and lycopene with seven concentration levels. The limit of quantification (LoQ) was 0.2 for lycopene and 0.4 ppm for α-carotene and β-carotene. The Z-isomers were quantified with the calibration curve of the corresponding E-form. The results were expressed as µg/g of fraction.

4.7. Statistical Analysis

The Shapiro–Wilk test was used to check data normality. The statistical differences between EVOO before and after cooking were analyzed by R v 3.4.4 using the *t*-test, and the differences between different fractions and tomato *sofrito* sauce by Dunn's Kruskal–Wallis multiple comparisons.

5. Conclusions

The use of olive oil in Mediterranean cuisine may improve the extractability of bioactive compounds such as polyphenols and carotenoids from food matrix. The migration of polyphenols, such as naringenin, ferulic acid, and quercetin, which are compounds not detected in olive oil, to the oil fraction during the *sofrito* preparation may enhance their bioacessibility and bioavailability. Carotenoids from tomato can also be transferred to the oil fraction, especially *Z*-isomers, due to their structure and solubility, and this could improve their bioacessibility. The presence of these compounds in oil fraction after the cooking process indicates their stability in oil matrix avoiding oxidation. The beneficial effects of the Mediterranean diet may be due not only to the consumption of certain foods, but also cooking techniques such as *sofrito*, which can help the extraction of carotenoids and phenolic compounds from food matrix, and could contribute to its bioacessibility, bioavailability, and health effects.

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References

1. Hoffman, R.; Gerber, M. Food Processing and the Mediterranean Diet. *Nutrients* **2015**, *7*, 7925–7964. [CrossRef] [PubMed]
2. Estruch, R.; Ros, E.; Salas-Salvadó, J.; Covas, M.-I.; Corella, D.; Arós, F.; Gómez-Gracia, E.; Ruiz-Gutiérrez, V.; Fiol, M.; Lapetra, J.; et al. Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts. *N. Engl. J. Med.* **2018**, *378*, e34. [CrossRef]
3. Martínez-González, M.A.; Salas-Salvadó, J.; Estruch, R.; Corella, D.; Fitó, M.; Ros, E. Benefits of the Mediterranean Diet: Insights From the PREDIMED Study. *Prog. Cardiovasc. Dis.* **2015**, *58*, 50–60. [CrossRef] [PubMed]
4. Murador, D.; Braqa, A.R.; Da Cunha, D.; de Rosso, V. Alterations in phenolic compound levels and antioxidant activity in response to cooking technique effects: A meta-analytic investigation. *Crit. Rev. Food Sci. Nutr.* **2018**, *58*, 169–177. [CrossRef] [PubMed]
5. Murador, D.C.; da Cunha, D.T.; de Rosso, V.V. Effects of cooking techniques on vegetable pigments: A meta-analytic approach to carotenoid and anthocyanin levels. *Food Res. Int.* **2014**, *65*, 177–183. [CrossRef]
6. De Alvarenga, J.F.R.; Lozano-Castellón, J.; Martínez-Huélamo, M.; Vallverdú-Queralt, A.; Lamuela-Raventós, R.M. Cooking practice and the matrix effect on the health properties of mediterranean diet: A study in tomato sauce. *ACS Symp. Ser.* **2018**, *1286*, 305–314. [CrossRef]
7. Vallverdú-Queralt, A.; De Alvarenga, J.F.R.; Estruch, R.; Lamuela-Raventos, R.M. Bioactive compounds present in the Mediterranean sofrito. *Food Chem.* **2013**, *141*, 3366–3372. [CrossRef]
8. Rodriguez-Rodriguez, R.; Jiménez-Altayó, F.; Alsina, L.; Onetti, Y.; Rinaldi de Alvarenga, J.F.; Claro, C.; Ogalla, E.; Casals, N.; Lamuela-Raventos, R.M. Mediterranean tomato-based sofrito protects against vascular alterations in obese Zucker rats by preserving NO bioavailability. *Mol. Nutr. Food Res.* **2017**, *61*, 1601010. [CrossRef]

9. Sandoval, V.; Rodríguez-Rodríguez, R.; Martínez-Garza, Ú.; Rosell-Cardona, C.; Lamuela-Raventós, R.M.; Marrero, P.F.; Haro, D.; Relat, J. Mediterranean Tomato-Based *Sofrito* Sauce Improves Fibroblast Growth Factor 21 (FGF21) Signaling in White Adipose Tissue of Obese ZUCKER Rats. *Mol. Nutr. Food Res.* **2018**, *21*, 1700606. [CrossRef]
10. Martínez-González, M.A.; García-Arellano, A.; Toledo, E.; Salas-Salvadó, J.; Buil-Cosiales, P.; Corella, D.; Covas, M.I.; Schröder, H.; Arós, F.; Gómez-Gracia, E.; et al. A 14-Item Mediterranean Diet Assessment Tool and Obesity Indexes among High-Risk Subjects: The PREDIMED Trial. *PLoS ONE* **2012**, *7*, e43134. [CrossRef]
11. Schro, H.; Corella, D.; Salas-salvado, J.; Lamuela-ravento, R.; Ros, E.; Salaverrı, I.; Vinyoles, E.; Go, E. A Short Screener Is Valid for Assessing Mediterranean Diet Adherence among Older. *J. Nutr.* **2011**, *141*, 1140–1145. [CrossRef] [PubMed]
12. Stojanovic, J.; Giraldi, L.; Arzani, D.; Pastorino, R.; Biondi, A.; Persiani, R.; Boccia, S.; Leoncini, E. Adherence to Mediterranean diet and risk of prostate cancer. *Eur. J. Cancer Prev.* **2017**, *26*, 491–496. [CrossRef]
13. Orsavova, J.; Misurcova, L.; Ambrozova, J.; Vicha, R.; Mlcek, J. Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids. *Int. J. Mol. Sci.* **2015**, *16*, 12871–12890. [CrossRef] [PubMed]
14. Medina, E.; de Castro, A.; Romero, C.; Brenes, M. Comparison of the Concentrations of Phenolic Compounds in Olive Oils and Other Plant Oils: Correlation with Antimicrobial Activity. *J. Agric. Food Chem.* **2006**, *54*, 4954–4961. [CrossRef]
15. Li, Q.; Li, T.; Liu, C.; Chen, J.; Zhang, R.; Zhang, Z.; Dai, T.; McClements, D.J. Potential physicochemical basis of Mediterranean diet effect: Ability of emulsified olive oil to increase carotenoid bioaccessibility in raw and cooked tomatoes. *Food Res. Int.* **2016**, *89*, 320–329. [CrossRef]
16. Lemmens, L.; Colle, I.; Van Buggenhout, S.; Palmero, P.; Van Loey, A.; Hendrickx, M. Carotenoid bioaccessibility in fruit- and vegetable-based food products as affected by product (micro)structural characteristics and the presence of lipids: A review. *Trends Food Sci. Technol.* **2014**, *38*, 125–135. [CrossRef]
17. Mutsokoti, L.; Panozzo, A.; Van Loey, A.; Hendrickx, M. Carotenoid transfer to oil during thermal processing of low fat carrot and tomato particle based suspensions. *Food Res. Int.* **2016**, *86*, 64–73. [CrossRef]
18. De Alvarenga, J.F.R.; Tran, C.; Hurtado-Barroso, S.; Martinez-Huélamo, M.; Illan, M.; Lamuela-Raventos, R.M. Home cooking and ingredient synergism improve lycopene isomer production in Sofrito. *Food Res. Int.* **2017**, *99*, 851–861. [CrossRef]
19. Bohm, V.; Puspitasari-Nienaber, N.L.; Ferruzzi, M.G.; Schwartz, S.J. Trolox equivalent antioxidant capacity of different geometrical isomers of α-carotene, β-carotene, lycopene, and zeaxanthin. *J. Agric. Food Chem.* **2002**, *50*, 221–226. [CrossRef] [PubMed]
20. Del Rio, D.; Rodriguez-Mateos, A.; Spencer, J.P.E.; Tognolini, M.; Borges, G.; Crozier, A. Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases. *Antioxid. Redox Signal.* **2013**, *18*, 1818–1892. [CrossRef] [PubMed]
21. Vallverdú-Queralt, A.; Regueiro, J.; Rinaldi De Alvarenga, J.F.; Torrado, X.; Lamuela-Raventos, R.M. Home cooking and phenolics: Effect of thermal treatment and addition of extra virgin olive oil on the phenolic profile of tomato sauces. *J. Agric. Food Chem.* **2014**, *62*, 3314–3320. [CrossRef] [PubMed]
22. Martínez-Huélamo, M.; Tulipani, S.; Estruch, R.; Escribano, E.; Illán, M.; Corella, D.; Lamuela-Raventós, R.M. The tomato sauce making process affects the bioaccessibility and bioavailability of tomato phenolics: A pharmacokinetic study. *Food Chem.* **2015**, *173*, 864–872. [CrossRef]
23. Martínez-Huélamo, M.; Vallverdú-Queralt, A.; Di Lecce, G.; Valderas-Martínez, P.; Tulipani, S.; Jáuregui, O.; Escribano-Ferrer, E.; Estruch, R.; Illan, M.; Lamuela-Raventós, R.M. Bioavailability of tomato polyphenols is enhanced by processing and fat addition: Evidence from a randomized feeding trial. *Mol. Nutr. Food Res.* **2016**, *60*, 1578–1589. [CrossRef] [PubMed]
24. Rohn, S.; Buchner, N.; Driemel, G.; Rauser, M.; Kroh, L.W. Thermal Degradation of Onion Quercetin Glucosides under Roasting Conditions. *J. Agric. Food Chem.* **2007**, *55*, 1568–1573. [CrossRef] [PubMed]
25. Cardona, F.; Andrés-Lacueva, C.; Tulipani, S.; Tinahones, F.J.; Queipo-Ortuño, M.I. Benefits of polyphenols on gut microbiota and implications in human health. *J. Nutr. Biochem.* **2013**, *24*, 1415–1422. [CrossRef]
26. Ramírez-Anaya, J.D.P.; Samaniego-Sánchez, C.; Castañeda-Saucedo, M.C.; Villalón-Mir, M.; De La Serrana, H.L.G. Phenols and the antioxidant capacity of Mediterranean vegetables prepared with extra virgin olive oil using different domestic cooking techniques. *Food Chem.* **2015**, *188*, 430–438. [CrossRef] [PubMed]

27. Carrasco-Pancorbo, A.; Cerretani, L.; Bendini, A.; Segura-Carretero, A.; Lercker, G.; Fernández-Gutiérrez, A. Evaluation of the influence oh thermal oxidation on the phenolic composition and on the antioxidant activity of extra-virgen olive oils. *J. Agric. Food Chem.* **2007**, *55*, 4771–4780. [CrossRef]
28. Tulipani, S.; Martinez Huelamo, M.; Rotches Ribalta, M.; Estruch, R.; Ferrer, E.E.; Andres-Lacueva, C.; Illan, M.; Lamuela-Raventós, R.M. Oil matrix effects on plasma exposure and urinary excretion of phenolic compounds from tomato sauces: Evidence from a human pilot study. *Food Chem.* **2012**, *130*, 581–590. [CrossRef]
29. Tomas, M.; Beekwilder, J.; Hall, R.D.; Sagdic, O.; Boyacioglu, D.; Capanoglu, E. Industrial processing versus home processing of tomato sauce: Effects on phenolics, flavonoids and in vitro bioaccessibility of antioxidants. *Food Chem.* **2017**, *220*, 51–58. [CrossRef] [PubMed]
30. Ban, C.; Park, S.J.; Lim, S.; Choi, S.J.; Choi, Y.J. Improving Flavonoid Bioaccessibility using an Edible Oil-Based Lipid Nanoparticle for Oral Delivery. *J. Agric. Food Chem.* **2015**, *63*, 5266–5272. [CrossRef]
31. Britton, G. SERIAL Structure function. *FASEB J.* **1995**, *9*, 1551–1558. [CrossRef] [PubMed]
32. Colle, I.J.P.; Lemmens, L.; Tolesa, G.N.; Van Buggenhout, S.; De Vleeschouwer, K.; Van Loey, A.M.; Hendrickx, M.E. Lycopene Degradation and Isomerization Kinetics during Thermal Processing of an Olive Oil/Tomato Emulsion. *J. Agric. Food Chem.* **2010**, *58*, 12784–12789. [CrossRef] [PubMed]
33. Colle, I.J.P.; Lemmens, L.; Van Buggenhout, S.; Van Loey, A.M.; Hendrickx, M.E. Modeling Lycopene Degradation and Isomerization in the Presence of Lipids. *Food Bioprocess Technol.* **2013**, *6*, 909–918. [CrossRef]
34. Honda, M.; Horiuchi, I.; Hiramatsu, H.; Inoue, Y.; Kitamura, C.; Fukaya, T.; Takehara, M. Vegetable oil-mediated thermal isomerization of (all-E)-lycopene: Facile and efficient production of Z -isomers. *Eur. J. Lipid Sci. Technol.* **2016**, *118*, 1588–1592. [CrossRef]
35. Lambelet, P.; Richelle, M.; Bortlik, K.; Franceschi, F.; Giori, A.M. Improving the stability of lycopene Z-isomers in isomerised tomato extracts. *Food Chem.* **2009**, *112*, 156–161. [CrossRef]
36. Vallverdú-Queralt, A.; Regueiro, J.; de Alvarenga, J.; Torrado, X.; Lamuela-Raventos, R. Carotenoid Profile of Tomato Sauces: Effect of Cooking Time and Content of Extra Virgin Olive Oil. *Int. J. Mol. Sci.* **2015**, *16*, 9588–9599. [CrossRef] [PubMed]
37. Cossignani, L.; Chiodelli, G.; Ghisoni, S.; Blasi, F.; Lucini, L.; Rocchetti, G.; Montesano, D.; Simonetti, M.S.; Baccolo, G. Changes in extra-virgin olive oil added with Lycium barbarum L. carotenoids during frying: Chemical analyses and metabolomic approach. *Food Res. Int.* **2017**, *105*, 507–516. [CrossRef]
38. Fielding, J.M.; Rowley, K.G.; Cooper, P.; O'Dea, K. Increases in plasma lycopene concentration after consumption of tomatoes cooked with olive oil. *Asia Pac. J. Clin. Nutr.* **2005**, *14*, 131–136.
39. Brown, M.J.; Ferruzzi, M.G.; Nguyen, M.L.; Cooper, D.A.; Eldridge, A.L.; Schwartz, S.J.; White, W.S. Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. *Am. J. Clin. Nutr.* **2004**, *80*, 396–403. [CrossRef]
40. Palmero, P.; Panozzo, A.; Simatupang, D.; Hendrickx, M.; Van Loey, A. Lycopene and β-carotene transfer to oil and micellar phases during in vitro digestion of tomato and red carrot based-fractions. *Food Res. Int.* **2014**, *64*, 831–838. [CrossRef]
41. Honda, M.; Kudo, T.; Kuwa, T.; Higashiura, T.; Fukaya, T.; Inoue, Y.; Kitamura, C.; Takehara, M. Isolation and spectral characterization of thermally generated multi- Z -isomers of lycopene and the theoretically preferred pathway to di-Z-isomers. *Biosci. Biotechnol. Biochem.* **2017**, *81*, 365–371. [CrossRef] [PubMed]
42. Li, H.; Deng, Z.; Liu, R.; Loewen, S.; Tsao, R. Ultra-performance liquid chromatographic separation of geometric isomers of carotenoids and antioxidant activities of 20 tomato cultivars and breeding lines. *Food Chem.* **2012**, *132*, 508–517. [CrossRef] [PubMed]
43. Lin, C.H.; Chen, B.H. Determination of carotenoids in tomato juice by liquid chromatography. *J. Chromatogr. A* **2003**, *1012*, 103–109. [CrossRef]
44. Kelebek, H.; Selli, S.; Kadiroğlu, P.; Kola, O.; Kesen, S.; Uçar, B.; Çetiner, B. Bioactive compounds and antioxidant potential in tomato pastes as affected by hot and cold break process. *Food Chem.* **2017**, *220*, 31–41. [CrossRef] [PubMed]
45. Becker, E.M.; Ntouma, G.; Skibsted, L.H. Synergism and antagonism between quercetin and other chain-breaking antioxidants in lipid systems of increasing structural organisation. *Food Chem.* **2007**, *103*, 1288–1296. [CrossRef]

46. Vallverdú-Queralt, A.; Medina-Remón, A.; Martínez-Huélamo, M.; Jáuregui, O.; Andres-Lacueva, C.; Lamuela-Raventos, R.M. Phenolic Profile and Hydrophilic Antioxidant Capacity as Chemotaxonomic Markers of Tomato Varieties. *J. Agric. Food Chem.* **2011**, *59*, 3994–4001. [CrossRef]
47. Palmero, P.; Lemmens, L.; Ribas-Agustí, A.; Sosa, C.; Met, K.; De Dieu Umutoni, J.; Hendrickx, M.; Van Loey, A. Novel targeted approach to better understand how natural structural barriers govern carotenoid in vitro bioaccessibility in vegetable-based systems. *Food Chem.* **2013**, *141*, 2036–2043. [CrossRef]
48. Capriotti, A.L.; Cavaliere, C.; Crescenzi, C.; Foglia, P.; Nescatelli, R.; Samperi, R.; Laganà, A. Comparison of extraction methods for the identification and quantification of polyphenols in virgin olive oil by ultra-HPLC-QToF mass spectrometry. *Food Chem.* **2014**, *158*, 392–400. [CrossRef]
49. Di Lecce, G.; Martínez-Huélamo, M.; Tulipani, S.; Vallverdú-Queralt, A.; Lamuela-Raventós, R.M. Setup of a UHPLC-QqQ-MS method for the analysis of phenolic compounds in cherry tomatoes, tomato sauce, and tomato juice. *J. Agric. Food Chem.* **2013**, *61*, 8373–8380. [CrossRef]
50. Suárez, M.; Macià, A.; Romero, M.-P.; Motilva, M.-J. Improved liquid chromatography tandem mass spectrometry method for the determination of phenolic compounds in virgin olive oil. *J. Chromatogr. A* **2008**, *1214*, 90–99. [CrossRef]
51. Hrvolová, B.; Martínez-Huélamo, M.; Colmán-Martínez, M.; Hurtado-Barroso, S.; Lamuela-Raventós, R.; Kalina, J. Development of an Advanced HPLC–MS/MS Method for the Determination of Carotenoids and Fat-Soluble Vitamins in Human Plasma. *Int. J. Mol. Sci.* **2016**, *17*, 1719. [CrossRef] [PubMed]

Sample Availability: Samples of the compounds are not available from the authors.

4.2.5 Publication 7. “Omics” allows to discover an unrevealed side of cooking: *cuisinomics*

José Fernando Rinaldi de Alvarenga, Paola Quifer-Rada, Sara Hurtado-Barroso, Montserrat Illan, Xavier Torrado-Prat, Rosa Maria Lamuela-Raventós.

Cooking made humans evolve. This habit has led humans to develop cooking techniques that extract maximum benefits from food resources and improve digestibility and nutrients absorption. However, is it not yet clear known how cooking impacts our health and the changes that promote on food. “Omics” is a new strategy that has been used for a global analysis of a complex system in which the discipline foodomics makes this approach to food to ensure food security and nutritional aspects. Our proposal is to bring foodomics closer to the gastronomy to better understand the culinary process. Five tomato *sofritos* formulations, differentiated by a fortification of extra virgin olive oil (EVOO) (10%), onion (40%), garlic (4%) and cooking time (60 min) were prepared and compared with a control (5% EVOO, 20% onion, 2% garlic and 30 min). Freeze-dried *sofritos* were extracted with different dissolvent (acetonitrile, methanol and water) and analyzed LC-LQT-Orbitrap-MS/MS. Data management (feature detection, peak alignment, retention time correction, filtering) were performed using XCMS-R. PCA Multivariate statistical analysis was performed to search differences between groups. Univariate statistical using Wilcox-Mann Whitney test ($p \leq 0.05$) was used to find significant features between groups. Metabolites that discriminate the groups were identified using online databases with exact mass matching and confirmed by MS/MS spectra. The PCA using two components revealed that the increase of the different ingredients modified the chemical profile, while an increase in cooking time did not, revealing that *sofrito* technique is not so aggressive to the sauce chemical profile. When a third component was added, the groups were separated by their composition. Using the univariate analysis, markers for the increase of olive oil (224), of onion (666), of garlic (276) and of cooking time (226) were detected that differ from the control sauce. New classes of compounds not explore in tomato products such as phytoprostanes are increased with more onion and garlic on the recipe. Other class such as hydroxycinnamic acid amides were detected with more cooking time. Amino acids, thiosulfates and phenolic compounds are also affected by ingredients and cooking time. Home-cooking is a complex system, in which recipe variation and cooking time impact the chemical profile of a dish, which influence the contribution of bioactive compounds. Studies should take into account cooking in the development of nutritional recommendations

OMICS ALLOW TO DISCOVER AN UNREVALED SIDE OF COOKING: *CUSINOMICS*

Running title: Use of OMICS to discover formation of new compounds during home cooking: cusinomics

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Abstract

Cooking played an important role in human evolution. Untargeted approach allows to detect thousands of compounds without any knowledge about sample composition. Using an untargeted omics technique (cusinomics), applied to different cooked preparations, that were made increasing minority ingredients or time, allowed to detect the presence of a unexplored compounds that may have a beneficial effect on health such as phytoprostanes, hydroxycinnamic acid amides and compounds such as 3,4 dihydroxyphenylglycone. Moreover, the effect on other known compounds, such as aminoacids, thioulfastes or phenolic has also been observed. Applying an untargeted approach allows to know the influence of ingredients and time on cooking that may help to advance on dietary recommendations to improve our diet, habits and health.

Keywords: Phytoprostanes, 3,4 dihydroxyphenylglycone Extra virgin olive oil, Mediterranean diet, Tomato, onion, garlic, *sofrito*, cooking time, cuisine

Cooking made humans evolve[1]. This habit played an important role in human evolution by reducing the intestine and the consumption of energy by digestion, allowing the development of a brain that could consume more energy[2]. For that, humans advance a range of cooking techniques to prove the maximum benefits from limited food resources. This habit changes the preference of our taste and consequently our health, since most of daily eaten foods are cooked[1]. Cooking makes nutrients and phytochemicals more easily absorbed[3]. Nowadays, efforts on research about how culinary techniques affect and preserve health-promote phytochemicals has been carried out[4,5], however they are focused on targeted compounds found in raw foods, completely forgetting about other chemical classes or formation of new compounds[6].

The "omics" era has been advancing significantly in the last years, particularly metabolomics by the advance in equipment, software and strategies. Applying an untargeted approach, thousands of compounds can be detected without any knowledge about sample composition[7]. Recently, the term foodomics was incorporated by Cifuentes and co-workers[8] for a global approach to integrating information regarding food science and nutrition applying advanced analytical techniques and bioinformatics. Foodomics have been applied in food safety and quality, personalized nutrition and human health, but not yet explored in cuisine, improving our knowledge about how daily cooked food impacts our diets and health.

Our findings demonstrate that exploring cooking with an omics approach "cuisinomics" provide unrevealed information about cooking. Studying the *sofrito*, a typical tomato-based sauce, of Mediterranean diet, different classes of compounds like phytoprostanes, hydroxycinnamic acid derivates and 3,4 dihydroxyphenylglycol seems to increase. This increment occurs when more amount of garlic or onion were used as an ingredient or prolonging cooking time. Other known compounds from vegetables such as polyphenol, thiosulfates and amino acids can evolve during the cooking process.

The use of an untargeted approach helps to explain the influence of cuisine: ingredient addition and cooking time on the chemical profile and allows to discover new ones, besides evaluating the profile changes of known bioactive compounds that may help to advance on the dietary recommendations for improve our diets and health.

Results

Data processing

The data processing with XCMS revealed 60164 features in negative ion mode during the 30 min of chromatogram using the same acquired range from chromatography analysis (100-1000 m/z) that was grouped in 4628 features. Applying filtering representativeness (80% rule), the number of features was reduced to 4451 (~96.18%) of the initial dataset. The intensity criteria reduced the feature numbers for 1314 (~29.52%) of the dataset after representativeness filtering. The CV% criteria do not reduce the number of features in the dataset. After data filtering, a Wilcox-Mann Whitney test adjusted by FDR were performed and the fold change of the mean intensity

between “control” vs “EVOO”, “control” versus “onion”, “control” versus “garlic” and “control” versus “time” were calculated, then features that presented adjusted p-value <0.05 and a fold change (FC) > 2 were selected.

During LC-MS analysis, a single metabolite is typically detected as a multiple metabolic feature having the same retention time but a different m/z. This variety of different ion types detected can be applied as an advantage in identification since it decreases the number of false positives identified by assigning an identification to an adduct or isotope[7]. Therefore, annotation is an essential step in metabolites identification. The selected features annotation was checked by CAMERA-R package and manually for isotopes and adducts confirmed by peak shape similarity, retention time and presence in full scan mass spectra. For EVOO, 224 features were selected and clustering in 73 group, for onion 666 features in 92 groups, for garlic 276 features in 40 groups and for time 226 features in 39 groups (Figure 1). Some of the features were not able to be grouped in the selected features list and the annotation was performed checking the full scan mass spectra.

Features that filled the criteria in the univariate analysis were putatively identified (Table 1), in which six compounds were related to higher content of EVOO, 12 to garlic, 16 to onion and seven with longer time of processing. The adducts and isotopes that corroborate the identification were showed in S1 (supplemental material 1). The unsupervised PCA analysis grouped the samples in 4 groups, in which no differences between longer cooking time versus control using two components, with a R2X = 0.50 and a Q2 = 0.33, indicating that *sofrito* technique and the ingredients in its recipe stabilized the profile of the sauce after 30 min extra of cooking (Figure 2). When a third component was added, a better model was constructed with R2X = 0.63 and a Q2 = 0.40, increasing differences between time and control with the quality of the model (Figure 3) and the univariate analysis revealed markers that were attributed to longer time of processing compared to control.

The untargeted approach revealed unexplored class of compounds, such as phytoprostanes and hydroxycinnamic acid amide or link to an amino acid in relation to the ingredient composition, cooking effect. Moreover, classical targeted compounds such as amino acids, sulfur and phenolic compound were found as markers of ingredients or time and it is still unclear what role these compounds could play on cooking process.

The larger processing time revealed presence of new markers such as p-coumaroyltyramine and 2feruloyl-1-(4-hydroxyphenyl)-1,2-ethanediol and the increase of garlic in the recipe showed N-caffeoyltyramine, an unexplored class of hydroxycinnamic derivate compounds. The hydroxycinnamic acid amides, such as p-coumaroyltyramine and N-caffeoyltyramine, are commonly found in flowering plants and are involved in a variety of plant physiological processes as signaling molecules in the cell wall structure for plant defense against microbial challenges and wound healing[9,10]. In food the presence describing these compounds is scarce, only in tomato, cherry tomato, pepper, paprika, onion, spinach[9] and in goji berry[10]. During cooking a catalytic oxidation process occurs, in which hydroxycinnamic acids can undergo

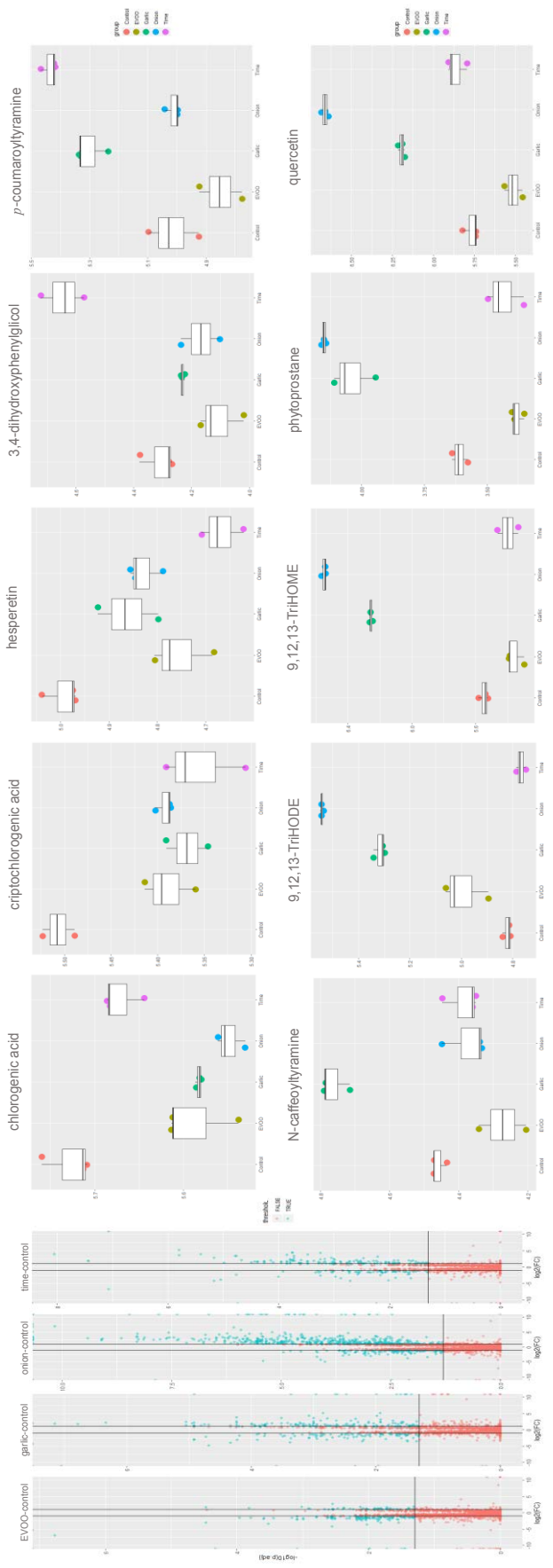

Figure 1. Volcano plot and boxplot of the features that discriminate the effect of ingredient addition and cooking time of the tomato *sofrito* sauce.

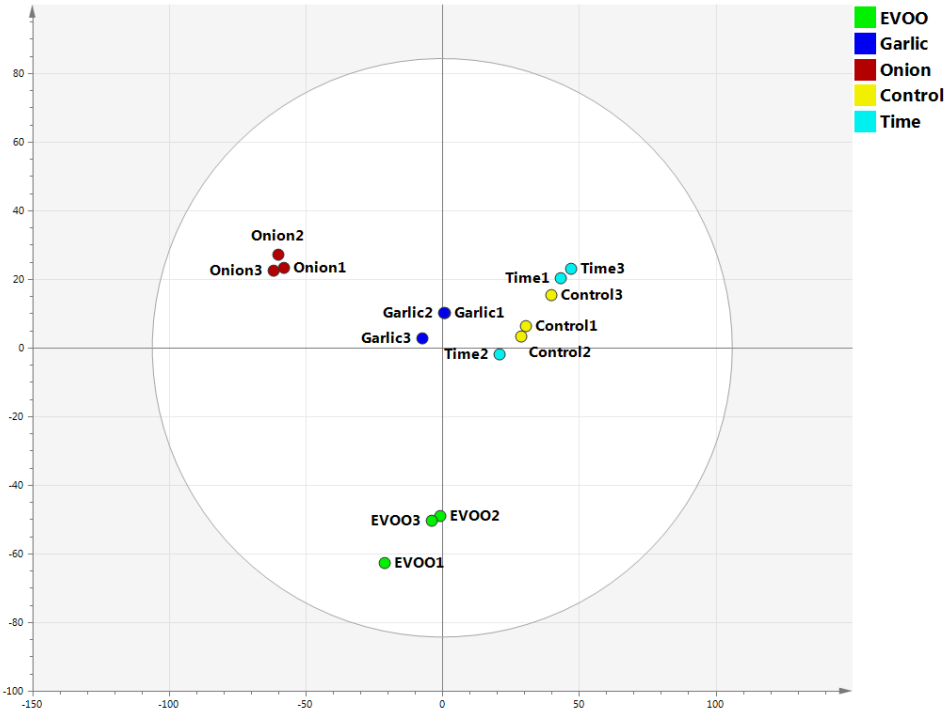

Figure 2. PCA analysis of different tomato *sofrito* sauces with an increment on ingredients and cooking time.

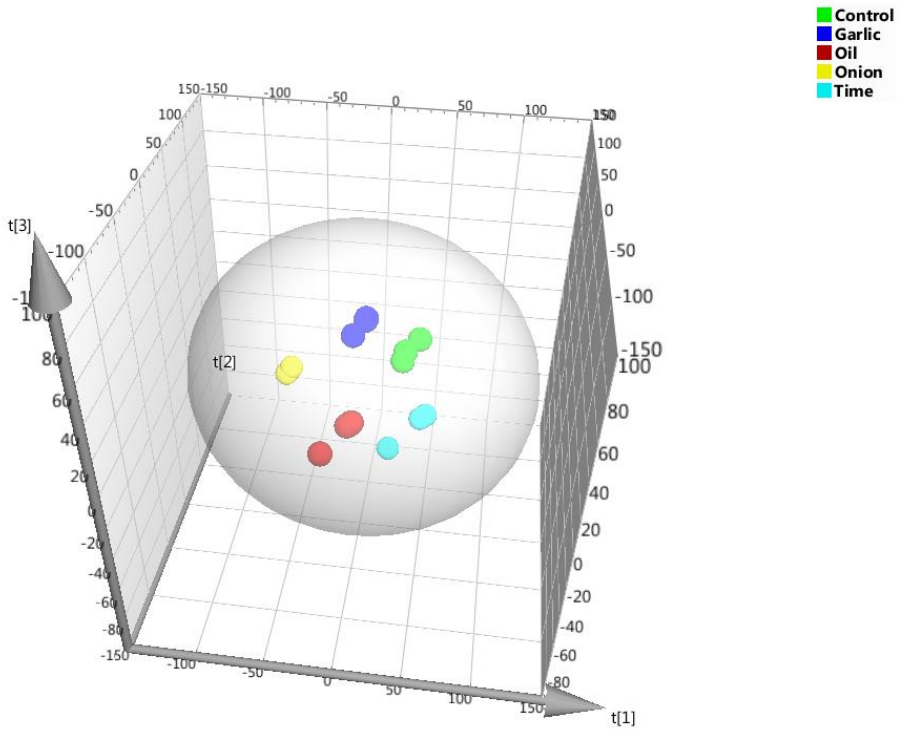

Figure 3. PCA analysis with three components of different tomato *sofrito* sauces with an increment on ingredients and cooking time.

Table 1. Annotated putative metabolites discriminating the effect of ingredient addition and cooking time on the chemical profile of tomato *sofrito* sauce.

Compound	Molecular Formula	rt	*m/z* detected	Annotation	MS^2 fragments	Error (ppm)	ID Level	*p*-value adjusted	Model
5-caffeoylquinic acid (chlorogenic acid)*	C16H18O9	8.54	353.0857	[M-H]	329.0130 (5) 191.0551 (100) 179.0340 (10) 135.0444 (5)	-2.516	I	0.0272	↓ E
4-caffeoylquinic acid (cryptochlorogenic acid)*	C16H18O9	8.98	353.0858	[M-H]	173.0447 (100) 179.0341 (60) 191.0552 (25) 135.0445 (10) 111.0446 (5)	-2.516	I	0.0145	↓ E
Naringenin-O-hexoside	C21H22O10	13.77	433.1116	[M-H]	271.0604 (100)	-5.103	II	0.0491	↓ E
Hesperetin*	C16H14O6	20.34	301.0699	[M-H]	151.0031(100) 177.0186(20) 221.1536(15) 283.1907(15)	-5.618	I	0.0145	↓ E
Oleuropein aglycone (3,4-DHPEA-EA)	C19H22O8	20.64	377.1221	[M-H]	345.0971(25) 333.1331(45) 307.0810(100) 275.0913(90)	-5.279	I	0.0498	↑ E
Ligstroside-aglycone	C19H22O7	21.14	361.1271	[M-H]	329.1009(20) 291.0861(85) 259.0967(100)	-6.22	II	0.0125	↑ E
N-gamma-Glutamyl-S-allylcysteine	C11H18O5N2S	4.63	289.0845	[M-H]	271.0744(45) 249.1088(5) 215.0663(20) 171.0772(10) 128.0347(100)	-6.907	III	0.0001	↑ G
N-gamma-L-Glutamyl-L-isoleucine / gamma-L-Glutamyl-L-leucine	C11H20N2O5	5.61	259.1283	[M-H]	241.1182(40) 223.1076(10) 128.0347(100)	-5.811	III	0.0001	↑ G
3/4-Hydroxybenzoic acid	C7H5O3	6.31	137.0235	[M-H]	93.0341(100)	-6.111	II	0.0182	↓ G
N-Caffeoyltyramine	C17H17NO2	13.83	298.1066	[M-H]	280.0964(100) 253.9980(20)	-0.874	II	0.0005	↑ G
Quercetin*	C15H10O7	19.29	301.0335	[M-H]	301.0337(100) 273.0389(15) 257.0440(10) 178.9976(90) 151.0028(70)	-0.899	I	0.0378	↑ G
Luteolin*	C15H10O6	20.40	285.0387	[M-H]	241.0492(100) 175.03395(90)	-6.705	I	0.0229	↑ G
9,12,13,TriHODE (Corchorifatty acid F)	C18H32O5	20.43	327.2158	[M-H]	327.2160(100) 309.2054(15) 191.1949(25) 199.1433(40) 171.1017(40)	-1.061	II	0.0000	↑ G
Isorhamnetin*	C16H12O7	20.53	315.0492	[M-H] [13C M-H]	315.0505(100) 300.0269(100) 151.0034(5)	-0.859	II	0.0337	↑ G
9,12,13-TriHOME (Pinellic acid)	C18H34O5	20.69	329.2315	[M-H]	329.2320(100) 311.2216(15) 293.2110(15) 229.1436(50) 211.1331(30) 171.1021(20)	-0.981	II	<0.0001	↑ G

16-A1-phytoprostane/ 16-B1-phytoprostane/ 16-J1-phytoprostane/ 9-A1-phytoprostane/ 9-B1-phytoprostane/ 9-J1-phytoprostane	C18H28O4	21.22	307.1895	[M-H]	315.1220(100) 163.0391(40) 145.0286 (20) 119.0496 (10)	-6.91	III	0.0009	↑ G
3-Oxo-2-(2-entenyl)cyclopentaneoctanoic acid	C18H30O3	21.71	293.2103	[M-H]	293.2107(100), 275.2000(10), 249.2212(20)	-0.941	III	0.0004	↑ G
Unknown / lipid	C18H32O4	22.01	311.2208	[M-H]	311.2210(100) 293.2104(90) 275.1999(25) 211.1327(40) 197.1171(35) 171.1017(35)	-0.666	III	0.0001	↑ G
S-Propyl thiosulfate	C3H8O3S2	4.78	154.9832	[M-H]	154.9836(20) 80.9648(100)	-0.118	II	<0.0001	↑ O
gamma-Glutamyl-leucine	C11H20N2O5	5.61	259.1283	[M-H]	241.1076(30) 223.1076(10) 128.0346(100)	-4.811	II	<0.0001	↑ O
Quercetin-dihexoside	C27H30O17	11.73	273.0863	[M-H]	625.1392(10) 463.0863(100) 301.0339(35)	-4.771	II	<0.0001	↑ O
Isorhamnetin-dihexoside	C28H32O17	13.03	639.1533	[M-H]	639.1541(100) 477.1018(35) 315.0495(40)	-5.23	II	0.0001	↑ O
Kaempferol hexoside	C21H20O11	16.76	447.0909	[M-H]	447.0912(35) 285.0390(100)	-5.11	II	<0.0001	↑ O
Isorhamnetin-O-hexoside	C22H22O12	17.30	*477.1014*	[M-H]	477.1021(80) 315.0496(100)	-4.484	II	-	↑ O
Quercetin*	C15H10O6	19.29	301.0335	[M-H]	301.0344 (100) 178.9980 (75) 151.0032 (60) 273.0395 (15) 193.0135 (5) 107.0135 (5)	-5.733	I	<0.0001	↑ O
Apiin	C26H28O14	19.52	563.1380	[M-H]	301.0870(100) 436.1738(20) 478.8416(20)	-4.401	II	<0.0001	↑ O
Kaempferol*	C15H10O6	20.40	285.0387	[M-H]	285.0389(100) 257.0442(10) 213.0548(10) 169.0653(10) 151.0027(10)	-0.534	I	<0.0001	↑ O
16-F1-phytoprostane / 9-F1-phytoprostane / 9,12,13,TriHODE (Corchorifatty acid F)	C18H32O5	20.43	327.2158	[M-H]	327.2164(100) 309.2058(10) 291.1952(20) 229.1435(30) 211.1330(20, 171.1019(25) 155.1071(5)	-0.921	II	<0.0001	↑ O
Isorhamnetin*	C16H12O7	20.53	315.0492	[M-H]	315.0497(100) 300.0262(90) 151.0033(10)	-0.549	I	<0.0001	↑ O
9,12,13-TriHOME (Pinellic acid)	C18H34O5	20.69	329.2315	[M-H]	329.2318(100) 311.2214(20) 293.2108(15) 229.1435(40) 211.1330(25) 171.1019(25)	-0.591	II	<0.0001	↑ O
16-A1-phytoprostane / 16-B1-phytoprostane /	C18H28O4	21.22	307.1895	[M-H]	259.1666(100) 279.1928(85)	-6.031	III	<0.0001	↑ O

16-D1-phytoprostane / 16-E1-phytoprostane / 16-H1-phytoprostane / 16-J1-phytoprostane / 9-A1-phytoprostane / 9-B1-phytoprostane / 9-D1-phytoprostane 9-D1-phytoprostane / 9-E1-phytoprostane / 9-H1-phytoprostane / 9-J1-phytoprostane					263.1638(40) 205.0866(20)				
9,12,13,TriHODE (Corchorifatty acid F)	C18H32O5	21.40	327.2158	[M-H]	327.2163 (100) 291.1952 (20) 229.1435 (30) 171.1019 (30)	-0.921	II	<0.0001	↑ O
LysoPE(0:0/18:3)/LysoPE(18:3/0:0)	C23H42NO7P	21.89	474.2601	[M-H]	274.2604(50) 277.2157(100) 214.0475(10) 196.0371(10)	-1.016	II	0.0023	↓ O
Lipid	C18H32O3	22.61	295.2260	[M-H]	295.2265(100) 277.2160(35) 221.2373(5) 195.1382(20) 171.1019(15)	-0.791	III	0.0031	↑ O
2/3/4-Methoxybenzoic acid	C8H8O3	5.48	157.0496	[M-H]	107.0496(100)	-6.67	II	0.0055	↑ T
(E)-S-1-Propenyl thiosulfate	C3H6O3S2	10.59	152.9676	[M-H]	152.9678(10) 123.0446(100) 109.0289(25) 89.0062(35) 80.9647(40) 79.9570(55)	-6.529	III	0.0043	↓ T
3,4-Dihydroxyphenylglycol	C8H10O4	2.26	169.0495	[M-H]	151.0394(100) 123.0447(45)	-6.874	III	0.0033	↑ T
N-trans-p-Coumaroyltyramine	C17H17NO3	17.96	282.1118	[M-H]	282.1121(100) 162.0552(10) 145.0287(20) 134.0604(10) 119.0496(15)	-6.05	III	0.0006	↑ T
Hesperetin*	C16H14O6	20.34	301.0699	[M-H]	151.0031(100) 286.0472(20)	-5.452	II	0.0031	↓ T
(R)-2-Feruloyl-1-(4-Hydroxyphenyl)-1,2-ethanediol	C18H18O6	19.535	329.1010	[M-H]	329.1017(15) 193.0498(100) 135.0445(60)	-5.899	III	<0.0001	↑ T
Luteolin 7-O-hexoside	C21H20O11	16.11	447.0910	[M-H]	285.0391(80) 284.0313 (70) 327.0496 (20) 255.0287 (5) 151.0028 (5)	-5.915	II	0.0118	↑ T

rt (retention time); E (control vs EVOO); O ((control vs onion), G ((control vs garlic); T ((control vs time).
*compounds confirmed by standards.

oxidation to reactive O-quinones, which interact with NH2 and SH groups of amino acids, that could produce these class of compounds[11,12]. Moreover, cooking process is responsible to promote disruption of cell walls that could contribute to increase the extractability of these compounds[4]. The association of a hydroxycinnamic acid with an amine has been related with an increase in the molecule antioxidant activity[13] and biological activities such as anti-inflammatory and anti-cancer properties[10,12,14]. N-caffeoyltyramine exhibit an inhibitory effect of NO production

in inflammatory cell models[10] and N-coumaroylserotonin and N-feuroylserotonin are able to ameliorate atherosclerosis and aortic wall distensibility in hypercholesteremic rabbits[15]. The presence of 2-feruloyl-1-(4-hydroxyphenyl)-1,2-ethanediol was annotated by FoodDB as originating from herbs and species. A theoretical explanation for this compound could be an esterification between 4-hydroxyphenylglycol (phenylpropanoidic from olive oil such as 3,4-dihydroxyphenylglycol) with a ferulic acid during the cooking process. Hydroxycinnamic acid ester derivates have characteristic like higher lipid solubility and antioxidant capacity compared to simple hydroxycinnamic acids which could improve their biological activities[12].

Another unexplored class of compounds are phytoprostanes, such as oxylipins like 9,12,12-TriHODE and 9,12,12-TriHOME that were identified as markers of onion and garlic increase on the recipe. Plant oxylipins are fatty acids derivate from a non-enzymatic lipid peroxidation resulted from the attack of reactive oxygens species to α-linoleic acid, being reported in several seed oils such as soybean, corn, canola and other products like olive oil and tree nuts[16,17] and recently was described in processed onions and garlics[18]. Gil-Izquierdo et al describe these compounds as a sensitive biomarker of the quality of nuts and vegetable oils. A recent publication has shown that hazelnuts oxylipins profile declines after a roasting process, indicating that processing may influence these compounds. Docking and structural-activity studies have been highlighted phytoprostanes by their similarity with human prostanes, which could show anti-inflammatory and antitumoral activities and being able to regulate biological functions[17]. However, the physiological effects of these compounds in in vivo studies and human body needs to be explored deeply.

A high content of EVOO in *sofrito* samples revealed markers that diminish compared to the control, such as chlorogenic acid, cryptochlorogenic acid, naringenin-O-hexoside and hesperetin, compounds that are present in tomato (Figure 1)[11,19,20]. Some markers also decreased like hesperetin with cooking time and 3/4-hydroxybenzoic acid with more garlic on the recipe (Figure 1). The EVOO markers showed an interesting effect on the pattern of tomato compounds. Naringenin-O-hexoside probably reduced by a hydrolysis of glucoside moiety, once the sugar blocks the hydroxyl function at de C-ring, showing higher stability toward oxidation compared to aglycones[21]. This hydrolysis could contribute to maintain tomato's aglycone content in the *sofrito* and help to stabilize the food system.

Chlorogenic and cryptochlorogenic acid reduced with the presence of more EVOO, however their hydrolysis products such as caffeic and quinic acid were not detected as a marker (Figure 1). This indicate that caffeic acid could react producing new compounds like hydroxycinnamic acid amides or caffeoylquinic acids could directly suffer oxidation. Recent publications have pointed out that small phenolics can produce carbonyl-phenol adducts, by trapping lipid-derived reactive carbonyl formed by lipid oxidation during cooking, decreasing oxidative stress to which *sofrito* technique could provoke [22,23]. The hydrolysis process that occurs during cooking could be an initial step on the production of new compounds.

Cooking process and ingredients also revealed phenolic compounds that increase its content as markers. The increment in the content of EVOO on the recipe increased oleuropein and ligstroside aglycone (Figure 1). There is a void in the literature about cooking process on the phenolic profile of extra virgin olive oil once normally they are focused on the raw product or on total polyphenols and more known EVOO compounds such as tyrosol and hydroxytyrosol [24]. Cerretani et al. (2009) studied the microwave cooking process on the phenolic profile of EVOO and observed a reduction of its content after 15 min of process. Oleuropein aglycone decreased during microwave process, however ligstroside aglycone increased or remain stable during the first 6 min of processing. Again, ester hydrolysis was pointed as the main cause for the stability of ligstroside aglycone for being the product of ligstroside derivates hydrolysis. The presence of more secoiridoids aglycone forms could be interesting to improve bioavailability of olive oil, since aglycones are more bioavailable than the glycoside forms[26].

A higher content of onion and garlic revealed phenolic markers in which quercetin and isorhamnetin, compounds already described in these two foods[18]. These ingredients increased the presence of flavonols and flavones content. The increase of only 2% more of garlic in *sofrito* formulation revealed as a marker quercetin, luteolin and isorhamnetin. Higher addition of onion in the recipe was able to increment quercetin, isorhamnertin, kaempferol and phenolics glucosilated forms such as apiin, isorhamnetin-hexoside, kaempferol-hexoside, isorhamnetin-dihexoside and quercetin-dihexoside.

Cooking time revealed as marker methoxybenzoic acid and 3,4 dihydroxyphenylglycol. These compounds increased with longer time of process probably by oxidation and hydrolysis reactions. 3,4 dihydroxyphenylglycol, found in olives products, increased its content during *alperujo* process by thermal treatment influence, being again hydrolysis reaction evidenced[27]. In the same way, cooking time may help to increase the content of 3,4 dihydroxyphenylglycol in the *sofrito* sauce (Figure 1). This compound showed antioxidant capacity higher than hydroxytyrosol, with anti-inflammatory properties and may also protect against platelet activation and aggregation[28,29], being a new target on olive oil research.

Onion and garlic increased the presence of amides and peptides such as gamma-glutamyl-S-allyl cysteine and gamma-glutamyl-S-allyl leucine for garlic and gamma-glutamyl-leucine for onion. S-propyl thiosulfate and S-1-propenyl thiosulfate were markers that increased by onion content and decrease with cooking time, respectively. These compounds have been already described in the literature as part of the composition of the Allium family and being related to several biological effects such as antioxidant, antimicrobial, antiasthmatic, antitumor and cancer, platelet-antiaggregating and reduction of hypercholesterolemia[18,30].

Discussion

The use of the omics approach to characterize a cooking technique showed an unrevealed side of cuisine. The unsupervised PCA analysis revealed that the amount of

ingredients of the recipe and cooking time are able to modify the chemical profile of tomato *sofrito* and also the grouping between control and time shows that *sofrito* culinary technique is able to almost maintain the phytochemical profile with a 30 min extra of cooking process follow the same recipe (Figure 1). An increment on the amount of onion and garlic in the recipe was able to increase the content of bioactive compounds like sulfur compounds and flavonols since these ingredients are an important dietary source.

An untargeted approach proved to be a reliable tool to reveal the presence of new compounds. Hydroxycinnamic acid amides, hydroxycinnamic acid derivates and phytoprostanes, which could be extracted or formed during cooking, are a class of compounds not exploited and apparently may exhibit relevant biological activities. Oxylipins such as 9,12,12-TriHODE and 9,12,12-TriHOME started now to be identified in foods and their effects on body are unknown. Database for foodomic studies are still recent and identification of new substances must be prioritized. Moreover, favoring of certain substances classes is evidenced by the greater number of phenolics markers compared to other classes, in which literature has more information. Food composition tables, that are necessary tools for epidemiological and nutritional studies, in general show their data in raw products. In addition, when there is data of cooked food, the information is focused on target compounds previously identified in raw food, forgetting how cook could modify substances. This aspect is important to be considered, since large parts of the vegetables consumed daily are processed and cooked or relevant information may be lost[6]. Epidemiological studies indicate that following a Mediterranean diet has beneficial effects on health that could be explained in part by the cuisine methodology used.

Methods

Chemicals and reagents

Acetonitrile, methanol and formic acid were purchased from AppliChem, Panreac Quimica SA (Barcelona, Spain). Chlorogenic acid, cryptochlorogenic acid, hesperetin, quercetin, luteolin, naringenin, epicatechin and isorhamnetin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Kaempferol was acquired from Extrasynthese (Genay, France), 4-hydroxyhipuric acid from Bachem (Bubendorf, Switzerland) and 3-hydroxybenzoic acid from Fluka (St. Louis, MO, USA). Ultrapure water was obtained using a Milli-Q purification system (Millipore, Bedford, MA, USA).

Tomato *sofrito* sauce cooking process

Tomatoes (*Licopersicon esculentum* Mill, c. v. Pera) of equal diameter from the same batch were bought from Grupo Almeria (La Cañada, Almeria, Spain). Onions and garlic were bought from Casa Ametller (Barcelona, Spain). Manuel Heredia Halcón (Cortijo De Suerte Alta, Albedin-Baena-Cordoba) supplied the extra virgin olive oil (EVOO).

Tomato *sofrito* sauces were prepared based on a traditional Mediterranean recipe in an industrial kitchen at Torribera Campus, University of Barcelona (Santa Coloma de Gramenet, Spain)[31]. In order to understand how a recipe could influence, the factors EVOO, onion, garlic content and cooking time were evaluated. Tomato *sofrito* formulations, differentiated by a fortification of increasing twice the ingredients: extra virgin olive oil (EVOO) (10%), onion (40%), garlic (4%) and the cooking time (60 min) were prepared and compared with a control with the following composition (5% EVOO, 20% onion, 2% garlic and 30 min). These samples were processed in triplicated and randomized for better reproducibility, resulting in a total of 15 samples[31].

Sample preparation and extraction

Frozen dried *sofrito* samples (0.5g) were homogenized with 5 mL of acetonitrile, vortexed for 1 min and sonicated for 10 min on ice and then centrifuged using 4000 rpm, for 20 min at 4 ºC. The supernatant was separated, and another extraction was performed using 5 mL of methanol under the same conditions. The supernatants were combined, evaporated under nitrogen flow until dry and suspended with 1 mL of acetonitrile. The residual pellet was extracted with 3 mL of ultrapure water (MilliQ) under the same conditions and combined with the acetonitrile extract. The final extract was suspended up to 5 mL in a volumetric flask with ultrapure water, filtered using a 0.22 µm into a 2 mL amber vial for HPLC analysis and stored at -80 ºC.

Liquid chromatography high-resolution mass spectometry

Liquid chromatography analysis was performed using a Accela chromatography (Thermo Scientific) equipped with a quaternary pump, a photodiode array detector and a thermostated autosamples. Chromatography separation was accomplished with an Atlantis T3 column 2.1 x 100mm, 3µm (Waters, Milford, MA, USA). *Sofrito* metabolites elution was carried out with water 0.1% formic acid (A) and acetonitrile 0.1% formic acid (B) using the follow conditions: 0 min, 2% B; 2 min, 2% B; 5 min, 8% B; 14 min, 20% B; 18 min, 30% B; 22 min, 100% B; 24 min, 100% B, 25 min, 2% B; 30 min, 2% B. Flow rate was 350 µL/min and the injection volume was 10 µL[32].

An LQT Orbitrap Velos mass spectometrer (Thermo Scientific, Hemel Hempstead, UK) equipped with an ESI source working in negative mode was applied for mass measurements. The metabolomics approach was performed only in negative ion mode in order to focus on phenolic compounds changes during *sofrito* cooking process. Mass spectra acquired conditions was performed in profile mode with a resolution of 30,000 at m/z 400. Operation conditions were: source voltage, 4kV, sheath gas, 20 (arbitrary units); auxiliary gas, 10 (arbitrary units); sweep gas, 2 (arbitrary units); capillary temperature, 275 ºC. Samples were analyzed in full scan mode with a resolution of 30,000 at m/z 400 and data-dependent scan MS/MS events at a resolving power of 15,000. The most intense ion detected during full scan MS triggered the data-depending scan. Ions that were not intense enough for automatic data-dependent scan were analyzed in MS2

experiments with a resolution also set at 15,000 at m/z 400, with an isolation width of 100 amu. The precursor ions were fragmented by a collision induced dissociation C-trap (CID) with a normalized collision energy of 35V and an activation time of 10 ms. The mass range in FTMS mode was from m/z 100 to 1000. Data analysis was achieved using XCalivur software v2.0.7 (Thermo Scientific, Hemel Hempstead, UK).

To ensure the quality of the analysis, an external quality controls were prepared by pooling 5 phenolic compounds in mobile phase: 4-hydroxyhipuric acid, epicatechin, quercetin, 3-hydroxybenzoic acid and naringenin. In addition, an equitable mixture of all samples was prepared as a quality control of the data. Quality controls were randomly injected before, during and once the sequence was finished, to control retention time shifts and mass accuracy. Intrabatch retention time shifts were lower than 0.04 min, peak area variation was lower than 12%, and mass accuracy was lower than 4 ppm. Additionally, principal component analysis was plotted with quality controls and samples to dismiss clustering by injection order and differences between quality controls.

Data processing and statistical analysis

The LC-MS raw files were converted to mzXML with the MSConvert utilizity included in ProteoWizard. Then, the mzXML files were processes with XCMS-R package that allows for obtaining data processing (feature detection, peak alignment and retention time correction) and the following parameters were set: mass tolerance 2.5 ppm, peak width range 10-60 s, three minimum scans, minimum intensity of 5000 arbitrary units. Peak detection was performed using “centWave” method, retention time corrected by “obiwarp” with a step size of 1 m/z and peak grouping using width of overlapping m/z slices of 0.015, a minimum fraction of sample per grouping of 0.5 and a bandwitdth of 5. The processed data obtained by XCMS in negative ion mode was filtered by samples representativeness using an 80% rule, that consists of retain in those features that are consistently found in 80% of the samples (n=2) or at least in one experimental group. A second filtering by intensity threshold was used at 30,000 arbitrary units as the minimum suitable intensity. A third filtering was applied using a coefficient of variation (CV%), accepting features that presented unleast a 20% of variation compared to a mix of all samples.

Afterward, univariate statistical analysis of the data was performed in other to evidence features with statistically different intensities between groups. Wilcox-Mann Whitney test analysis was performed with R to compare the “control” with plus EVOO, plus onion, plus garlic and plus time. After that, p-values for all features was corrected for multiple testing by Benjamini and Hochberg False Rate Discovery (FDR) with a significance level of 0.05.

Features discriminating between plus EVOO, plus onion, plus garlic and plus time versus control that presented an adjusted p-valeu <0.05 and a fold change (FC) > 2 were putatively identified by exact mass matching using PhytoHub, FooDB and METLIN with a accuracy of <10 ppm. Metabolites identification was performed by the detected pseudo-molecular ion that must

differed from the exact mass of proposed molecular formula in a maximum value of 5 ppm (mass accuracy ≤5 ppm) and isotopic pattern was checked with theoretical isotope profile. Identification was confirmed by MS/MS experiments and comparing the spectra with databases and literature. Isotopes and adducts were annotated by CAMERA R-package. Metabolites were classified according to metabolomics guidelines using four levels of identification: Level I, confidently identified compounds comparing the proposed structure with reference standard; Level II, putatively annotated compound comparing physicochemical proprieties and/or spectral similarity with spectral libraries; Level III, putatively annotated compound class comparing physicochemical proprieties and/or spectral similarity to known compounds of a chemical class; Level IV, unknown compounds.

The data was exported to SIMCA 13.0.3 software (Umetrics) to perform multivariate analysis. An unsupervised PCA analysis was used to see metabolomics differences between groups and the data was scaled by Pareto. The quality of the model was evaluated using the goodness-of-fit (R2X) and the predictive ability parameter (Q2).

Data availability

Full identification of the isotopes and adducts are available in Supplementary Table 1. All relevant data are available from the corresponding author upon reasonable request.

References

1. Pellegrini, N. & Fogliano, V. Cooking, industrial processing and caloric density of foods. Curr. Opin. Food Sci. 14, 98–102 (2017).

2. Carmody, R. N., Weintraub, G. S. & Wrangham, R. W. Energetic consequences of thermal and nonthermal food processing. Proc. Natl. Acad. Sci. 108, 19199–19203 (2011).

3. Hoffman, R. & Gerber, M. Food Processing and the Mediterranean Diet. Nutrients 7, 7925–7964 (2015).

4. Murador, D., Braqa, A. R., Cunha, D. Da & Rosso, V. de. Alterations in phenolic compound levels and antioxidant activity in response to cooking technique effects: A meta-analytic investigation. Crit. Rev. Food Sci. Nutr. 8398, 00–00 (2016).

5. Murador, D. C., da Cunha, D. T. & de Rosso, V. V. Effects of cooking techniques on vegetable pigments: A meta-analytic approach to carotenoid and anthocyanin levels. Food Res. Int. 65, 177–183 (2014).

6. Nicoli, M. ., Anese, M. & Parpinel, M. Influence of processing on the antioxidant properties of fruit and vegetables. Trends Food Sci. Technol. 10, 94–100 (1999).

7. Dunn, W. B. et al. Mass appeal : metabolite identification in mass spectrometry-focused untargeted metabolomics. 44–66 (2013). doi:10.1007/s11306-012-0434-4

8. Cifuentes, A. Food analysis and Foodomics. J. Chromatogr. A 1216, 7109 (2009).

9. Ly, D. et al. HPLC Analysis of Serotonin, Tryptamine, Tyramine, and the Hydroxycinnamic Acid Amides of Serotonin and Tyramine in Food Vegetables. J. Med. Food 11, 385–389 (2008).

10. Wang, S., Suh, J. H., Zheng, X., Wang, Y. & Ho, C.-T. Identification and Quantification of Potential Anti-inflammatory Hydroxycinnamic Acid Amides from Wolfberry. J. Agric. Food Chem. 65, 364–372 (2017).

11. Vallverdú-Queralt, A., Regueiro, J., Rinaldi de Alvarenga, J. F., Torrado, X. & Lamuela-Raventos, R. M. Home Cooking and Phenolics: Effect of Thermal Treatment and Addition of Extra Virgin Olive Oil on the Phenolic Profile of Tomato Sauces. J. Agric. Food Chem. 62, 3314–3320 (2014).

12. Razzaghi-Asl, N., Garrido, J., Khazraei, H., Borges, F. & Firuzi, O. Antioxidant properties of hydroxycinnamic acids: a review of structure- activity relationships. Curr. Med. Chem. 20, 4436–50 (2013).

13. Marinova, E., Georgiev, L., Totseva, I., Seizova, K. & Milkova, T. Antioxidant activity and mechanism of action of some synthesised phenolic acid amides of aromatic amines. Czech J. Food Sci. 31, 5–13 (2013).

14. Wang, S. et al. Use of UHPLC-TripleQ with synthetic standards to profile anti-inflammatory hydroxycinnamic acid amides in root barks and leaves of Lycium barbarum. J. Food Drug Anal. 26, 572–582 (2018).

15. Katsuda, S. et al. Safflower seed polyphenols (N-(p-coumaroyl)serotonin and N-feruloylserotonin) ameliorate atherosclerosis and distensibility of the aortic wall in Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits. Hypertens. Res. 32, 944–949 (2009).

16. Napolitano, A., Cerulli, A., Pizza, C. & Piacente, S. Multi-class polar lipid profiling in fresh and roasted hazelnut (Corylus avellana cultivar “Tonda di Giffoni”) by LC-ESI/LTQOrbitrap/MS/MS n. Food Chem. 269, 125–135 (2018).

17. Ferreres, F., Gil-Izquierdo, Á., Medina, S., Domínguez-Perles, R. & Durand, T. Structural/Functional Matches and Divergences of Phytoprostanes and Phytofurans with Bioactive Human Oxylipins. Antioxidants 7, 165 (2018).

18. Farag, M. et al. Phytochemical Profiles and Antimicrobial Activities of Allium cepa Red cv. and A. sativum Subjected to Different Drying Methods: A Comparative MS-Based Metabolomics. Molecules 22, 761 (2017).

19. Vallverdú-Queralt, A. et al. Phenolic Profile and Hydrophilic Antioxidant Capacity as Chemotaxonomic Markers of Tomato Varieties. J. Agric. Food Chem. 59, 3994–4001 (2011).

20. Gómez-Romero, M., Segura-Carretero, A. & Fernández-Gutiérrez, A. Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. Phytochemistry 71, 1848–1864 (2010).

21. Makris, D. P. & Rossiter, J. T. Heat-Induced, Metal-Catalyzed Oxidative Degradation of Quercetin and Rutin (Quercetin 3- O -Rhamnosylglucoside) in Aqueous Model Systems. J. Agric. Food Chem. 48, 3830–3838 (2000).

22. Hidalgo, F. J., Delgado, R. M. & Zamora, R. Protective effect of phenolic compounds on carbonyl-amine reactions produced by lipid-derived reactive carbonyls. Food Chem. 229, 388–395 (2017).

23. Hidalgo, F. J. & Zamora, R. Characterization of Carbonyl–Phenol Adducts Produced by Food Phenolic Trapping of 4-Hydroxy-2-hexenal and 4-Hydroxy-2-nonenal. J. Agric. Food Chem. 67, 2043–2051 (2019).

24. Santos, C. S. P., Cruz, R., Cunha, S. C. & Casal, S. Effect of cooking on olive oil quality attributes. Food Res. Int. 54, 2016–2024 (2013).

25. Cerretani, L., Bendini, A., Rodriguez-Estrada, M. T., Vittadini, E. & Chiavaro, E. Microwave heating of different commercial categories of olive oil: Part I. Effect on chemical oxidative stability indices and phenolic compounds. Food Chem. 115, 1381–1388 (2009).

26. Suárez, M. et al. Bioavailability of phenols from a phenol-enriched olive oil. Br. J. Nutr. 106, 1691–1701 (2011).

27. Lama-Muñoz, A., Rodríguez-Gutiérrez, G., Rubio-Senent, F., Palacios-Díaz, R. & Fernández-Bolaños, J. A study of the precursors of the natural antioxidant phenol 3,4-dihydroxyphenylglycol in olive oil waste. Food Chem. 140, 154–160 (2013).

28. de Roos, B. et al. Anti-platelet effects of olive oil extract: in vitro functional and proteomic studies. Eur. J. Nutr. 50, 553–562 (2011).

29. Rodríguez, G., Rodríguez, R., Fernández-Bolaños, J., Guillén, R. & Jiménez, A. Antioxidant activity of effluents during the purification of hydroxytyrosol and 3,4-dihydroxyphenyl glycol from olive oil waste. Eur. Food Res. Technol. 224, 733–741 (2007).

30. Lanzotti, V. The analysis of onion and garlic. J. Chromatogr. A 1112, 3–22 (2006).

31. Rinaldi de Alvarenga, J. F. et al. Home cooking and ingredient synergism improve lycopene isomer production in Sofrito. Food Res. Int. 99, 851–861 (2017).

32. Heras, R. M. Las, Quifer-Rada, P., Andrés, A. & Lamuela-Raventós, R. Polyphenolic profile of persimmon leaves by high resolution mass spectrometry (LC-ESI-LTQ-Orbitrap-MS). J. Funct. Foods 23, 370–377 (2016).

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Competing interest

RLR reports having received lecture fees from Adventia and Cerveceros de España. The other authors declare that they have no competing interest

Author contributions

Conceptualization: JFRA, PQR, SHB, MI, XTP and RLR; methodology: JFRA, PQR, MI, XTP and RLR, investigation: JFRS, PQP, SHB; wrinting-original draft: JFRA; wrinting-review and editing: JFRA, PQR and RLR; funding acquisition: RLR; supervision: PQP and RLR.

Supplemental material

Supplemental Material I. Annotated putative metabolites, isotopes and adducts discriminating the effect of ingredient addition and cooking time on the chemical profile of tomato *sofrito* sauce.

Compound	Molecular Formula	rt	*m/z* detected	Annotation	MS^2 fragments	Error (ppm)	ID Level	Model
5-caffeoylquinic acid (chlorogenic acid)*	C16H18O9	8.54	**353.0857** 354.0890 707.1789	[M-H] [^{13}C M-H] [M-H]+354.0932[M]	329.0130 (5) 191.0551 (100) 179.0340 (10) 135.0444 (5)	-2.516	I	↓ E
4-caffeoylquinic acid (cryptochlorogenic acid)*	C16H18O9	8.98	**353.0858** 354.0891 707.1793 708.1826	[M-H] [^{13}C M-H] [M-H]+354.0935[M] [^{13}C M-H]+354.0935[M]	173.0447 (100) 179.0341 (60) 191.0552 (25) 135.0445 (10) 111.0446 (5)	-2.516	I	↓ E
Naringenin-*O*-hexoside	C21H22O10	13.77	**433.1116**	[M-H]	271.0604 (100)	-5.103	II	↓ E
Hesperidin*	C16H14O6	20.34	**301.0699** 302.0734	[M-H] [^{13}C M-H]	151.0031(100) 177.0186(20) 221.1536(15) 283.1907(15)	-5.618	I	↓ E
Oleuropein aglycone (3,4-DHPEA-EA)	C19H22O8	20.64	**377.1221** 399.1040 345.0962 755.2513	[M-H] [M-H]+21,9819 [Na] [M-H]-32,0259[CH3OH] [M-H]+378,1292[M]	345.0971(25) 333.1331(45) 307.0810(100) 275.0913(90)	-5.279	I	↑ E
Ligstroside-aglycone	C19H22O7	21.14	**361.1271** 362.1305 407.1325 291.0856 292.0889 259.0960	[M-H] [^{13}C M-H] [M-H]+46,0054[HCOOH] [M-H]-70,0416[C4H6O] [^{13}C M-H]-70,0416[C4H6O] [M-H]-70,0416[C4H6O]-31,9896[O2]	329.1009(20) 291.0861(85) 259.0967(100)	-6.22	II	↑ E
N-gamma-Glutamyl-*S*-allylcysteine	C11H18O5N2S	4.63	**289.0845** 579.1775 601.1593 602.1626 623.1412 624.1443	[M-H] [M-H]+M [M-H]+M+21.9818[Na] [^{13}C M-H]+M+21.9818[Na] [M-H]+M+21.9818[Na]+21.9818[Na] [^{13}C M-H]+M+21.9818[Na]+21.9818[Na]	271.0744(45) 249.1088(5) 215.0663(20) 171.0772(10) 128.0347(100)	-6.907	III	↑ G
N-gamma-L-Glutamyl-L-isoleucine / gamma-L-Glutamyl-L-leucine	C11H20N2O5	5.61	**259.1283** 519.2647 541.2467 563.2285 585.2102	[M-H] [M-H]+M [M-H]+M+21.9818[Na] [M-H]+M+21.9818[Na]+21.9818[Na] [M-H]+M+21.9818[Na]+21.9818[Na] +21.9818[Na]	241.1182(40) 223.1076(10) 128.0347(100)	-5.811	III	↑ G
3/4-Hydroxybenzoic acid	C7H5O3	6.31	**137.0235**	[M-H]	93.0341(100)	-6.111	II	↓ G
N-Caffeoyltyramine	C17H17NO2	13.83	**298.1066**	[M-H]	280.0964(100) 253.9980(20)	-0.874	II	↑ G

Quercetin*	C15H10O7	19.29	**301.0335** 358.9920 369.0207 370.0241 391.0027 392.0061 437.0079	[M-H] [M-H]+57.9585[NaCl] [M-H]+67.987[HCOONa] [^{13}C M-H]+67.987[HCOONa] [M-H]+67.987[HCOONa]+21.982[Na] [^{13}C M-H]+67.987[HCOONa]+21.982[Na] [M-H]+67.987[HCOONa]+21.982[Na] +46.0054[HCOOH]	301.0337(100) 273.0389(15) 257.0440(10) 178.9976(90) 151.0028(70)	- 0.899	I	↑ G
Luteolin*	C15H10O6	20.40	**285.0387**	[M-H]	241.0492(100) 175.03395(90)	- 6.705	I	↑ G
Corchorifatty acid F [(10E,15Z)-9,12,13-Trihydroxy-10,15-octadecadienoic]	C18H32O5	20.43	**327.2158** 328.2190 395.2030 463.1902	[M-H] [^{13}C M-H] [M-H]+67.987[HCOONa] [M-H]+67.987[HCOONa]+67.987[HCOONa]	327.2160(100) 309.2054(15) 191.1949(25) 199.1433(40) 171.1017(40)	- 1.061	II	↑ G
Isorhamnetin	C16H12O7	20.53	315.0492 316.0525	[M-H] [13C M-H]	315.0505(100) 300.0269(100) 151.0034(5)	- 0.859	II	↑ G
Pinellic acid (9,12,13-TriHOME / 9S,12S,13S-trihydroxy-10E-octadecenoic acid)	C18H34O5	20.69	**329.2315** 330.2348 331.2376 332.2391 351.2133 352.2169 387.1899 389.1870 397.2187 398.2221 399.2248 465.2059 466.2093 601.1807 533.1933 534.1968 669.1670 682.4561 749.4399 750.4429 817.4270	[M-H] [^{13}C M-H] [^{13}C ^{13}C M-H] [^{13}C ^{13}C ^{13}C M-H] [M-H]+21.9818[Na] [^{13}C M-H]+21.9818[Na] [M-H]+57.9584[NaCl] [^{13}C M-H]+57.9584[NaCl] [M-H]+67.9872[HCOONa] [^{13}C M-H] +67.9872[HCOONa] [^{13}C ^{13}C M-H]+67.9872[HCOONa] [M-H]+67.9872[HCOONa]+67.9872[HCOONa] [^{13}C M-H+67.9872[HCOONa]+67.9872[HCOONa] [M-H]+67.9872[HCOONa]+67.9872[HCOONa] +67.9872[HCOONa] [M-H]+67.9872[HCOONa]+67.9872[HCOONa] +67.9872[HCOONa] [^{13}C M-H+67.9872[HCOONa]+67.9872[HCOONa] +67.9872[HCOONa] [M-H]+67.9872[HCOONa]+67.9872[HCOONa] +67.9872[HCOONa]+67.9872[HCOONa] [^{13}C M-H]+330.2348 [M] [M-H]+330.2348 [M]+67.987[HCOONa] [^{13}C M-H]+330.2348 [M]+67.987[HCOONa] [M-H]+330.2348 [M]+67.987[HCOONa]	329.2320(100) 311.2216(15) 293.2110(15) 229.1436(50) 211.1331(30) 171.1021(20)	- 0.981	II	↑ G

			818.4300	+67.987[HCOONa] [^{13}C M-H]+330.2348 [M]+67.987[HCOONa]				
			885.4144	+67.987[HCOONa] [M-H]+330.2348[M]+67.987[HCOONa] +67.987[HCOONa]+67.987[HCOONa]				
16-A1-phytoprostane/ 16-B1-phytoprostane/ 16-J1-phytoprostane/ 9-A1-phytoprostane/ 9-B1-phytoprostane/ 9-J1-phytoprostane	C18H28O4	21.22	**307.1895**	[M-H]	315.1220(100) 163.0391(40) 145.0286 (20) 119.0496 (10)	-6.91	III	↑ G
3-Oxo-2-(2- entenyl)cyclopentaneoctanoic acid	C18H30O3	21.71	**293.2103** 315.1921	[M-H] [M-H]+21.9818[Na]	293.2107(100), 275.2000(10), 249.2212(20)	- 0.941	III	↑ G
Unknown / lipid	C18H32O4	22.01	**311.2208** 312.2241 379.2080 380.2113 447.1952 583.1700	[M-H] [^{13}C M-H] [M-H]+67.9871[HCOONa] [^{13}C M-H]+67.9871[HCOONa] [M-H]+67.9871[HCOONa]+67.9871[HCOONa] [M-H]+67.9871[HCOONa]+67.9871[HCOONa] +67.9871[HCOONa]+67.9871[HCOONa]	311.2210(100) 293.2104(90) 275.1999(25) 211.1327(40) 197.1171(35) 171.1017(35)	- 0.666	III	↑ G
S-Propyl thiosulfate	C3H8O3S2	4.78	**154.9832**	[M-H]	154.9836(20) 80.9648(100)	- 0.118	II	↑ O
gamma-Glutamyl-leucine	C11H20N2O5	5.61	**259.1283** 260.1315 519.2647 541.2467 563.2285 585.2102 241.1178	[M-H] [^{13}C M-H] [M-H]+M [M-H+]+M+21.9818[Na] [M-H]+M+21.9818[Na]+21.9818[Na] [M-H]+M+21.9818[Na]+21.9817[Na]+21.9818[Na] [M-H]-18.0105[H2O]	241.1076(30) 223.1076(10) 128.0346(100)	- 4.811	II	↑ O
Quercetin-dihexoside	C27H30O17	11.73	**273.0863** 274.0894 229.0968	[M-H] [^{13}C M-H] [M-H]-43.9895[CO2]	625.1392(10) 463.0863(100) 301.0339(35)	- 4.771	II	↑ O
Isorhamnetin-dihexoside	C28H32O17	13.03	**639.1533** 640.1568 641.1594	[M-H] [^{13}C M-H] [^{13}C ^{13}C M-H]	639.1541(100) 477.1018(35) 315.0495(40)	-5.23	II	↑ O
Kaempferol hexoside	C21H20O11	16.76	**447.0909** 448.0942 449.0964 515.0783	[M-H] [^{13}C M-H] [^{13}C ^{13}C M-H] [M-H]+67.9874[HCOONa]	447.0912(35) 285.0390(100)	-5.11	II	↑ O
Isorhamnetin-O-hexoside	C22H22O12	17.30	***477.1014*** 955.2100	[M-H] [M-H]+M	477.1021(80) 315.0496(100)	- 4.484	II	↑ O

			956.2136	[^{13}C M+H]+M					
			957.2156	[^{13}C ^{13}C M+H]+M					
			958.2189	[^{13}C ^{13}C M+H]+M					
			977.1917	[M+H]+M+21.9817[Na]					
			301.0335	[M+H]					
			302.0368	[^{13}C M+H]					
			303.0382	[^{13}C ^{13}C M+H]					
			304.0409	[^{13}C ^{13}C M+H]					
			323.0154	[M+H]+21.9819[Na]					
			358.9920	[M+H]+57.9585[NaCl]					
			369.0207	[M+H]+67.9871[HCOONa]					
			370.0241	[^{13}C M+H]+67.9871[HCOONa]					
			391.0027	[M+H]+67.9871[HCOONa]+21.9820[Na]	301.0344 (100)				
			392.0061	[^{13}C M+H]+67.9871[HCOONa]+21.9820[Na]	178.9980 (75)				
	Quercetin*	C15H10O6	19.29	415.0260	[M+H]+67.9871[HCOONa]+46.0053[HCOOH]	151.0032 (60)	5.733	I	↑ O
				437.0079	[M+H]+67.9871[HCOONa]+46.0053[HCOOH] +21.9819[Na]	273.0395 (15)			
				458.9898	[M+H]+67.9871[HCOONa]+46.0053[HCOOH]+21.9819[Na]+21.9819[Na]	193.0135 (5)			
				504.9950	[M+H]+67.9871[HCOONa]+46.0053[HCOOH] +67.987[HCOONa]	107.0135 (5)			
				603.0753	[M+H]+M				
				604.0788	[^{13}C M+H]+M				
				605.0813	[^{13}C ^{13}C M+H]+M				
				625.0572	[M+H]+M+21.98[Na]				
				647.0389	[M+H]+M+21.98[Na]+21.98[Na]				
				648.0425	[^{13}C M+H]+M+21.98[Na]+21.98[Na]				
	Apiin	C26H28O14	19.52	**563.1380**	[M-H]	301.0870(100)	-	II	↑ O
				564.1415	[^{13}C M-H]	436.1738(20)	4.401		
						478.8416(20)			
						285.0389(100)			
				285.0387	[M-H]	257.0442(10)	-	I	↑ O
	Kaempferol*	C15H10O6	20.40	286.0420	[^{13}C M-H]	213.0548(10)	0.534		
				353.0258	[M+H]+67.9871[HCOONa]	169.0653(10)			
						151.0027(10)			
						327.2164(100)			
				327.2158	[M-H]	309.2058(10)			
	9,12,13,TriHODE (corchorifatty acid F)	C18H32O5	20.43	328.2190	[^{13}C M-H]	291.1952(20)	-	II	↑ O
				349.1976	[M+H]+21.9818[Na]	229.1435(30)	0.921		
				395.2030	[M+H]+67.9872[HCOONa]	211.1330(20,			
				396.2063	[^{13}C M+H]+67.9872[HCOONa]	171.1019(25)			
						155.1071(5)			
				315.0492	[M-H]	315.0497(100)	-	I	↑ O
	Isorhamnetin*	C16H12O7	20.53	316.0525	[^{13}C M-H]	300.0262(90)	0.549		
				383.0364	[M+H]+67.9872[HCOONa]	151.0033(10)			
				384.0398	[^{13}C M-H]+67.9872[HCOONa]				

			451.0235	[M-H]+67.9872[HCOONa]+67.9871[HCOONa]						
			631.1065	[M-H]+M						
			632.1099	[^{13}C M-H]+M						
			329.2315	[M-H]						
			330.2348	[^{13}C M-H]						
			331.2376	[^{13}C ^{13}C M-H]						
			332.2391	[^{13}C ^{13}C ^{13}C M-H]						
			351.2133	[M-H]+21.9817[Na]						
			352.2169	[^{13}C M-H]+21.9817[Na]						
			387.1899	[M-H]+57.9584[NaCl]						
			397.2187	[M-H]+67.9872[HCOONa]						
			398.2221	[^{13}C M-H]+67.9872[HCOONa]						
			399.2248	[^{13}C ^{13}C M-H]+67.9872[HCOONa]						
			439.2294	[M-H]+67.9872[HCOONa]+42.0108[COCH2]				329.2318(100)		
			465.2059	[M-H]+67.9872[HCOONa]+67.9872[HCOONa]				311.2214(20)		
9,12,13-TriHOME (Pinellic acid)	C18H3405	20.69	466.2093	[^{13}C M-H]+67.9872[HCOONa]+67.9872[HCOONa]				293.2108(15)	-	
			533.1933	[M-H]+67.9872[HCOONa]+67.9872[HCOONa] +67.9874[HCOONa]				229.1435(40) 211.1330(25) 171.1019(25)	0.591	II ↑O
			534.1968	[^{13}C M-H]+67.9872[HCOONa]+67.9872[HCOONa] +67.9874[HCOONa]						
			659.4710	[M-H]+M						
			660.4745	[^{13}C M-H]+M						
			681.4527	[M-H]+M+21.9816[Na]						
			682.4561	[^{13}C M-H]+M+21.9816[Na]						
			749.4399	[M-H]+M+21.9816[Na]+67.9871[HCOONa]						
			750.4429	[^{13}C M-H]+M+21.9816[Na]+67.9871[HCOONa]						
			885.4144	[M-H]+M+21.9816[Na]+67.9871[HCOONa] +67.9873[HCOONa]+67.9878[HCOONa]						
16-A1-phytoprostane / 16-B1-phytoprostane / 16-D1-phytoprostane / 16-E1-phytoprostane / 16-H1-phytoprostane / 16-J1-phytoprostane / 9-A1-phytoprostane / 9-B1-phytoprostane / 9-D1-phytoprostane 9-D1-phytoprostane / 9-E1-phytoprostane / 9-H1-phytoprostane / 9-J1-phytoprostane	C18H2804	21.22	**307.1895**	[M-H]				259.1666(100) 279.1928(85) 263.1638(40) 205.0866(20)	- 6.031	III ↑O
			327.2163 (100)							
9,12,13,TriHODE (corchorifatty acid F)	C18H3205	21.40	**327.2158**	[M-H]				291.1952 (20) 229.1435 (30) 171.1019 (30)	- 0.921	II ↑O

			474.2601	[M-H]	274.2604(50)					
			475.2636	[^{13}C M-H]	277.2157(100)	-	II	↓ O		
LysoPE(0:0/18:3)/LysoPE(18:3/0:0)	C23H42NO7P	21.89	542.2476	[M-H]+67.9875[HCOONa]	214.0475(10)	1.016				
			543.2511	[^{13}C M-H]+67.9875[HCOONa]	196.0371(10)					
			610.2344	[M-H]+67.9875[HCOONa]+67.9875[HCOONa]						
			295.2260	[M-H]						
			296.2294	[^{13}C M+H]+13C						
			363.2132	[M+H]+67.9871[HCOONa]						
			364.2164	[^{13}C M+H]+67.9871[HCOONa]+13C	295.2265(100)					
			431.2003	[M+H]+67.9871[HCOONa]+67.9872[HCOONa]	277.2160(35)	-	III	↑ O		
Lipid	C18H32O3	22.61	499.1876	[M+H]+67.9871[HCOONa]+67.9872[HCOONa] +67.9874[HCOONa]	221.2373(5)	0.791				
			567.1747	[M+H]+67.9871[HCOONa]+67.9872[HCOONa] +67.9874[HCOONa]+67.987[HCOONa]	195.1382(20) 171.1019(15)					
			635.1616	[M+H]+67.9871[HCOONa]+67.9872[HCOONa] +67.9874[HCOONa]+67.987[HCOONa]+67.9869[HCOONa]						
			277.2154	[M+H]-18.0106[H2O]						
2/3/4-Methoxybenzoic acid	C8H8O3	5.48	**157.0496**	[M-H]	107.0496(100)	-6.67	II	↑ T		
					152.9678(10)					
					123.0446(100)					
					109.0289(25)	-	III	↓ T		
(E)-S-1-Propenyl thiosulfate	C3H6O3S2	10.59	**152.9676**	[M-H]	89.0062(35)	6.529				
					80.9647(40)					
					79.9570(55)					
			169.0495	[M-H]	151.0394(100)	-	III	↑ T		
3,4-Dihydroxyphenylglycol	C8H10O4	2.26	123.0443	[M-H]-46.0052[HCOOH]	123.0447(45)	6.874				
					282.1121(100)					
					162.0552(10)					
			282.1118	[M-H]	145.0287(20)	-6.05	III	↑ T		
N-trans-p-Coumaroyltyramine	C17H17NO3	17.96	283.1151	[^{13}C M-H]	134.0604(10)					
					119.0496(15)					
			301.0699	[M-H]	151.0031(100)	-	II	↓ T		
Hesperetin	C16H14O6	20.34	302.0734	[^{13}C M-H]	286.0472(20)	5.452				
			329.1010	[M-H]	329.1017(15)	-	III	↑ T		
(R)-2-Feruloyl-1-(4-Hydroxyphenyl)-1,2-ethanediol	C18H18O6	19.535	330.1044	[^{13}C M-H]	193.0498(100)	5.899				
			397.0883	[M-H]+67.9873[HCOONa]	135.0445(60)					
					285.0391(80)					
					284.0313 (70)	-	II	↑ T		
Luteolin 7-*O*-glucoside	C21H20O11	16.11	**447.0910**	[M-H]	327.0496 (20)	5.915				
					255.0287 (5)					
					151.0028 (5)					

rt (retention time); LogFC (log Fold Change); E (control vs EVOO); O ((control vs onion), G ((control vs garlic); T ((control vs time).
*compounds confirmed by standards.

4.3 Section III: Health effect of tomato *sofrito* intake

4.3.1 Publication 8. Mediterranean tomato-based *sofrito* protects against vascular alterations in obese Zucker rats by preserving NO bioavailability

Rosalia Rodriguez-Rodriguez, Francesc Jimenez-Altayó, Laia Alsina, Yara Onetti, **José Fernando Rinaldi de Alvarenga**, Carmen Claro, Elena Ogalla, Núria Casals, Rosa Maria Lamuela-Raventós. *Molecular Nutrition and Food Research*, 2017, 61, 9, 1601010

Obesity is a multifactorial disease that affects all physiological functions of the body. It increases the risk of highly lethal chronic complications such as diabetes mellitus and cardiovascular diseases which have negative effects on quality of life and healthcare costs. Traditional dietary approaches, like Mediterranean diet, have proven successful as part of the treatment of obesity and cardiovascular pathologies. The aim of this study was to explore the potential beneficial effects of *sofrito* dietary intake on obesity-related cardiovascular alterations in obese Zucker rats. Eight weeks old obese and lean Zucker rats were fed with a control diet supplemented or not with 2% (w/w) *sofrito* for 8 weeks. Body weight and food intake were evaluated weekly. Blood samples were analyzed by glucose, total cholesterol and triglycerides and tissues (liver, visceral and epididymal adipose tissue) were weight. Vascular function was evaluated in aorta in organ baths. Dihydroethidium staining and immunofluorescence were used to determinate aortic superoxide and peroxynitrite production, respectively. Food and caloric intake was significant higher in obese *sofrito* group compared to obese control, however no differences in body weight gain and organ weights was observed. *Sofrito* intake attenuated phenylephrine contractions in aorta for obese and lean without differences between groups. In the presence of phenylephrine and L-NAME (NO synthesis inhibitor), obese control rats demonstrated that obesity is associated with a loss of NO influence on aortic contraction. However, this effect was not seen in the obese rats supplemented with *sofrito*, indicating a restore of the endothelial NO influence contraction. Comparing obese groups, *sofrito* was able to reduce NO metabolites, vascular iNOS expression and superoxide production, which contribute to a better inflammatory state. In addition, obese *sofrito* group showed higher levels of nitrotyrosine compared to obese control group that normalize vascular levels of NO and O_2- improving vascular functions. *Sofrito* was able to restore and normalize vascular levels of reactive oxygen and nitrogen species, vascular iNOS expression and systemic levels of NO metabolites. A short to medium term daily intake of *sofrito* prevents vascular alterations that could precede cardiometabolic complications associated with obesity.

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RESEARCH ARTICLE

Mediterranean tomato-based *sofrito* protects against vascular alterations in obese Zucker rats by preserving NO bioavailability

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Scope: *Sofrito*, a key component of the Mediterranean diet, provides nutritional interest due to its high content in bioactive compounds from tomato and olive oil, and especially to the lipid matrix in which these compounds are found. In this study, the potential beneficial effects of dietary intake of *sofrito* on obesity-related vascular alterations were explored in obese Zucker rats.

Methods and results: Obese and lean rats were fed a control diet supplemented or not with 2% w/w *sofrito* for 8 weeks. Vascular function was evaluated in aorta in organ baths. Dihydroethidium staining and immunofluorescence was used to determine aortic superoxide and peroxynitrite production, respectively. Despite food and caloric intake was higher in *sofrito*-fed obese rats, no differences were appreciated on body weight compared to control rats. *Sofrito* attenuated phenylephrine-induced vasoconstriction. This effect was associated with preservation of nitric oxide on vasoconstriction and normalization of serum nitric oxide metabolites, vascular inducible nitric oxide synthase and vascular superoxide and peroxynitrite levels.

Conclusion: This is the first evidence of tomato-based *sofrito* protection against vascular alterations that could precede major cardiometabolic complications in obesity. These results contribute to explain the therapeutic properties of the Mediterranean diet in obesity-related disorders. Therefore, *sofrito* is an attractive dietary approach against vascular alterations in obesity.

Keywords:
Mediterranean diet / Nitric oxide / Obesity / *Sofrito* / Vascular function

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1 Introduction

Obesity is one of the leading public health problems in developing countries. Besides its direct impact on life quality, obesity is a major risk factor for highly lethal chronic complications such as insulin resistance and cardiovascular disease

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Abbreviations: ACh, acetylcholine; **DHE**, dihydroethidium; **eNOS**, endothelial NOS; **iNOS**, inducible NOS; **L-NAME**, *N*-nitro-L-arginine methyl ester; **NO**, nitric oxide; **NOS**, NO synthase; **Phe**, phenylephrine

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[1]. Particularly, the pathophysiology of obesity-related vascular disorders is an important target for developing new therapeutic strategies aimed to prevent or ameliorate cardiovascular complications in obesity [2].

Although traditional dietary approaches have proven successful as part of the treatment of obesity and cardiovascular pathologies in clinical trials, the exact role and importance of the individual components of the dietary pattern on specific aspects of these diseases need further evaluation [3, 4]. Research has therefore turned its attention to functional foods and nutraceutics as main components of a healthy prototype diet that could have the potential ability to modulate physiological and pathophysiological molecular mechanisms, thus resulting in favorable health outcomes [4]. In this sense, the Mediterranean dietary pattern has been shown to reduce the burden or even prevent the development of cardiovascular disease, cancer and obesity, among other pathologies [3, 5, 6]. Within the Mediterranean diet, tomato and tomato sauces are typical. In fact, tomato sauce is the most commonly consumed tomato product worldwide and particularly in Spain, where it represents almost 40.8% of all sauce consumption [7]. In addition, consumption of tomato products has been consistently associated with a lower risk of several types of cancer and coronary heart disease, an effect mainly attributable to its high content in bioactive compounds such as polyphenols, carotenoids, and vitamin C [8–11].

Tomato-based *sofrito*, a key component of the Mediterranean diet, is particularly interesting for its high content in bioactive compounds, not only from tomato but also from onion and virgin olive oil, and especially for the lipid matrix in which these compounds are found [12, 13]. Regarding the latter, recent investigations have demonstrated that mechanical and thermal treatments, as well as oil matrix addition during tomato and *sofrito* sauce processing, may increase the bioaccessibility, extractability, and bioavailability of phenolic compounds from tomato [14, 15]. Once they reach the adequate bioavailability, predominant phytochemicals present in *sofrito*, especially polyphenols and carotenoids, can exert their health-promoting effects [16–18]. Epidemiological and preclinical studies have demonstrated that fruits, vegetables, and beverages rich in carotenoids (e.g., lycopene and beta-carotene) and polyphenols (e.g., rutin and naringenin) delay the onset of atherosclerosis and exhibit anti-inflammatory and antiplatelet activities [19]. Additionally, these phytochemicals are able to reduce neointimal thickening by inhibiting proliferation of vascular smooth muscle cells and can also prevent vascular dysfunction by improving nitric oxide (NO) availability [20]. In particular, recent evidence suggests that carotenoids and polyphenols are key players not only in the treatment of inflammation and vascular complications, but also in restoring normal adipocyte function in obese subjects [4, 21, 22]. Interestingly, an inverse association has been established between plasmatic levels of carotenoids and inflammatory and oxidative stress markers in obese patients [23]. The fact that obesity is an inflammatory state that contributes to oxidative stress and vascular complications [2] indicates that a dietary source of carotenoids and polyphenols could represent an attractive therapeutic strategy to prevent or ameliorate the obesity-related complications.

In the present study, the potential preventive effects of a short- to middle-term dietary intake of Mediterranean *sofrito* were explored for the first time in an animal model of obesity during initial stages of the disease. Concretely, we used the Zucker rat that is an obesity model extremely useful to explore vascular and metabolic complications associated with obesity [24]. Thus, this study is an attempt to evidence the potential contribution of *sofrito* consumption in the healthy properties of the Mediterranean diet.

2 Materials and methods

2.1 Standards and reagents

The standards of carotenoids and polyphenols were purchased from Extrasynthese (Genay, France) (ethylgallate, hydroxytyrosol, naringenin-7-o-glucoside), Sigma-Aldrich (St. Louis, MO, USA) (all-*E*-α-carotene, all-*E*-β-carotene, all-*E*-lycopene, apigenin, caffeic acid, chlorogenic acid, o-coumaric acid, p-coumaric acid, ferulic acid, naringenin, oleuropein, pinoresinol, protocatechic acid, quercetin, rutin, vanillic acid), and HWI Analitik (Rülzheim, Germany) (verbascoside).

2.2 Animals and diets

Six-week-old male obese Zucker rats and their lean littermate controls were purchased from Charles River (Charles River Laboratories, Barcelona, Spain). At 8 weeks of age, obese and lean rats were randomly assigned to the following groups ($n = 8$): lean rats fed control chow diet (LC), obese rats fed control chow diet (OC), lean rats fed chow diet supplemented in 2% (w/w) *sofrito* (LS), and obese rats fed chow diet supplemented in 2 % (w/w) *sofrito* (OS). Control (standard) chow diet (Teklad Global 2018) was provided by Harlan Laboratories (Milan, Italy). *Sofrito* (Gallina Blanca-Star, Barcelona, Spain) was characterized as described in the following sections and content of polyphenols and carotenoids is shown in Table 1. *Sofrito* supplementation was calculated according to previous unpublished studies in humans in which 2.25 g/kg *sofrito* peer week was administered. Lycopene content was used as a biomarker, and 2% *sofrito*-supplemented diet provides 3.61 ± 0.23 μg/g lycopene.

Body weight and food intake was weekly evaluated. After 8 weeks of administration of the experimental diets, animals were killed by decapitation. Blood samples were immediately collected and thoracic aorta, liver, visceral (perirenal + retroperitoneal), and epididymal adipose tissues were dissected.

The protocol for animal handling and experimentation agreed with the European Union guidelines for the ethical

Table 1. Characterization of polyphenol and carotenoids content of the Mediterranean *sofrito* used in the study expressed in μg/g of fresh weight. The *sofrito* is mainly composed by tomato (50%) (pulp and concentrated), onion (37%), extra virgin olive oil (12%), and salt

Polyphenols		Carotenoids	
3,4-DHPEA-EDA	0.052 ± 0.002	all-*E*-ζ-Carotene	3.6 ± 0.3
Caffeic acid	0.74 ± 0.02	all-*E*-α-Carotene	4.0 ± 0.2
Caffeic hexoside I	1.77 ± 0.04	all-*E*-β-Carotene	9.1 ± 0.2
Chlorogenic acid	3.6 ± 0.3	13-*Z*-β-Carotene	3.31 ± 0.05
Coumaric hexoside I	0.51 ± 0.01	15-*Z*-Lycopene	7.3 ± 0.5
DHOA	0.046 ± 0.001	13-*Z*-Lycopene	33 ± 3
Dicaffeoylquinic acid	0.20 ± 0.00	9-*Z*-Lycopene	34 ± 1
Dihydroxytyrosol	0.105 ± 0.001	all-*E*-Lycopene	142 ± 3
Eneloic acid	0.074 ± 0.001	5-*Z*-Lycopene	28.6 ± 0.2
Ferulic acid	0.51 ± 0.04		
Ferulic hexoside	2.16 ± 0.04		
HCM-EA	0.34 ± 0.01		
HDCM-OA	1.1 ± 0.1		
HOA	0.26 ± 0.01		
Hydroxyelenoic acid	0.26 ± 0.03		
Hydroxytyrosol	1.00 ± 0.06		
Lariciresinol	0.32 ± 0.03		
Luteolin	1.5 ± 0.2		
Naringenin	9.6 ± 0.9		
Naringenin-7-glucoside	0.87 ± 0.01		
Oleuropein I	0.063 ± 0.002		
p-Coumaric acid	0.27 ± 0.02		
Pinoresinol	1.1 ± 0.1		
Protocatechic acid	0.28 ± 0.02		
Rutin	4.5 ± 0.7		
Vanillic acid	0.39 ± 0.02		

Data are mean ± SD.
3,4-DHPEA-EDA, decarboxyl methyl oleuropein aglycone; DHOA, dihydroxyoleuropein aglycone; HCM-EA, hydroxycarboxymethyl elenoic acid; HDCM-OA, hydroxydecarboxymethyl oleuropein aglycone; HOA, hydroxyoleuropein aglycone.

management of animals and was approved by the Committee of Ethical Experimentation of the Universitat of Barcelona (557/16).

2.3 Extraction of carotenoids and polyphenols

The bioactive compounds extractions were performed under light with UV filters and ice to minimize the oxidation and isomerization of the compounds. All the samples were extracted in triplicate.

2.3.1 Extraction of carotenoids and polyphenols from *sofrito*

Sofrito (0.5 g) was homogenized with ethanol:n-hexane (4:3, v/v) for the carotenoid extraction. The homogenate was sonicated for 10 min on ice and then centrifuged (2486 × *g*, 20 min at 4°C). The apolar phase was separated and the extraction was repeated until colorless. The supernatants were combined and evaporated under nitrogen flow and the residue was reconstituted with MTBE and filtered using a 0.22 μm PTFE filter and stored at –80°C until analysis [12]. The same weight of *sofrito* was extracted with ethanol: water (80:20; v/v) for polyphenols extraction. The homogenate was sonicated, centrifuged, and extracted in the same conditions. The residue was suspended up to 2 mL of ultrapure water with 0.1% of formic acid and filtered through a 0.22 μm PTFE filter storage at −80°C [12].

2.3.2 Extraction of carotenoids from rat liver tissues

Liver sections (50 mg) were weighed and homogenized with ethanol:water (50:50, v/v) containing 100 mM PMSF and 1% BHT. Then, 600 μL of hexane was added, homogenized, sonicated for 5 min, and centrifuged (6000 × *g*, 5 min at 4°C). The apolar phase was transferred into a flask and the extraction was repeated. After evaporation, the extract was reconstituted and transferred into an amber vial for HPLC analysis [25].

2.4 Carotenoids analysis by HPLC-DAD

The carotenoid analysis was performed using an HPLC system (HP1100 HPLC system; Hewlett-Packard, Waldbronn,

Germany) equipped with a quaternary pump and autosampler, coupled to a diode array detector DAD G1315B. Separation was carried out on a C30 250 × 4.6 mm, 5 μm column (YMCTM; Water Co., Milford, MA, USA) and the conditions were based on the procedure described by Vallverdú-Queralt et al. [12]. Identification of the carotenoids was confirmed by retention time, chromatography with standards, UV/VIS absorption spectrum (λ_{max}, spectral fine structure (%III/II), peak cis intensity (%Ab/AII)), characteristics compared to the standards analyzed under the same conditions, and the literature [13, 26]. Quantification was performed using external calibration curves for all-*E*-α-carotene, all-*E*-β-carotene, and all-*E*-lycopene with seven concentration levels. The Z-isomers were quantified using the same calibration curve corresponding to the all-*E*-form and all-*E*-ζ-carotene using a β-carotene curve. The results were expressed as μg/g of *sofrito* oryes tissue.

2.5 Polyphenols analysis by HPLC-MS/MS

The identification and quantification of the phenolic compounds typically presented in *sofrito* were carried out by UHPLC-ESI-QqQ-MS/MS analysis using the conditions of a validated method by Di Lecce et al. [27] for tomato and a method described by Capriott et al. [28] for olive oil. LC was performed with a UHPLC Acquity system equipped with a binary pump and an autosampler from Waters (Milford, MA, USA). A BEH C18 column (50 mm × 2.1 mm) i.d., 1.7 μm (Waters, Milford, MA, United States) was used. An API 3000 triple quadrupole mass spectrometer (ABSciex, Concord, Ontario, Canada) equipped with Turbo Ionspray source in negative-ion mode was used for the MS/MS data analysis. Quantification of polyphenols was performed using multiple reactions monitoring mode tracking the transition of parent ion and product ions specific for each compound. The quantification was carried out by the internal standard method using ethyl gallate. The polyphenols were quantified related to their corresponding standard or with a compound corresponding at the same class. The results were expressed as μg/g of *sofrito*.

2.6 Blood biochemical assays

Serum samples were obtained from blood by centrifugation (2000 × *g*, 5 min). Glucose, total cholesterol, and triglycerides levels were determined using enzymatic-based assays following manufacturer's instructions (CatBio Laboratories, Barcelona, Spain). Serum levels of NO metabolites (NO_x) were determined based on the colorimetric Griess reaction [29]. Absorbance was measured spectrophotometrically at 540 nm [30].

2.7 Tissue preparation

Thoracic aorta was cleaned in Krebs-Henseleit solution (in mmol/L: NaCl 112; KCl 4.7; $NaHCO_3$ 25; $MgSO_4$ 1.2; $CaCl_2$ 2.5; KH_2PO_4 1.2; and glucose 11.1; pH 7.4). For detection of superoxide anion (O_2^-), aortic rings were maintained in 30% sucrose- Krebs-Henseleit solution overnight, placed into cryomolds with embedding medium, and immediately frozen for storage at −80°C [31]. For immunofluorescence studies, aortas were fixed in 4% phosphate-buffered paraformaldehyde for 1 h and then washed in PBS. Afterward, arterial segments were placed in 30% sucrose PBS overnight, transferred to cryomolds, and kept at −80°C until analysis [31].

2.8 Vascular reactivity experiments

Aortic rings were disposed in organ baths, and vascular function was measured as previously described [31]. Contractile capacity of the vessels was assessed with KCl 100 mmol/L. After stabilization, endothelial-dependent vasodilatations were studied by evaluating the relaxation induced by acetylcholine (ACh; 0.001–10 μmol/L) in vessels previously contracted with phenylephrine (Phe). To investigate contractile responses, concentration–response curves to Phe (0.001–100 μmol/L) were performed in the absence or presence of the nonselective NO synthase (NOS) inhibitor *N*-nitro-L-arginine-methyl-ester (L-NAME, 300 μmol/L).

2.9 *In situ* detection of superoxide anion

The oxidative fluorescent dye dihydroethidium (DHE) was used to evaluate production of arterial O_2^-, as described [31]. Briefly, 14 μm thick aortic sections were incubated with DHE (2 μmol/L) in Krebs-HEPES buffer. Preparations were viewed by laser scanning confocal microscope (TCS SP2; Leica, Heidelberg, Germany). DHE fluorescence was abolished by the O_2^- scavenger, Mn(III)tetrakis(1-methyl-4-pyridyl)porphyrin, indicating the specificity of this reaction. Integrated optical densities were quantified using MetaMorph Image Analysis Software (Molecular Devices, Sunnyvale, CA, USA). The fluorescence signal per area was measured in at least two rings of each animal, and the results were expressed as arbitrary units.

2.10 Immunofluorescence

The *in situ* production of peroxynitrite ($ONOO^-$) was measured indirectly by analyzing nitrotyrosine levels, whereas inducible NOS (iNOS) expression was analyzed by inmunofluorescence, as previously described [32]. Frozen transverse sections (14 μm thick) were incubated with rabbit polyclonal antibody against nitrotyrosine (1:100; Merck

Millipore, Darmstadt, Germany) or iNOS (1:50; Thermo Scientific, Rockford, IL, USA) [32]. Preparations were then incubated with the secondary antibody IgG conjugated to cyanine 3 (1:200; Jackson ImmunoResearch Laboratories Inc, West Grove, PA, USA). Signals were viewed using a laser scanning confocal microscope (TCS SP2; Leica). Cyanine 3 labeled antibody was visualized by excitation at 561 nm and detection at 600–700 nm. The specificity of the immunostaining was evaluated by omission of the primary antibody and processed as described above. Optical density of immunofluorescence was assayed as described in Section 2.9.

2.11 Data analysis and statistics

Results were shown as mean ± SD. Vasoconstrictor responses were expressed as a percentage of tone generated by KCl 100 mmol/L. Vasodilator responses were expressed as a percentage of the previous tone generated by Phe. Area under the curve was calculated from each individual concentration–response curve and illustrated as arbitrary units. Two-way ANOVA with repeated measurements on the concentration factor followed by Bonferroni's posttest was used for comparisons. Data analysis was carried out using GraphPad Prism Software 5.0 (San Diego, CA, USA).

3 Results

3.1 Effect of *sofrito*-supplemented diet on body weight gain, food intake, organ weights, and serum biochemical determinations

Lycopene content in liver samples was used as a biomarker of the intake of *sofrito*-supplemented diets, showing significantly higher content in LS and OS groups compared to control groups (Supporting Information Table 1). As illustrated in Table 2, obese rats gained more body weight, and liver and white adipose tissue weights were higher compared to lean rats. Eight weeks administration of *sofrito*-supplemented diet was not able to modify body weight gain and organ weights in obese rats. Moreover, it is important to mention that, despite food and caloric intake in OS group was significantly higher than in OC rats, this hyperphagia did not imply higher body weight gain or higher values of liver and white adipose tissue weights in *sofrito*-fed rats (Table 2). According to the obese phenotype of Zucker rats, serum analysis revealed higher levels of total cholesterol, triglycerides, and glucose in obese compared with lean rats, and *sofrito*-fed groups showed similar levels of these parameters compared to their respective lean or obese control groups (Table 2).

3.2 Effect of *sofrito*-supplemented diet on vascular reactivity

Evaluation of endothelium-dependent ACh-induced vasodilatation in isolated aorta did not show endothelial dysfunction to ACh in obese compared to lean rats (Fig. 1A and B). Conversely, concentration-dependent contractions to Phe were slightly increased in aortic rings from OC compared to LC rats (Fig. 1C and D). Remarkably, in the present study, administration of *sofrito* in the diet significantly attenuated Phe contractions in aorta from either obese or lean rats, eliminating differences between groups (Fig. 1C and D).

It has been suggested that one of the mechanisms underlying the enhanced vascular contraction in obesity could be closely related to reductions in arterial NO bioavailability [31, 33]. Therefore, we investigated whether the attenuation of Phe-induced responses in aortas from *sofrito*-fed rats was dependent on the increased NO from endothelial cells. In the presence of L-NAME, vasoconstriction to Phe was increased in aortic rings from lean rats, irrespective of the type of diet (Fig. 2A and B). On the other hand, L-NAME did not restored Phe-induced vasoconstriction in OC rats, demonstrating that obesity is associated with a loss of NO influence on aortic contraction (Fig. 2C and D). Interestingly, the *sofrito*-supplemented diet was able to restore NO influence on contraction in obese rats, since the presence of L-NAME now significantly increased Phe-induced contractions (Fig. 2C and D). Altogether, these results suggest that aortas from obese rats have lost the NO negative feedback on Phe contraction, and that a *sofrito*-enriched diet can restore it.

3.3 Effect of *sofrito*-supplemented diet on serum levels of NO metabolites and vascular iNOS expression

In agreement with previous publications, nontreated obese Zucker rats showed increased serum levels of NO metabolites (NO_x) compared to lean rats (Fig. 3A), which is associated with the inflammatory state in early obesity. Obese animals fed a *sofrito*-supplemented diet showed a significant attenuation in serum NO_x levels (Fig. 3A), suggesting that this dietary approach is capable of restoring physiological circulating levels of NO metabolites in obesity.

Although endothelial NOS (eNOS) produces relatively low quantities of NO, the iNOS isoform has the highest capacity to generate NO, thus contributing to the NO_x pool in cardiometabolic diseases with an important inflammatory status [34]. Particularly, iNOS expression is markedly induced in obese rats, and iNOS-mediated NO production is a preliminary cause of obesity-linked insulin resistance [34]. According with this and previous observations, iNOS expression was slightly upregulated ($p = 0.0559$) in aortic sections from obese rats, whereas this expression remained significantly lower in arteries from *sofrito*-fed obese rats (Fig. 3B).

Table 2. Body weight, food intake, organ weights, and serum biochemical determinations

	LC	LS	OC	OS
Body weight (g)	383.6 ± 28.7	378.4 ± 20.7	518.3 ± 49.6*	517.3 ± 46.1+
Food intake (g/day/rat)	20.00 ± 3.73	23.61 ± 4.43	28.64 ± 5.32*	34.44 ± 3.39+
Caloric intake (kcal)	61.99 ± 11.50	73.71 ± 13.80	88.79 ± 16.50*	107.55 ± 10.57+,#
Organ weight (g/100 g b.w.)				
Liver	3.78 ± 0.43	3.46 ± 0.14	5.11 ± 0.86*	5.44 ± 0.92+
VAT	0.78 ± 0.07	0.91 ± 0.07	1.58 ± 0.23*	1.35 ± 0.19+
EAT	0.75 ± 0.14	0.77 ± 0.26	1.94 ± 0.21*	1.89 ± 0.24+
Serum parameters				
Glucose (mmol/L)	7.1 ± 0.26	7.633 ± 0.20	8.433 ± 0.81*	9.4 ± 2.26+
Triglycerides (mmol/L)	1.496 ± 0.31	1.327 ± 0.25	5.684 ± 1.21*	4.76 ± 0.62+
Cholesterol (mmol/L)	3.494 ± 0.20	3.523 ± 0.23	6.09 ± 0.95*	7.07 ± 0.78+

Data are mean ± SD.
*$p < 0.05$ versus LC; +$p < 0.05$ versus LS; #$p < 0.05$ versus OC.
LC, lean rats fed a control diet; LS, lean rats fed a *sofrito*-supplemented diet; OC, obese rats fed a control diet; OS, obese rats fed a *sofrito*-supplemented diet; VAT, visceral perirenal + retroperitoneal adipose tissue; EAT, epididymal adipose tissue.

3.4 Effect of *sofrito*-supplemented diet on vascular levels of reactive oxygen and nitrogen species

In agreement with previous findings [31], aortas from obese rats showed higher levels of vascular O_2^- compared to those from lean rats, whereas obese rats fed a *sofrito*-supplemented diet did not evidence this increase (Fig. 4A). We also determined protein tyrosine nitration in the vascular wall by immunofluorescence, as an indirect measurement of $ONOO^-$ production. The results indicated that nitrotyrosine staining was lower in aortas from OC compared to LC rats (Fig. 4B). As illustrated in Fig. 4B, nitrotyrosine levels in aorta from

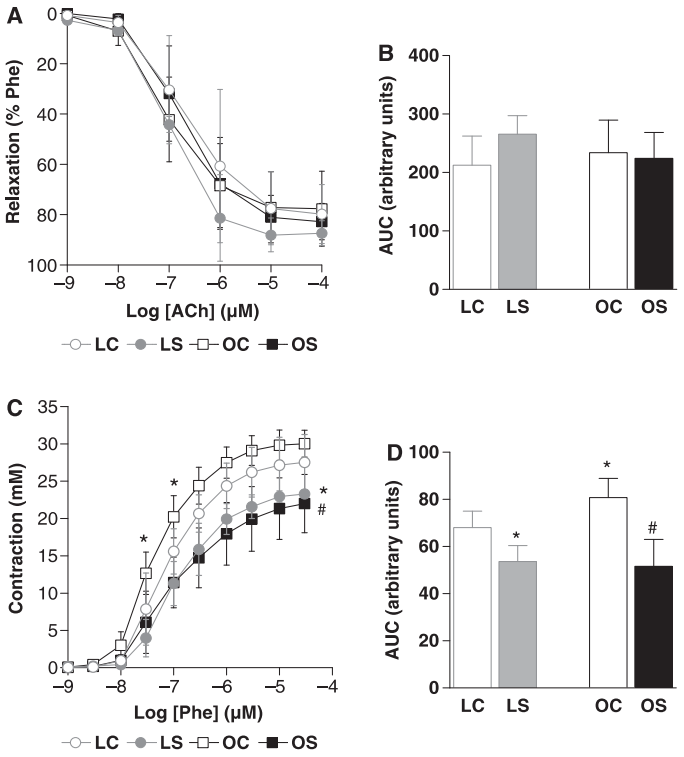

Figure 1. Concentration–response curves to acetylcholine (ACh) (A and B) and phenylephrine (Phe) (C and D) in aortic rings from lean and obese Zucker rats. LC: lean rats fed a control diet; LS: lean rats fed a *sofrito*-supplemented diet; OC: obese rats fed a control diet; OS: obese rats fed a *sofrito*-supplemented diet. Data are mean ± SD ($n = 5$–6). *$p < 0.05$ versus LC; #$p < 0.05$ versus OC.

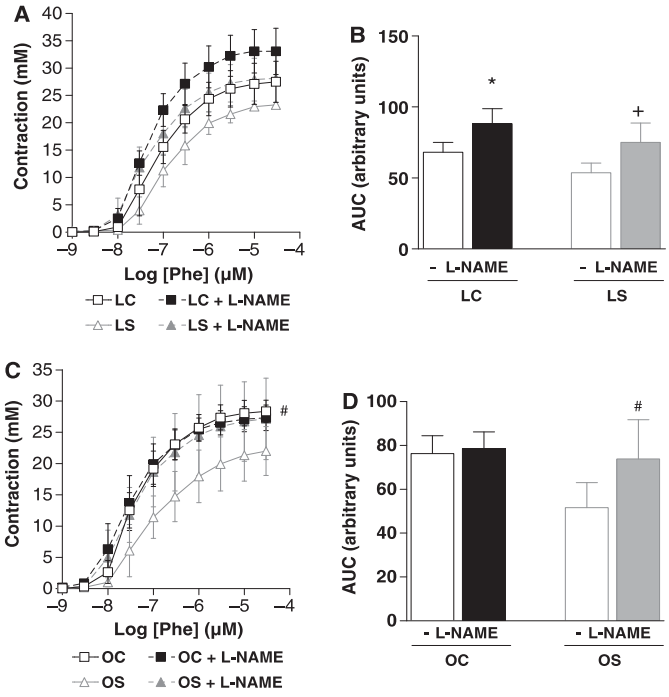

Figure 2. Concentration–response curves to phenylephrine (Phe) in the absence or presence of the NO synthesis inhibitor L-NAME in aortic rings from lean (A and B) and obese Zucker (C and D) rats. LC: lean rats fed a control diet; LS: lean rats fed a *sofrito*-supplemented diet; OC: obese rats fed a control diet; OS: obese rats fed a *sofrito*-supplemented diet. Data are mean ± SD ($n = 5$–6). *$p < 0.05$ versus LC; +$p < 0.05$ versus LS; #$p < 0.05$ versus OS.

OS rats were similar to those observed in lean rats. These results suggest that administration of *sofrito* could normalize vascular levels of NO and O_2^-, contributing to the effects on vascular function.

4 Discussion

The present study demonstrates for the first time that short- to medium-term daily intake of *sofrito*, a key component of the Mediterranean diet, prevents vascular alterations that could precede major cardiometabolic complications associated with obesity in rats. Particularly, the administration of a diet supplemented in 2% (w/w) *sofrito* normalized the adrenergic vasoconstriction response in aorta by a NO-dependent mechanism, and restored serum NO metabolites, vascular iNOS expression, and superoxide and peroxynitrite levels in obese Zucker rats.

Despite food and caloric intake was higher in *sofrito*-fed obese rats, no differences were appreciated on body weight gain and organ weights compared to obese control rats. According to the obese phenotype of Zucker rats, serum analysis revealed higher levels of total cholesterol, triglycerides, and glucose in obese compared with lean rats, and *sofrito* administration did not modify these parameters. It is known that chronic administration of tomato-based diets and polyphenols- or carotenoids-enriched products in the diet is able to improve lipid profile in animal models of obesity by reducing lipid peroxidation and circulating levels of cholesterol and triglycerides [35]. However, compared to our investigation, in these previous studies, the period of administration of the bioactive compounds was longer and/or used higher concentrations of polyphenols and carotenoids. Furthermore, in some of these studies the polyphenols or extracts were orally administered by gavage instead of in the diet. According to serum glucose levels, the effects of polyphenols or carotenoids from tomato in glucose metabolism are variable and strictly dependent on the type of bioactive compound and experimental design of the study [36, 37]. In addition, evidence suggests that the obese Zucker rat is not an appropriate model of diabetes or glucose metabolism alterations, especially at early stages of the disease [24, 30]. Therefore, the lack of significant alterations in glucose or lipid profile in obese rats treated with *sofrito* are intriguing data that would need further evaluation and probably longer period of administration of the dietary approach would be needed to appreciate significant modifications of these parameters, according to the literature.

Despite *sofrito*-fed rats did not experience any detectable change in body and organ weight and in serum metabolic parameters compared to control diet-fed animals, administration of a *sofrito*-supplemented diet was able to modify alterations in vascular reactivity and alterations linked to early stages of obesity. A remarkable result from this study was

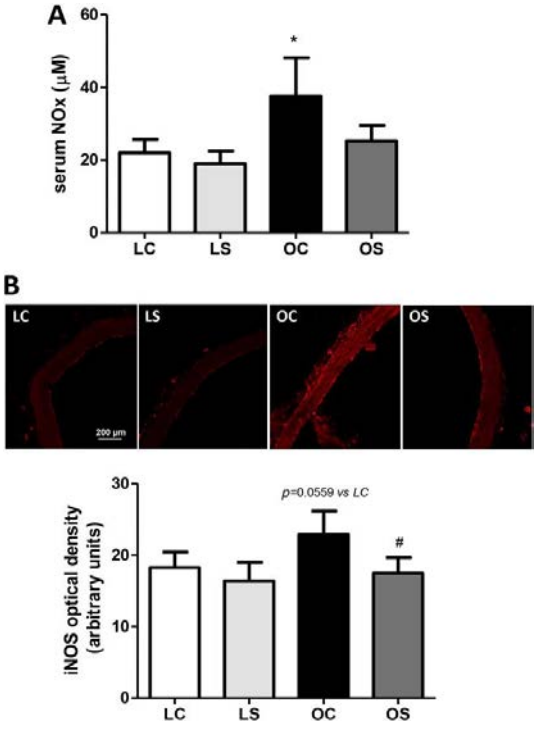

Figure 3. Serum NO metabolites (nitrates and nitrites, NOx) levels (A) and aortic iNOS protein expression (B) after *sofrito*-supplemented diet administration in lean and obese Zucker rats. LC: lean rats fed a control diet; LS: lean rats fed a *sofrito*-supplemented diet; OC: obese rats fed a control diet; OS: obese rats fed a *sofrito*-supplemented diet. Data are mean ± SD (n = 4–6). *$p < 0.05$ versus LC; #$p < 0.05$ versus OC.

that *sofrito* attenuated the aortic vasoconstriction to adrenergic stimulation, which is altered in obese animals. This increased vascular contraction in obese rats had previously been related to an elevated endothelin-dependent signaling [38] and an increased sympathetic nervous system activity [31, 39, 40]. Thus, in the development of obesity, an augmented adrenergic activity is frequently observed, which can have a long-term impact on tissue perfusion and accelerate onset and progression of cardiovascular disease. Evidence has revealed that NO exerts an opposed modulatory effect on vasoconstriction induced by endothelin [33] or Phe [41]. It has been suggested that one of the mechanisms underlying the enhanced vascular contraction in obesity could be closely related to reductions in arterial NO bioavailability [31, 33]. Data obtained in the presence of L-NAME revealed a significant restoration of vasoconstriction in aorta from lean (LC and LS) and remarkably in OS rats. On the one hand, these results suggest that *sofrito*-supplemented diet was able to restore endothelial NO influence on contraction in obese rats, since the presence of L-NAME significantly increased Phe-induced contractions, in contrast to that observed in OC rats. The arterial NO-restoring effects of *sofrito* are particularly evident in a low endothelial NO situation—associated with the hypercontractility in aorta from obese rats [31, 39, 40]—needed to provide a normal vasonconstriction. However, in a situation of appropriate arterial NO levels (such as in lean rats), *sofrito* is still attenuating vasoconstriction, but probably by additional mechanisms apart from NO (e.g., cyclooxygenase-derived factors). Both experimental and clinical studies indicate that polyphenol- and carotenoid-rich foods and supplements provide vascular protection, not only by their antioxidant effects but also by promoting NO release thus protecting vascular function [42]. These activities are suggested to be mediated by several molecular mechanisms such eNOS phosphorylation, increased endothelial calcium levels, activation of the PI3-kinase/Akt pathway, inhibition of smooth muscle p38 MAPK pathway, and downregulation of vascular NADPH oxidase isoforms expression, among others [42–45]. In the present study, the precise mechanisms by which components from Mediterranean *sofrito* preserves NO availability in aorta from obese rats would need additional investigation.

Increases in NO bioavailability could be caused by augmented NO production and/or by reduced NO breakdown (i.e., throughout a decrease in ROS production). To evaluate the mechanisms by which *sofrito* could impact obesity, we also measured levels of NO metabolites in serum. NO_x measurement is a suitable method for assessment of NO synthesis in vivo and a high correlation between endogenous NO production and serum NO_x levels has been reported [46]. Although beneficial effects of NO concentrations covering the physiological range have been documented [47], high NO_x levels are positively correlated with cardiovascular disease, hyperlipidemia, diabetes, and metabolic syndrome [48]. While still under debate, increased serum NO_x levels in obese subjects is a potential biomarker for assessing cardiometabolic disturbances in obesity [49]. Although eNOS produces relatively low quantities of NO, the inducible iNOS isoform has the highest capacity to generate NO, thus contributing to the NO_x pool in cardiometabolic diseases [34]. In agreement with this and previous observations, obese rats showed increased levels of serum NO_x and increased iNOS expression in aortic sections. A likely explanation for increased circulating NO_x levels is the inflammatory state that accompanies obesity, which induces the expression of iNOS by macrophages [34], thus contributing to the development of insulin resistance [34, 50]. Administration of *sofrito* significantly prevented these alterations, suggesting that this dietary approach is capable of restoring biomarkers of the initial inflammation that precedes major cardiometabolic complications in obese rats.

In addition to the inflammatory state, an altered redox status is also crucial in NO metabolism disturbances and the subsequent development of complications in obesity [51]. Nitrooxidative stress in the vessel wall has been strongly related to a proinflammatory-mediated increase in iNOS and NO formation [52]. Our findings suggest that the intake of

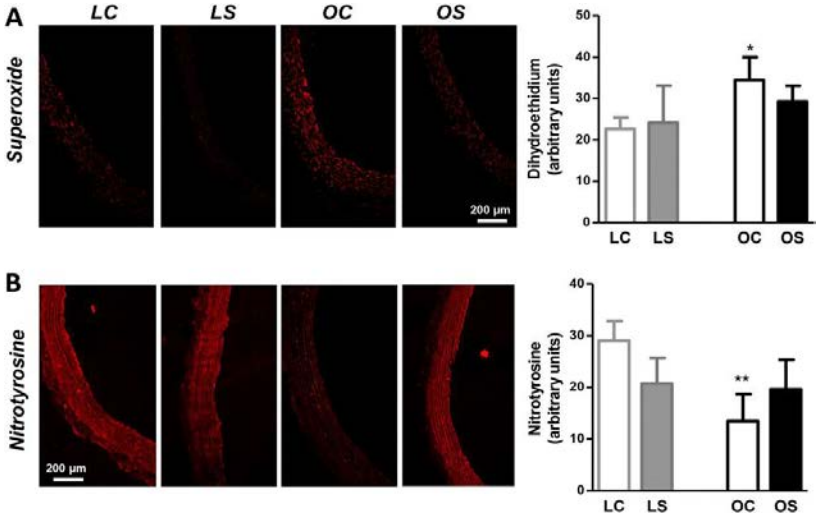

Figure 4. Representative photomicrographs and quantification of dihydroethidium-derived (an indirect indicator of superoxide anion) fluorescence (A) and nitrotyrosine (an indirect indicator of peroxynitrite) immunofluorescence (B) levels in confocal microscopic aortic sections from lean and obese Zucker rats. LC: lean rats fed a control diet; LS: lean rats fed a *sofrito*-supplemented diet; OC: obese rats fed a control diet; OS: obese rats fed a *sofrito*-supplemented diet. Data are mean ± SD ($n = 5$–7). *$p < 0.05$; **$p < 0.01$ versus LC.

sofrito normalized vascular production of superoxide anion and protein tyrosine nitration, which can be associated with its capacity to improve vascular function in obesity. Despite of these beneficial vascular changes, these effects are not enough to, at this point of the dietary administration, restore serum glucose and lipid profile. In this sense, longer period of administration should be evaluated. However, the prevention of vascular alterations in obesity has been associated with an early prevention of major cardiometabolic complications related to the disease [2, 33, 49, 53].

Altogether, these effects of *sofrito* could be mainly attributable to the high content on polyphenols and carotenoids from tomato, onion, and olive oil, which have separately demonstrated vasoprotection in animal models and humans with obesity and metabolic syndrome [4, 20, 35]. Remarkably, *sofrito* is particularly interesting due to the lipid matrix in which these compounds are found [12]. Although the mechanisms underlying this vasoprotection are probably multiple, in our study we demonstrate for the first time that *sofrito* is able to restore and normalize vascular levels of reactive oxygen and nitrogen species, vascular iNOS expression, and systemic levels of NO metabolites. Obesity is associated with an inflammatory state that likely contributes to oxidative stress and vascular complications [2, 34]. In addition, it is important to mention that higher food and caloric intake in *sofrito*-fed obese group did not imply higher body weight gain. The results of the present study could contribute to explain the therapeutic beneficial effect against human obesity-related complications attributable to the Mediterranean diet and suggest that *sofrito* is one of the main active subcomponents of this healthy dietary pattern. Therefore, Mediterranean *sofrito* is an attractive dietary approach against obesity and its cardiovascular health risks, at least particularly in the early stages of obesity.

R.R.-R., F. J.-A., and R.M.L.-R. designed the research; R.R.-R., F.J.-A., L.A., and Y.O. conducted the experiments and data analysis; J.F.A. and R.M.L.-R. performed the sofrito characterization and carotenoids and polyphenols determinations; C.C. and E.O. prepared the experimental diets; R.R.-R. wrote the paper; R.R.-R., F. J.-A., R.M.L.-R., and N.C. participated in the discussion and interpretation of the results. All authors read and approved the final manuscript.

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The authors have declared no conflict of interest.

5 References

[1] Haslam, D. W., James, W. P. T., Obesity. *Lancet (London, England)* 2005, *366*, 1197–1209.

[2] Tesauro, M., Cardillo, C., Obesity, blood vessels and metabolic syndrome. *Acta Physiol.* 2011, *203*, 279–286.

[3] Grosso, G., Mistretta, A., Marventano, S., Purrello, A. et al., Beneficial effects of the Mediterranean diet on metabolic syndrome. *Curr. Pharm. Des.* 2014, *20*, 5039–5044.

[4] Ríos-Hoyo, A., Cortés, M. J., Ríos-Ontiveros, H., Meaney, E. et al., Obesity, metabolic syndrome, and dietary

therapeutical approaches with a special focus on nutraceuticals (polyphenols): a mini-review. *Int. J. Vitam. Nutr. Res.* 2014, *84*, 113–123.

[5] Buckland, G., Travier, N., Cottet, V., González, C. A. et al., Adherence to the Mediterranean diet and risk of breast cancer in the European prospective investigation into cancer and nutrition cohort study. *Int. J. Cancer* 2013, *132*, 2918–2927.

[6] Estruch, R., Ros, E., Salas-Salvadó, J. et al., Primary prevention of cardiovascular disease with a Mediterranean diet. *N. Engl. J. Med.* 2013, *368*, 1279–1290.

[7] Martínez-Huélamo, M., Tulipani, S., Estruch, R., Escribano, E. et al., The tomato sauce making process affects the bioaccessibility and bioavailability of tomato phenolics: a pharmacokinetic study. *Food Chem.* 2015, *173*, 864–872.

[8] Grieb, S. M. D., Theis, R. P., Burr, D., Benardot, D. et al., Food groups and renal cell carcinoma: results from a case-control study. *J. Am. Diet. Assoc.* 2009, *109*, 656–667.

[9] Silaste, M.-L., Alfthan, G., Aro, A., Kesäniemi, Y. A. et al., Tomato juice decreases LDL cholesterol levels and increases LDL resistance to oxidation. *Br. J. Nutr.* 2007, *98*, 1251–1258.

[10] Agarwal, S., Rao, A. V., Tomato lycopene and its role in human health and chronic diseases. *CMAJ* 2000, *163*, 739–744.

[11] Burton-Freeman, B. M., Sesso, H. D., Whole food versus supplement: comparing the clinical evidence of tomato intake and lycopene supplementation on cardiovascular risk factors. *Adv. Nutr.* 2014, *5*, 457–485.

[12] Vallverdú-Queralt, A., de Alvarenga, J. F. R., Estruch, R., Lamuela-Raventos, R. M., Bioactive compounds present in the Mediterranean sofrito. *Food Chem.* 2013, *141*, 3365–3372.

[13] Rinaldi de Alvarenga, J. F., Tran, C., Hurtado-Barroso, S., Martinez-Huélamo, M. et al., Home cooking and ingredient synergism improve lycopene isomer production in Sofrito. *Food Res. Int.* 2017, https://doi.org/10.1016/j.foodres.2017.01.009.

[14] Tulipani, S., Martinez Huelamo, M., Rotches Ribalta, M., Estruch, R. et al., Oil matrix effects on plasma exposure and urinary excretion of phenolic compounds from tomato sauces: evidence from a human pilot study. *Food Chem.* 2012, *130*, 581–590.

[15] Arranz, S., Martinez-Huelamo, M., Vallverdu-Queralt, A., Valderas-Martinez, P. et al., Influence of olive oil on carotenoid absorption from tomato juice and effects on postprandial lipemia. *Food Chem.* 2015, *168*, 203–210.

[16] Tresserra-Rimbau, A., Rimm, E. B., Medina-Remón, A., Martínez-González, M. A. et al., Polyphenol intake and mortality risk: a re-analysis of the PREDIMED trial. *BMC Med.* 2014, *12*, 77.

[17] Hanske, L., Loh, G., Sczesny, S., Blaut, M. et al., The bioavailability of apigenin-7-glucoside is influenced by human intestinal microbiota in rats. *J. Nutr.* 2009, *139*, 1095–1102.

[18] Martínez-Huélamo, M., Vallverdú-Queralt, A., Di Lecce, G., Valderas-Martínez, P. et al., Bioavailability of tomato polyphenols is enhanced by processing and fat addition: evidence from a randomized feeding trial. *Mol. Nutr. Food Res.* 2016, *60*, 1578–1589.

[19] Wang, H., Chen, Y. E., Eitzman, D. T., Imaging body fat: techniques and cardiometabolic implications. *Arterioscler. Thromb. Vasc. Biol.* 2014, *34*, 2217–2223.

[20] Upadhyay, S., Dixit, M., Role of polyphenols and other phytochemicals on molecular signaling. *Oxid. Med. Cell. Longev.* 2015, *2015*, 1–15.

[21] Luisa Bonet, M., Canas, J. A., Ribot, J., Palou, A., Carotenoids and their conversion products in the control of adipocyte function, adiposity and obesity. *Arch. Biochem. Biophys.* 2015, *572*, 112–125.

[22] Siriwardhana, N., Kalupahana, N. S., Cekanova, M., LeMieux, M. et al., Modulation of adipose tissue inflammation by bioactive food compounds. *J. Nutr. Biochem.* 2013, *24*, 613–623.

[23] Markovits, N., Ben Amotz, A., Levy, Y., The effect of tomato-derived lycopene on low carotenoids and enhanced systemic inflammation and oxidation in severe obesity. *Isr. Med. Assoc. J.* 2009, *11*, 598–601.

[24] Aleixandre de Artiñano, A., Miguel Castro, M., Experimental rat models to study the metabolic syndrome. *Br. J. Nutr.* 2009, *102*, 1246–1253.

[25] Yonekura, L., Kobayashi, M., Terasaki, M., Nagao, A., Keto-carotenoids are the major metabolites of dietary lutein and fucoxanthin in mouse tissues. *J. Nutr.* 2010, *140*, 1824–1831.

[26] Rodriguez, D., A Guide to Carotenoid Analysis in Foods, ILSI Press, Whashington, USA, 2001.

[27] Di Lecce, G., Martínez-Huélamo, M., Tulipani, S., Vallverdú-Queralt, A. et al., Setup of a UHPLC–QqQ-MS Method for the analysis of phenolic compounds in cherry tomatoes, tomato sauce, and tomato juice. *J. Agric. Food Chem.* 2013, *61*, 8373–8380.

[28] Capriotti, A. L., Cavaliere, C., Crescenzi, C., Foglia, P. et al., Comparison of extraction methods for the identification and quantification of polyphenols in virgin olive oil by ultra-HPLC-QToF mass spectrometry. *Food Chem.* 2014, *158*, 392–400.

[29] Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L. et al., Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Anal. Biochem.* 1982, *126*, 131–138.

[30] Justo, M. L., Rodriguez-Rodriguez, R., Claro, C. M., Alvarez De Sotomayor, M. et al., Water-soluble rice bran enzymatic extract attenuates dyslipidemia, hypertension and insulin resistance in obese Zucker rats. *Eur. J. Nutr.* 2013, *52*, 789–797.

[31] Justo, M. L., Candiracci, M., Dantas, A. P., de Sotomayor, M. A. et al., Rice bran enzymatic extract restores endothelial function and vascular contractility in obese rats by reducing vascular inflammation and oxidative stress. *J. Nutr. Biochem.* 2013, *24*, 1453–1461.

[32] Onetti, Y., Dantas, A. P., Pérez, B., Cugota, R. et al., Middle cerebral artery remodeling following transient brain ischemia is linked to early postischemic hyperemia: a target of uric acid treatment. *Am. J. Physiol. Heart Circ. Physiol.* 2015, *308*, H862–H874.

[33] Barton, M., Baretella, O., Meyer, M. R., Obesity and risk of vascular disease: importance of endothelium-dependent vasoconstriction. *Br. J. Pharmacol.* 2012, *165*, 591–602.

[34] Sansbury, B. E., Hill, B. G., Regulation of obesity and insulin resistance by nitric oxide. *Free Radic. Biol. Med.* 2014, *73*, 383–399.

[35] Wang, S., Moustaid-Moussa, N., Chen, L., Mo, H. et al., Novel insights of dietary polyphenols and obesity. *J. Nutr. Biochem.* 2014, *25*, 1–18.

[36] Assini, J. M., Mulvihill, E. E., Burke, A. C., Sutherland, B. G. et al., Naringenin prevents obesity, hepatic steatosis, and glucose intolerance in male mice independent of fibroblast growth factor 21. *Endocrinology* 2015, *156*, 2087–2102.

[37] Babu, P. V. A., Liu, D., Gilbert, E. R., Recent advances in understanding the anti-diabetic actions of dietary flavonoids. *J. Nutr. Biochem.* 2013, *24*, 1777–1789.

[38] Wu, S. Q., Hopfner, R. L., McNeill, J. R., Wilson, T. W. et al., Altered paracrine effect of endothelin in blood vessels of the hyperinsulinemic, insulin resistant obese Zucker rat. *Cardiovasc. Res.* 2000, *45*, 994–1000.

[39] Stepp, D. W., Frisbee, J. C., Augmented adrenergic vasoconstriction in hypertensive diabetic obese Zucker rats. *Am. J. Physiol. Heart Circ. Physiol.* 2002, *282*, H816–H280.

[40] Frisbee, J. C., Delp, M. D., Vascular function in the metabolic syndrome and the effects on skeletal muscle perfusion: lessons from the obese Zucker rat. *Essays Biochem.* 2006, 145–161.

[41] Martin, W., Furchgott, R. F., Villani, G. M., Jothianandan, D., Depression of contractile responses in rat aorta by spontaneously released endothelium-derived relaxing factor. *J. Pharmacol. Exp. Ther.* 1986, *237*, 529–538.

[42] Stoclet, J. C., Chataigneau, T., Ndiaye, M., Oak, M. H. et al., Vascular protection by dietary polyphenols. *Eur. J. Pharmacol.* 2004, *500*, 299–313.

[43] Schini-Kerth, V. B., Auger, C., Kim, J.-H., Etienne-Selloum, N. et al., Nutritional improvement of the endothelial control of vascular tone by polyphenols: role of NO and EDHF. *Pflugers Arch.* 2010, *459*, 853–862.

[44] Di Tomo, P., Canali, R., Ciavardelli, D., Di Silvestre, S. et al., β-Carotene and lycopene affect endothelial response to TNF-α reducing nitro-oxidative stress and interaction with monocytes. *Mol. Nutr. Food Res.* 2012, *56*, 217–227.

[45] Perez-Ternero, C., Rodriguez-Rodriguez, R., Parrado, J., Alvarez de Sotomayor, M., Grape pomace enzymatic extract restores vascular dysfunction evoked by endothelin-1 and DETCA via NADPH oxidase downregulation and SOD activation. *J. Funct. Foods* 2013, *5*, 1673–1683.

[46] Tsikas, D., Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: appraisal of the Griess reaction in the L-arginine/nitric oxide area of research. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* 2007, *851*, 51–70.

[47] Colasanti, M., Suzuki, H., The dual personality of NO. *Trends Pharmacol. Sci.* 2000, *21*, 249–252.

[48] Zahedi Asl, S., Ghasemi, A., Azizi, F., Serum nitric oxide metabolites in subjects with metabolic syndrome. *Clin. Biochem.* 2008, *41*, 1342–1347.

[49] Perreault, M., Marette, A., Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. *Nat. Med.* 2001, *7*, 1138–1143.

[50] Hotamisligil, G. S., Shargill, N. S., Spiegelman, B. M., Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 1993, *259*, 87–91.

[51] Caimi, G., Lo Presti, R., Montana, M., Noto, D. et al., Lipid peroxidation, nitric oxide metabolites, and their ratio in a group of subjects with metabolic syndrome. *Oxid. Med. Cell. Longev.* 2014, *2014* , 824756.

[52] Kossmann, S., Hu, H., Steven, S., Schönfelder, T. et al., Inflammatory monocytes determine endothelial nitric-oxide synthase uncoupling and nitro-oxidative stress induced by Angiotensin II. *J. Biol. Chem.* 2014, *289*, 27540–27550.

[53] Frisbee, J. C., Delp, M. D., Vascular function in the metabolic syndrome and the effects on skeletal muscle perfusion: lessons from the obese Zucker rat. *Essays Biochem.* 2006, *42*, 145–161.

GLOBAL DISCUSSION

5. GLOBAL DISCUSSION

5.1 Methodological considerations

In this study, two main methodologies were used to explore the effects of Mediterranean cuisine on bioactive compounds: factorial designs and foodomics. As mentioned in the introduction, factorial designs are useful exploratory analytical tools to verify which factors may influence a process[43]. Moreover, first-order models are the main tools chosen for a primary screening of which factors should be selected and be exploited in a process optimization[43,46]. In our case, the factors to be explored were the proportion of the ingredients onion, garlic, and olive oil and the cooking time could influence the bioactive compounds content in the tomato *sofrito* sauce.

In **publications 1** and **2**, a fractional factorial design was applied, in which the effect of olive oil content (5% and 10%) on different cooking times (15, 30, 45, 60 min) was first evaluated in order to understand if olive oil could help in the extraction of lipophilic compounds such as carotenoids and more polar compounds like polyphenols. This approximation indicated that olive oil act as a food excipient for both apolar and polar compounds from the food matrix. In addition, it has been indicated that depending on the nature of a compound, more and lees processing time may be a critical factor to preserve or increase the content and extraction thereof. Unfortunately, this experiment design did not allows to fully understand the role of olive oil, since it was not possible to create mathematical models from it. In addition, *tomate frito* (tomato with olive oil) is used as an ingredient and is not a representative model of Mediterranean cuisine. From these results, new hypothesis were postulated: how could olive oil extract the compounds from food matrix? Are these compounds incorporated into the oil matrix? In a more complex and real culinary process, would these results also be reproducible? Could there be a synergistic effect between compounds/ingredients that may increase the content of bioactive compounds? Could be new compounds formed during cooking process?

Firstly, to explore the Mediterranean cuisine, considering its plurality, one should choose something that is used in the daily life of that populations and its consumption is easily identified. The questionnaire of adherence to the Mediterranean diet has as a question “How many times per week do you consume vegetables, pasta, rice, or other dishes seasoned with tomato-based *sofrito*?”. The *sofrito* is a basis of many dishes of the Mediterranean diet, it consists of lightly frying onion and garlic in olive oil, to start preparing a dish or a sauce, such as tomato *sofrito* sauce. This product is known by the population, is part of the food habit and is available in commercial products. Therefore, tomato *sofrito* sauce is an excellent candidate for study how Mediterranean cuisine may influence the consumption of bioactive compounds. However, when searching in the literature for the *sofrito* composition, bioactive compounds profile and nutritional value, no results could be found. Therefore, the starting point was to characterize the composition of bioactive compounds present in commercial *sofrito* that resulted in the **publication 3**. With this

information obtained it was possible to establish the factors and domain that could influence the *sofrito* bioactive compounds composition and also the variable response that should be analyzed.

The next step was to enlarge our study incorporated a food more complex and closer to reality, with Mediterranean tomato *sofrito* sauce, a full factorial design was performed variating the ingredients content such as onion (20% and 40%), olive oil (5% and 10%) and garlic (2% and 4%) and time of cooking (30 and 60 min).With this new design, the effect of the ingredients and cooking time on the content of tomato carotenoids (**Publication 4**) and polyphenols (**Publication 5**) were studied, being a useful tool to characterize the *sofrito* technique. However this tool shows its limitations, since interaction between factors appears, with great influence in the calculated mathematical model, a second-order experimental design may be necessary to describe with precision the studied process[43]. An example is the graph describing processing time and oil content in the production of lycopene isomers (**Publication 4**), in which the principle of distortion of the graph surface. However, this does not eliminate the validity of the first-order model, the identified factors and the synergism between them.

Unfortunately, a factorial design is applied only to identify factors that influence a response pre-determinate[43,45], in our case, polyphenols and carotenoids already identified in tomato *sofrito* sauce. This approach did not allow us to obtain a global view of the changes during the cooking process. For this, the use of a foodomics approach was applied to continue exploring the Mediterranean cuisine, resulting in the **publication 7**. Using mass spectrometry combined with bioinformatics it was possible to identify markers that cause a perturbation in the chemical profile with increasing the content of each ingredient and cooking time in the *sofrito*. During cooking, ingredients may help to produce new compounds or increase compounds from other ingredients. However, foodomics is a very young science and the libraries used for identifications still need to be improved, in which most of the detected markers could not be identified.

The approximation between Mediterranean cuisine and health was done through a study using Zucker rats prone to develop obesity and metabolic syndrome with their respective controls. This model was chosen for the option of having obese and healthy groups with similar characteristic, in which the main difference would be the addition of 2% of tomato *sofrito* sauce in the diet. In this way, the *sofrito* could modify the clinical state of unhealthy individuals and at the same time its effects do not compromise the status on healthy individuals. Moreover, the *sofrito* content added in the animals feed was calculated based on the recommendation of the United States Department of Agriculture, which reported the measure of a cup of tomato sauce as 245g. According to this reason and previously studies[38,103–105,108] that establish a dose of 240g of tomato sauce for an average weight of 70 kg , the addition of 2% of *sofrito* was calculated so that intake corresponds to rats that have a minimum of 100g of weight.

5.2 Discussion of results

To promote cooking as a dietary strategy it is necessary to understand how cooking impacts the nutritional content of food, especially phytochemicals related to exert health effects, to arrive to any recommendation[12–15]. Since cooking is an essential part of our daily life, different factors such as culture, dietary pattern and culinary methods increase the complexity of the prepared dishes which can modify the bioactive compounds consumed. The MD is characterized by a high intake of phytochemicals and this diet has been correlated with improvements in metabolic and cardiovascular health[26,27]. However, the health outcomes of a MD are reportedly difficult to reproduce in non-Mediterranean populations, indicating that food preparation is one of the causes of this lack of sucess[26]. Therefore, efforts should be done to characterize Mediterranean cuisine, including its culinary process, ingredients and recipes to bring closer to Mediterranean diet and broaden the understanding of beneficial health effects. The aim of this thesis has been to study the role of traditional Mediterranean cuisine, especially home-made preparations, impact the content of bioactive compounds of tomato products, with a special focus on the *sofrito* technique, and its contribution to health effects.

In **Publication 1** and **Publication 2** of this thesis, we evaluated the role of cooking with olive oil on the extraction of bioactive compounds in tomato sauce using different content of olive oil and time of processing. The **Publication 1** showed that the presence of olive oil and thermal treatment are important for extracting carotenoids from the food matrix. Moreover, with the increase of cooking time, from 15 to 60 min, an increase in the content of α-carotene, β-carotene, lycopene *Z*-isomers and total carotenoids were observed. All-*E*-lycopene was the only carotenoid that decrease during the cooking process due to the isomerization reactions[76]. In this publication 5% of olive oil proved to extract more carotenoids compared to 10%, indicating that not only solubilization of these apolar substances in the oil is involved on the extraction from food matrix. **Publication 2** revealed also that olive oil is able to extract polar compounds such as polyphenols. Unlike the carotenoids, with a longer cooking time a decrease in polyphenol content occurs. Only caffeic acid and tyrosol showed an improvement with cooking time probably by hydrolysis reactions of glucoside phenolics[109]. The use of 10% of olive oil on tomato sauce preparation was able to extract more phenolic compounds compared to 5%. These works reveal the importance of olive oil, the main source of fat on Mediterranean diet, for Mediterranean culinary process. Literature has been exploring the consumption of olive oil, its composition and beneficial effects to health, but forgets that the main olive oil consumption form by Mediterranean's is in the dishes elaborated with this product.

Revealing the importance of the role of Mediterranean cuisine that could play the beneficial effects of the Mediterranean diet, it was proposed to explore deeper the main characteristic of this cuisine, choosing as a key element the tomato *sofrito* sauce. The starting point was to characterize the bioactive compounds present in commercial *sofrito* described in the **publication 3**. A full range of phenolic compounds found were characterized in ten different

commercial *sofritos* by high-resolution mass spectrometry in order to comprehend the phenolic diversity. 40 different polyphenols were identified and confirmed by exact mass, molecular formula, product ion scan experiments and standards, in which luteolin-*O*-hexoside, sinapic acid-*O*-hexoside, sinapic acid, quercetin-*O*-dihexoside and isoharmnetin-*O*-hexoside are reported for the first time in tomato-based products. The identification of these new compounds was probably by the addition of ingredients such as onion and garlic. A targeted approach was used to quantify phenolic compounds by LC-QqQ-MS/MS and carotenoids by LC-DAD and LC-QqQ-MS/MS revealed that ingredients impact the content of bioactive compounds and Principal Component Analysis was able to discriminate the different *sofrito* formulation by the presence of garlic, onion and olive oil. Therefore, this work allowed to identify the bioactive compounds found in *sofrito* and to identify the factors that contribute to the presence of such polyphenols and carotenoids which turned to be olive oil, onion and garlic ingredients.

Joining the results obtained from the first three publications, we observe that ingredients and cooking time can modify the profile of phytochemicals during a culinary process. To understand better how the *sofrito* technique could affect bioactive compounds a full factorial design was applied. The effect of the factors EVOO (5% and 10%), onion (20% and 40%), garlic (2% and 4%) and cooking time (30 and 60 min) were explored on carotenoid (**Publication 4**) and polyphenols (**Publication 5**) composition of tomato-*sofrito* sauce. Mathematical models were constructed in order to verify how ingredients and cooking time could affect the phytochemical content. Among carotenoids, an isomerization reaction was verified for lycopene, in which during the cooking process the content of 5, 9 and 13-*Z*-lycopene were increased. From these results, mathematical models were constructed revealing the factors that affect the isomerization process. Time was the factor that most influence lycopene isomers production and a combination of more cooking time with a high content of onion improved lycopene isomerization process. An increase in the content of EVOO seems to help to extract more lycopene from food matrix, but the combined effect of more EVOO with longer time decreases the production of these isomers, according to the result found in **Publication 1**. Unfortunately, it was no possible to construct a mathematical model for all-*E*-lycopene due to its multi-isomerization, which makes it difficult to predict its behavior[76,77]. Other carotenoids such as β-carotene and α-carotene did not showed the statistical requirements to be evaluated by mathematical models. Therefore the *sofrito* technique proved to be interesting for the production of *Z*-lycopene isomers, in which the literature has shown interest due to better bioavailability and antioxidant activity compared to the *E*-form[110,111]. Among polyphenols, 16 different polyphenols typical from tomato were evaluated, being ferulic acid, caffeic acid and caffeic acid hexoside I and II discarded for being present in the ingredients which would interfere in the evaluation of the mathematical models. An increase in the content of chlorogenic acid, naringenin and ferulic acid hexoside were found and mathematical models were constructed in order to verify how factors are involved. Again, time was the factor that most affect polyphenols, decreasing with more cooking time. The presence of a higher content of EVOO and onion was able to increase or maintain the content of these

polyphenols during cooking. EVOO is pointed out as responsible for the extraction of these compounds from the food matrix, while both onion and EVOO provide antioxidants that can prevent the oxidation of tomato compounds. Therefore, the *sofrito* technique, together with its ingredients, proved to be important for the extraction of phytochemicals from the food matrix and also in preserving them during cooking.

The extraction process of phytochemicals from the food matrix, in particular by the use of olive oil and applying thermal process, has been correlated with an increase in the bioaccessibility and bioavailability of these compounds. Martinez-Huélamo and co-workers[103] compared the bioavailability of polyphenols from raw tomato, tomato sauce and tomato sauce with refined olive oil and reported an increase in the bioavailability of polyphenols, especially naringenin glucuronide and quercetin with the presence of an oil matrix. Moreover, a double peak was found on plasma pharmacokinetics profile of naringenin glucuronide, quercetin and ferulic acid glucuronide with the sauce prepared with oil, indicating that an oil matrix favors the re-absorption of the compound through enterohepatic circulation, prolonging its permanence in the body. Arranz and co-workers[110] also reported an increase in the absorption of carotenoids, especially *E*-lycopene and 5-*Z*-lycopene after a consumption of tomato juice with oil compared to tomato juice. The role of olive oil in the extraction of polyphenols and carotenoids during cooking process influence the bioaccessibility and bioavailability of these phytochemicals, although this process has not yet been clarified. The **Publication 6** was designed to verify whether phytochemicals are incorporated into the oil matrix and this phenomenon would aid in the bioaccessibility of these compounds. A tomato *sofrito* was centrifuged and different fractions were isolated: insoluble, water and oil fraction. These fractions were analyzed by targeted approach for polyphenols composition from ingredients using a LC-QqQ-MS/MS and for carotenoids profile by LC-DAD and LC-QqQ-MS/MS. Among polyphenols, the insoluble fraction showed the higher content of phenolic compounds, follow by the oil and water fraction, indicating that most part of phenolics are still bound to the food matrix of tomato, onion and garlic. However, the presence of polyphenols in oil that were not present in EVOO before cooking, such as naringenin, ferulic acid and quercetin, shows that part of the polyphenols are incorporated into the oily matrix (Figure 6). Precisely these compounds are those that were detected by Martinez-Huélamo and co-workers[103] for tomato sauce with oil. Among carotenoids, again insoluble fraction showed the highest content of total carotenoids, follow by oil and water fraction, but it is detachable that carotenoids were not detected in EVOO before cooking. Oil matrix exhibited a high content of lycopene *Z*-isomers attributed by the structure and size of these molecules that favors its solubilization on oil[61] (Figure 6). Moreover, di-*Z*-isomers of lycopene was detected, very unstable molecules, indicating that the oil fraction in a Mediterranean dish could be a stable environment for antioxidants. The presence of polyphenols and carotenoids in the oil fraction, especially those that are detected in bioavailability studies with tomato products, may indicate that cooking with olive oil favors the absorption of phytochemicals[103,110].

Promoting a step forward in the study of Mediterranean cuisine, an untargeted approach by foodomics was carried out as the ingredients and cooking time could cause a change in the chemical profile of tomato *sofrito* and could also be contributing new compounds not yet explored by literature. The **Publication 7** perform the *sofrito* analysis by LC-LQT-Orbitrap-MS/MS allied to bioinformatics treatments in order to find differences in the chemical profile of these sauces by ingredients and cooking time. The multivariate analysis applying a Principal Component Analysis using two components revealed that the increase of the different ingredients modified the chemical profile, while an increase in cooking time did not, revealing that *sofrito* technique is not so aggressive to the composite profile. Using the univariate analysis, markers for 10% of olive oil (224), 40% of onion (666), 4% of garlic (276) and 60 min of cooking time (226) were detected that differ from the control sauce (5% EVOO, 20% onion, 2% garlic and 30 min cooking time). New classes of compounds not explore in tomato products such as phytoprostanes and hydroxycinnamic acid amides were detected and which are not exploited in relation to their bioactivity. Other compounds such as amino acids, thiosulfates and phenolics also are affected (Figure 7). This approach allows to know the influence of ingredients and time on cooking in a large number of chemical classes, opening up the opportunity to explore new compounds to be correlated with the beneficial effects of the Mediterranean cuisine, diet and to improve nutritional recommendations.

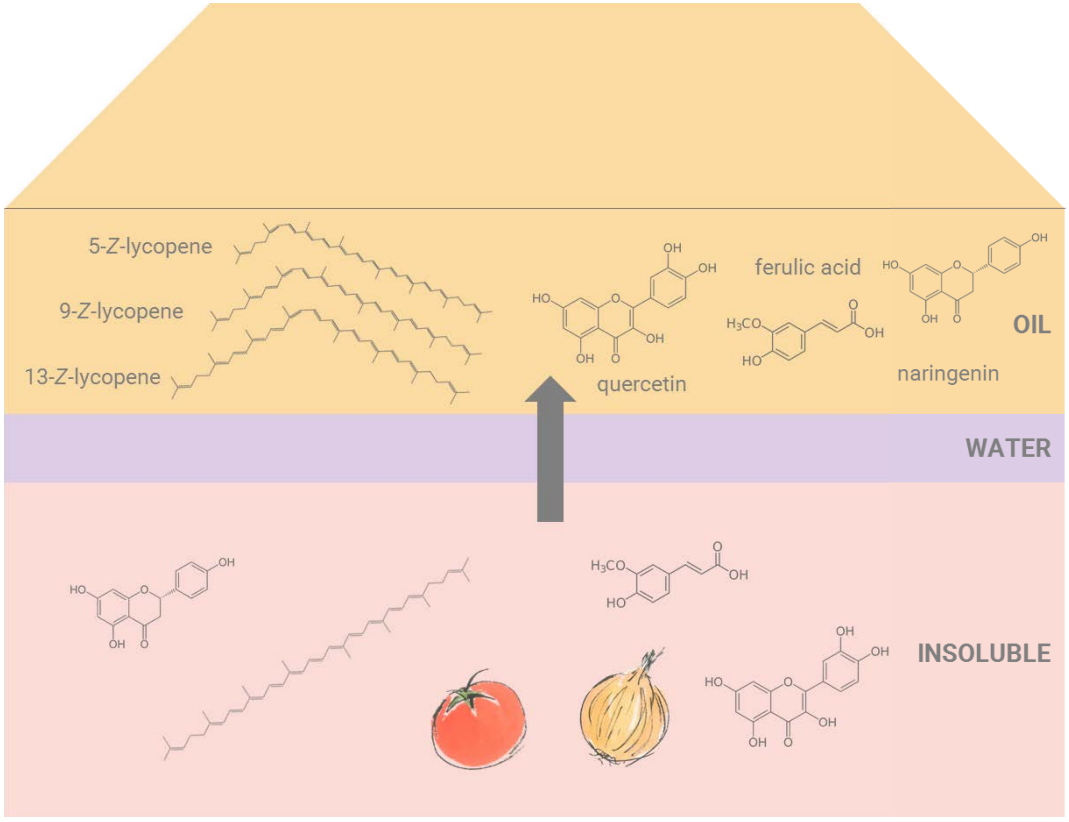

Figure 6. Carotenoid and polyphenols incorporation to olive oil from food matrix during *sofrito* cooking process (**Publication 6**)

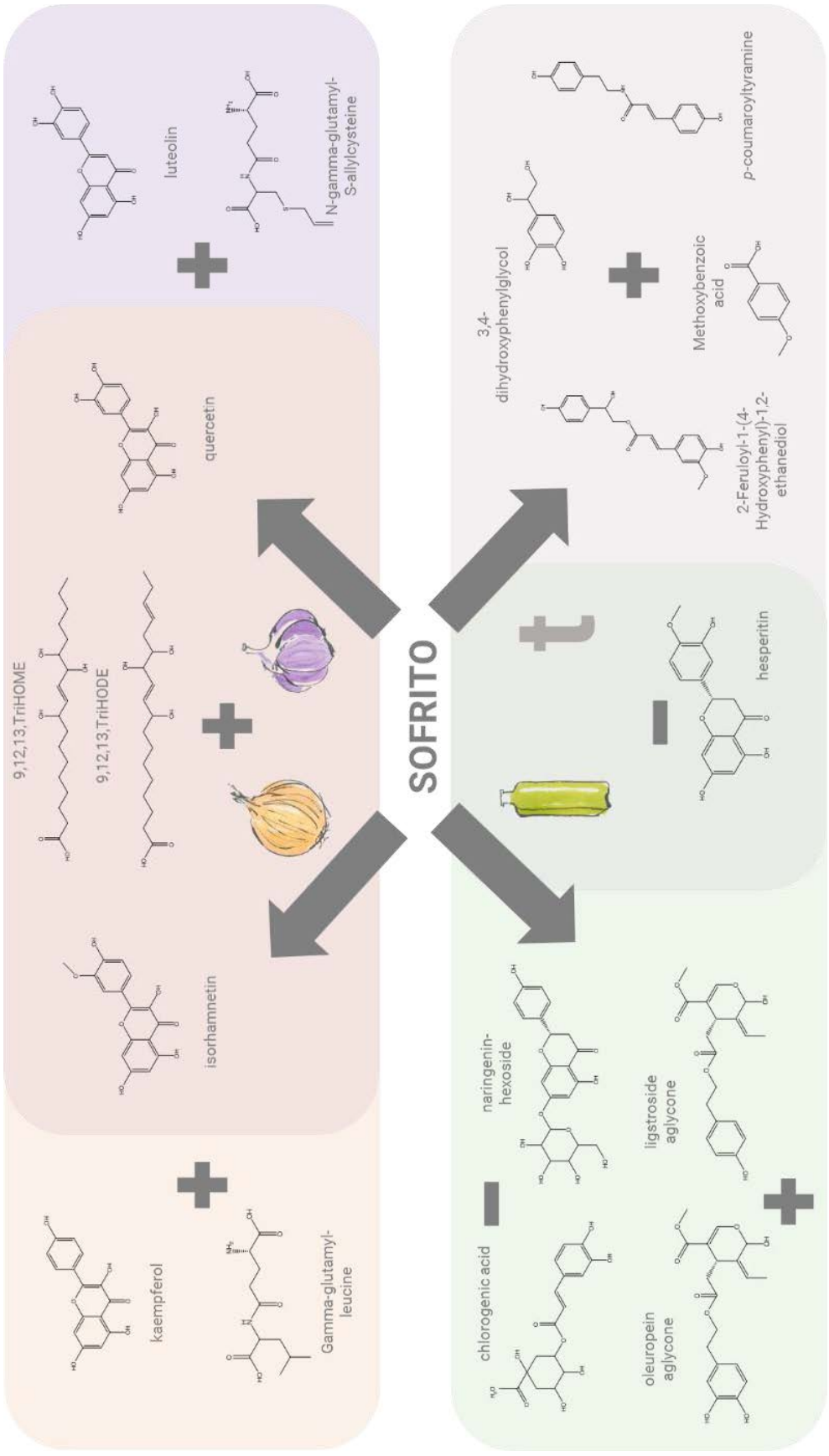

Figure 7. Effect of ingredients addition and cooking time on the chemical profile of tomato *sofrito* sauce (**Publication 7**).

The multiapproach was able to identify different mechanisms by which Mediterranean cuisine could contribute to the beneficial effects of the Mediterranean diet and open new approaches to be explored in gastronomy such as foodomics. However, a more concrete approximation between the Mediterranean cuisine and health should be carried on. Previous studies have shown that adding olive oil in tomato sauce was able to decrease inflammatory biomarkers related to atherosclerosis by increasing lycopene absorption[108] and regulate lipid profile and improve cardiovascular health[38]. The **Publication 8** explores the effect of *sofrito* intake on vascular parameters in obesity rats compared with their healthy control. The obese group, whose diet contained 2% of *sofrito*, showed hyperphagia and a high calories intake, however no differences were observed in weight gain compared to the obese control. The *sofrito* consumption was able to restore the effect of NO on aorta contraction process that is lost due to obesity. In addition, there has been a decrease in reactive species of N_2 and O_2 that help to decrease the inflammatory state provoke by obesity. For that, a short to medium term daily intake of *sofrito* prevents vascular alterations that could precede cardiometabolic complications associated with obesity. Understand the Mediterranean cuisine, taking into accounts its products, culinary techniques and culture brings us closer to a global vision of the Mediterranean Diet and its beneficial effects to health.

CONCLUSION

6. CONCLUSION

1. Addition of olive oil combined with thermal processing helps to extract polyphenols and carotenoids from tomato sauce.

2. A longer cooking time with a smaller amount of olive oil provided higher content of carotenoids in tomato sauce with olive oil, however the opposite conditions is applied to improve polyphenols at the same matrix.

3. Tomato *sofrito* sauce, a key element of Mediterranean diet, was characterized for the first time and its content of polyphenol and carotenoids quantified.

4. A recipe, varying the content of the ingredients and cooking time applying a full factorial design, impacts the chemical profile of the tomato *sofrito* sauce.

5. For carotenoids, time was the factor that most contributed to its increase. The presence of olive oil helps the extraction, however high content of oil with long time diminish its content. Onion was the ingredient that increased lycopene isomerization.

6. For polyphenols, time was the factor that most contributed to its decrease. The presence of olive oil and onion helps to preserve polyphenols from tomato during the *sofrito* cooking process.

7. Olive oil acts as a vehicle, in which polyphenols and carotenoids from other matrices are incorporated, which could increase the bioaccessibility and bioavailability of these compounds.

8. The identification of compounds such as di-*Z*-lycopenes, which are difficult to found in food, indicate a high stability of the oil fraction from tomato *sofrito*.

9. Foodomics unveiled chemical classes of compounds extracted/formed during the *sofrito* cooking process that open new ideas to explore about cooking and bioactive compounds.

10. Chronic consumption of *sofrito* helps to prevent vascular alterations that could result in cardiovascular diseases associated with obesity.

7. REFERENCES

1. Carmody RN, Weintraub GS, Wrangham RW. Energetic consequences of thermal and nonthermal food processing. *Proc Natl Acad Sci*. 2011;108(48):19199-19203. doi:10.1073/pnas.1112128108
2. Armelagos GJ. Brain Evolution, the Determinates of Food Choice, and the Omnivore's Dilemma. *Crit Rev Food Sci Nutr*. 2014;54(10):1330-1341. doi:10.1080/10408398.2011.635817
3. Pellegrini N, Fogliano V. Cooking, industrial processing and caloric density of foods. *Curr Opin Food Sci*. 2017;14:98-102. doi:10.1016/j.cofs.2017.02.006
4. van Boekel M, Fogliano V, Pellegrini N, et al. A review on the beneficial aspects of food processing. *Mol Nutr Food Res*. 2010;54(9):1215-1247. doi:10.1002/mnfr.200900608
5. Mills S, White M, Brown H, et al. Health and social determinants and outcomes of home cooking: A systematic review of observational studies. *Appetite*. 2017;111:116-134. doi:10.1016/j.appet.2016.12.022
6. Möser A. Food preparation patterns in German family households. An econometric approach with time budget data. *Appetite*. 2010;55(1):99-107. doi:10.1016/j.appet.2010.04.008
7. Mills S, Brown H, Wrieden W, White M, Adams J. Frequency of eating home cooked meals and potential benefits for diet and health: cross-sectional analysis of a population-based cohort study. *Int J Behav Nutr Phys Act*. 2017;14(1):109. doi:10.1186/s12966-017-0567-y
8. Lobato JCP, Costa AJL, Sichieri R. Food intake and prevalence of obesity in Brazil: an ecological analysis. *Public Health Nutr*. 2009;12(11):2209-2215. doi:10.1017/S1368980009005527
9. Tiwari A, Aggarwal A, Tang W, Drewnowski A. Cooking at Home: A Strategy to Comply With U.S. Dietary Guidelines at No Extra Cost. *Am J Prev Med*. 2017;52(5):616-624. doi:10.1016/j.amepre.2017.01.017
10. Chu YL, Storey KE, Veugelers PJ. Involvement in Meal Preparation at Home Is Associated With Better Diet Quality Among Canadian Children. *J Nutr Educ Behav*. 2014;46(4):304-308. doi:10.1016/j.jneb.2013.10.003
11. da Rocha Leal FM, de Oliveira BMPM, Pereira SSR. Relationship between cooking habits and skills and Mediterranean diet in a sample of Portuguese adolescents. *Perspect Public Health*. 2011;131(6):283-287. doi:10.1177/1757913911419909
12. Murador D, Braqa AR, Cunha D Da, Rosso V de. Alterations in phenolic compound levels and antioxidant activity in response to cooking technique effects: A meta-analytic investigation. *Crit Rev Food Sci Nutr*. 2016;8398(ja):00-00. doi:10.1080/10408398.2016.1140121
13. Murador DC, da Cunha DT, de Rosso VV. Effects of cooking techniques on vegetable pigments: A meta-analytic approach to carotenoid and anthocyanin levels. *Food Res Int*. 2014;65(PB):177-183. doi:10.1016/j.foodres.2014.06.015
14. Palermo M, Pellegrini N, Fogliano V. The effect of cooking on the phytochemical content of vegetables. *J Sci Food Agric*. 2014;94(6):1057-1070. doi:10.1002/jsfa.6478
15. Zhao C, Liu Y, Lai S, et al. Effects of domestic cooking process on the chemical and biological properties of dietary phytochemicals. *Trends Food Sci Technol*. 2019;85(December 2017):55-66. doi:10.1016/j.tifs.2019.01.004
16. González Turmo I. Chapter 5. The Mediterranean Diet: consumption, cuisine and food habits. In: *MediTERRA 2012 The Mediterranean Diet for Sustainable Regional Development*. Vol 2012. ; 2012:115-132. http://www.cairn.info/article.php?ID_ARTICLE=SCPO_CHEA_2012_02_0115.

17. Caporaso N, Formisano D. Developments, applications, and trends of molecular gastronomy among food scientists and innovative chefs. *Food Rev Int*. 2016;32(4):417-435. doi:10.1080/87559129.2015.1094818

18. This H. Molecular Gastronomy, a Scientific Look at Cooking. *Acc Chem Res*. 2009;42(5):575-583. doi:10.1021/ar8002078

19. Aguilera JM. Relating Food Engineering to Cooking and Gastronomy. *Compr Rev Food Sci Food Saf*. 2018;17(4):1021-1039. doi:10.1111/1541-4337.12361

20. Vega C, Ubbink J. Molecular gastronomy: a food fad or science supporting innovative cuisine? *Trends Food Sci Technol*. 2008;19(7):372-382. doi:10.1016/j.tifs.2008.01.006

21. Barham P, Skibsted LH, Bredie WLP, et al. Molecular Gastronomy: A New Emerging Scientific Discipline. *Chem Rev*. 2010;110(4):2313-2365. doi:10.1021/cr900105w

22. Aguilera JM. The emergence of gastronomic engineering. *Innov Food Sci Emerg Technol*. 2017;41:277-283. doi:10.1016/j.ifset.2017.03.017

23. This H. The science of molecular gastronomy and the art of innovative cooking. *FEBS Lett*. April 2019:1873-3468.13373. doi:10.1002/1873-3468.13373

24. This H. Molecular Gastronomy in France. *J Culin Sci Technol*. 2011;9(3):140-149. doi:10.1080/15428052.2011.600212

25. Nicoli M., Anese M, Parpinel M. Influence of processing on the antioxidant properties of fruit and vegetables. *Trends Food Sci Technol*. 1999;10(3):94-100. doi:10.1016/S0924-2244(99)00023-0

26. Hoffman R, Gerber M. Food Processing and the Mediterranean Diet. *Nutrients*. 2015;7(9):7925-7964. doi:10.3390/nu7095371

27. Estruch R, Ros E, Salas-Salvadó J, et al. Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts. *N Engl J Med*. 2018;378(25):e34. doi:10.1056/NEJMoa1800389

28. Martínez-González MA, Salas-Salvadó J, Estruch R, Corella D, Fitó M, Ros E. Benefits of the Mediterranean Diet: Insights From the PREDIMED Study. *Prog Cardiovasc Dis*. 2015;58(1):50-60. doi:10.1016/j.pcad.2015.04.003

29. Czerner M, Agustinelli SP, Guccione S, Yeannes MI. Effect of different preservation processes on chemical composition and fatty acid profile of anchovy (Engraulis anchoita). *Int J Food Sci Nutr*. 2015;66(8):887-894. doi:10.3109/09637486.2015.1110687

30. Sacchi R, Paduano A, Savarese M, Vitaglione P, Fogliano V. Extra Virgin Olive Oil: From Composition to "Molecular Gastronomy." In: *Advances in Nutrition and Cancer*. Vol 26. ; 2014:325-338. doi:10.1007/978-3-642-38007-5_19

31. Giambanelli E, Verkerk R, Fogliano V, Capuano E, D'Antuono LF, Oliviero T. Broccoli glucosinolate degradation is reduced performing thermal treatment in binary systems with other food ingredients. *RSC Adv*. 2015;5(82):66894-66900. doi:10.1039/C5RA11409H

32. Ramírez-Anaya JDP, Samaniego-Sánchez C, Castañeda-Saucedo MC, Villalón-Mir M, De La Serrana HLG. Phenols and the antioxidant capacity of Mediterranean vegetables prepared with extra virgin olive oil using different domestic cooking techniques. *Food Chem*. 2015;188:430-438. doi:10.1016/j.foodchem.2015.04.124

33. Ahn YY, Ahnert SE, Bagrow JP, Barabási AL. Flavor network and the principles of food pairing. *Sci Rep*. 2011;1:1-7. doi:10.1038/srep00196

34. Tomas M, Beekwilder J, Hall RD, Sagdic O, Boyacioglu D, Capanoglu E. Industrial processing versus home processing of tomato sauce: Effects on phenolics, flavonoids and in vitro bioaccessibility of antioxidants. *Food Chem*. 2017;220:51-58. doi:10.1016/j.foodchem.2016.09.201

35. Agricultura DE. Informe del consumo de alimentación en España.

36. Rodriguez-Rodriguez R, Jiménez-Altayó F, Alsina L, et al. Mediterranean tomato-based sofrito protects against vascular alterations in obese Zucker rats by preserving NO bioavailability. *Mol Nutr Food Res*. 2017;61(9):1601010. doi:10.1002/mnfr.201601010

37. Agarwal S, Rao AV. Tomato lycopene and its role in human health and chronic diseases. 2000;163(6):23-26.

38. Valderas-Martinez P, Chiva-Blanch G, Casas R, et al. Tomato sauce enriched with olive oil exerts greater effects on cardiovascular disease risk factors than raw tomato and tomato sauce: A randomized trial. *Nutrients*. 2016;8(3):1-14. doi:10.3390/nu8030170

39. Vallverdú-Queralt A, Alvarenga JFR De, Estruch R, Lamuela-Raventos RM. Bioactive compounds present in the Mediterranean sofrito. *Food Chem*. 2013;141(4):3366-3372. doi:10.1016/j.foodchem.2013.06.032

40. Martínez-González MA, García-Arellano A, Toledo E, et al. A 14-Item Mediterranean Diet Assessment Tool and Obesity Indexes among High-Risk Subjects: The PREDIMED Trial. Peiró C, ed. *PLoS One*. 2012;7(8):e43134. doi:10.1371/journal.pone.0043134

41. Sandoval V, Rodríguez-Rodríguez R, Martínez-Garza Ú, et al. Mediterranean Tomato-Based Sofrito Sauce Improves Fibroblast Growth Factor 21 (FGF21) Signaling in White Adipose Tissue of Obese ZUCKER Rats. *Mol Nutr Food Res*. 2018;62(4):1700606. doi:10.1002/mnfr.201700606

42. Stojanovic J, Giraldi L, Arzani D, et al. Adherence to Mediterranean diet and risk of prostate cancer. *Eur J Cancer Prev*. 2017;26(6):491-496. doi:10.1097/CEJ.0000000000000371

43. Tarley CRT, Silveira G, dos Santos WNL, et al. Chemometric tools in electroanalytical chemistry: Methods for optimization based on factorial design and response surface methodology. *Microchem J*. 2009;92(1):58-67. doi:10.1016/j.microc.2009.02.002

44. Seyed Shahabadi SM, Reyhani A. Optimization of operating conditions in ultrafiltration process for produced water treatment via the full factorial design methodology. *Sep Purif Technol*. 2014;132:50-61. doi:10.1016/j.seppur.2014.04.051

45. Hanrahan G, Lu K. Application of Factorial and Response Surface Methodology in Modern Experimental Design and Optimization. *Crit Rev Anal Chem*. 2006;36(3-4):141-151. doi:10.1080/10408340600969478

46. Arteaga GE, Li-Chan E, Vazquez-Arteaga MC, Nakai S. Systematic experimental designs for product formula optimization. *Trends Food Sci Technol*. 1994;5(8):243-254. doi:10.1016/0924-2244(94)90017-5

47. Cifuentes A. Food analysis and Foodomics. *J Chromatogr A*. 2009;1216(43):7109. doi:10.1016/j.chroma.2009.09.018

48. Herrero M, Simó C, García-Cañas V, Ibáñez E, Cifuentes A. Foodomics: MS-based strategies in modern food science and nutrition. *Mass Spectrom Rev*. 2012;31(1):49-69. doi:10.1002/mas.20335

49. Braconi D, Bernardini G, Millucci L, Santucci A. Foodomics for human health: current status and perspectives. *Expert Rev Proteomics*. 2018;15(2):153-164. doi:10.1080/14789450.2018.1421072

50. Ulaszewska MM, Weinert CH, Trimigno A, et al. Nutrimetabolomics: An Integrative Action for Metabolomic Analyses in Human Nutritional Studies. *Mol Nutr Food Res*. 2019;63(1):1800384. doi:10.1002/mnfr.201800384

51. Andjelković U, Gajdošik MŠ, Gašo-Sokač D, Martinović T, Josić D. Foodomics and food safety: Where we are. *Food Technol Biotechnol*. 2017;55(3):290-307. doi:10.17113/ftb.55.03.17.5044

52. Cuadros-Rodríguez L, Ruiz-Samblás C, Valverde-Som L, Pérez-Castaño E, González-Casado A. Chromatographic fingerprinting: An innovative approach for food “identitation” and food authentication – A tutorial. *Anal Chim Acta*. 2016;909:9-23. doi:10.1016/j.aca.2015.12.042

53. Vallverdú-Queralt A, Lamuela-Raventós RM. Foodomics: A new tool to differentiate between organic and conventional foods. *Electrophoresis*. 2016;37(13):1784-1794. doi:10.1002/elps.201500348

54. Bordoni A, Laghi L, Babini E, et al. The foodomics approach for the evaluation of protein bioaccessibility in processed meat upon in vitro digestion. *Electrophoresis*. 2014;35(11):1607-1614. doi:10.1002/elps.201300579

55. Carbonell-Capella JM, Buniowska M, Barba FJ, Esteve MJ, Frígola A. Analytical Methods for Determining Bioavailability and Bioaccessibility of Bioactive Compounds from Fruits and Vegetables: A Review. *Compr Rev Food Sci Food Saf*. 2014;13(2):155-171. doi:10.1111/1541-4337.12049

56. Aguilera JM. The food matrix: implications in processing, nutrition and health. *Crit Rev Food Sci Nutr*. 2018;0(0):1-18. doi:10.1080/10408398.2018.1502743

57. Chacón-Ordóñez T, Carle R, Schweiggert R. Bioaccessibility of carotenoids from plant and animal foods. *J Sci Food Agric*. 2019;99(7):3220-3239. doi:10.1002/jsfa.9525

58. Kopec RE, Failla ML. Recent advances in the bioaccessibility and bioavailability of carotenoids and effects of other dietary lipophiles. *J Food Compos Anal*. 2018;68(January 2017):16-30. doi:10.1016/j.jfca.2017.06.008

59. Reboul E. Absorption of Vitamin A and Carotenoids by the Enterocyte: Focus on Transport Proteins. *Nutrients*. 2013;5(9):3563-3581. doi:10.3390/nu5093563

60. Tyssandier V, Choubert G, Grolier P, Borel P. Carotenoids, Mostly the Xanthophylls, Exchange Between Plasma Lipoproteins. *Int J Vitam Nutr Res*. 2002;72(5):300-308. doi:10.1024/0300-9831.72.5.300

61. Britton G. SERIAL Structure function. *FASEB J*. 1995;9(15):1551-1558.

62. Castenmiller JJM, West CE. BIOAVAILABILITY AND BIOCONVERSION OF CAROTENOIDS. *Annu Rev Nutr*. 1998;18(1):19-38. doi:10.1146/annurev.nutr.18.1.19

63. Boileau TW-M, Boileau AC, Erdman JW. Bioavailability of all-trans and cis–Isomers of Lycopene. *Exp Biol Med*. 2002;227(10):914-919. doi:10.1177/153537020222701012

64. Schweiggert RM, Carle R. Carotenoid deposition in plant and animal foods and its impact on bioavailability. *Crit Rev Food Sci Nutr*. 2017;57(9):1807-1830. doi:10.1080/10408398.2015.1012756

65. Schweiggert RM, Mezger D, Schimpf F, Steingass CB, Carle R. Influence of chromoplast morphology on carotenoid bioaccessibility of carrot, mango, papaya, and tomato. *Food Chem*. 2012;135(4):2736-2742. doi:10.1016/j.foodchem.2012.07.035

66. Schweiggert RM, Kopec RE, Villalobos-Gutierrez MG, et al. Carotenoids are more bioavailable from papaya than from tomato and carrot in humans: a randomised cross-over study. *Br J Nutr*. 2014;111(03):490-498. doi:10.1017/S0007114513002596

67. Cervantes-Paz B, Victoria-Campos CI, Ornelas-Paz J de J. Absorption of Carotenoids and Mechanisms Involved in Their Health-Related Properties. In: Vol 79. ; 2016:415-454. doi:10.1007/978-3-319-39126-7_16

68. Livny O, Reifen R, Levy I, et al. β-carotene bioavailability from differently processed carrot meals in human ileostomy volunteers. *Eur J Nutr*. 2003;42(6):338-345. doi:10.1007/s00394-003-0430-6

69. Verrijssen TAJ, Christiaens S, Verkempinck SHE, et al. In vitro β-Carotene Bioaccessibility and Lipid Digestion in Emulsions: Influence of Pectin Type and Degree of Methyl-Esterification. *J Food Sci*. 2016;81(10):C2327-C2336. doi:10.1111/1750-3841.13408

70. Sahlin E, Savage G., Lister C. Investigation of the antioxidant properties of tomatoes after processing. *J Food Compos Anal*. 2004;17(5):635-647. doi:10.1016/j.jfca.2003.10.003

71. Rinaldi de Alvarenga JF, Tran C, Hurtado-Barroso S, Martinez-Huélamo M, Illan M, Lamuela-Raventos RM. Home cooking and ingredient synergism improve lycopene isomer

production in Sofrito. *Food Res Int*. 2017;99:851-861. doi:10.1016/j.foodres.2017.01.009

72. Vallverdú-Queralt A, Regueiro J, de Alvarenga J, Torrado X, Lamuela-Raventos R. Carotenoid Profile of Tomato Sauces: Effect of Cooking Time and Content of Extra Virgin Olive Oil. *Int J Mol Sci*. 2015;16(12):9588-9599. doi:10.3390/ijms16059588

73. Mayeaux M, Xu Z, King JM, Prinyawiwatkul W. Effects of Cooking Conditions on the Lycopene Content in Tomatoes. *J Food Sci*. 2006;71(8):C461-C464. doi:10.1111/j.1750-3841.2006.00163.x

74. Boon CS, McClements DJ, Weiss J, Decker EA. Factors Influencing the Chemical Stability of Carotenoids in Foods. *Crit Rev Food Sci Nutr*. 2010;50(6):515-532. doi:10.1080/10408390802565889

75. Rodriguez EB, Rodriguez-Amaya DB. Lycopene Epoxides and Apo-Lycopenals Formed by Chemical Reactions and Autoxidation in Model Systems and Processed Foods. *J Food Sci*. 2009;74(9):C674-C682. doi:10.1111/j.1750-3841.2009.01353.x

76. Colle IJP, Lemmens L, Knockaert G, Van Loey A, Hendrickx M. Carotene Degradation and Isomerization during Thermal Processing: A Review on the Kinetic Aspects. *Crit Rev Food Sci Nutr*. 2016;56(11):1844-1855. doi:10.1080/10408398.2013.790779

77. Colle IJP, Lemmens L, Van Buggenhout S, Van Loey AM, Hendrickx ME. Modeling Lycopene Degradation and Isomerization in the Presence of Lipids. *Food Bioprocess Technol*. 2013;6(4):909-918. doi:10.1007/s11947-011-0714-4

78. De Sá MC, Rodriguez-Amaya DB. Carotenoid composition of cooked green vegetables from restaurants. *Food Chem*. 2003;83(4):595-600. doi:10.1016/S0308-8146(03)00227-9

79. Mutsokoti L, Panozzo A, Van Loey A, Hendrickx M. Carotenoid transfer to oil during thermal processing of low fat carrot and tomato particle based suspensions. *Food Res Int*. 2016;86:64-73. doi:10.1016/j.foodres.2016.05.019

80. Lemmens L, Colle I, Van Buggenhout S, Palmero P, Van Loey A, Hendrickx M. Carotenoid bioaccessibility in fruit- and vegetable-based food products as affected by product (micro)structural characteristics and the presence of lipids: A review. *Trends Food Sci Technol*. 2014;38(2):125-135. doi:10.1016/j.tifs.2014.05.005

81. Palmero P, Panozzo A, Simatupang D, Hendrickx M, Van Loey A. Lycopene and β-carotene transfer to oil and micellar phases during in vitro digestion of tomato and red carrot based-fractions. *Food Res Int*. 2014;64:831-838. doi:10.1016/j.foodres.2014.08.022

82. Kamiloglu S, Demirci M, Selen S, Toydemir G, Boyacioglu D, Capanoglu E. Home processing of tomatoes (Solanum lycopersicum): effects on in vitro bioaccessibility of total lycopene, phenolics, flavonoids, and antioxidant capacity. *J Sci Food Agric*. 2014;94(11):2225-2233. doi:10.1002/jsfa.6546

83. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. *Am J Clin Nutr*. 2004;79(5):727-747. http://www.ncbi.nlm.nih.gov/pubmed/15113710. Accessed December 1, 2017.

84. Tsao R. Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients*. 2010;2(12):1231-1246. doi:10.3390/nu2121231

85. Del Rio D, Rodriguez-Mateos A, Spencer JPE, Tognolini M, Borges G, Crozier A. Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases. *Antioxid Redox Signal*. 2013;18(14):1818-1892. doi:10.1089/ars.2012.4581

86. Spencer JPE, Chowrimootoo G, Choudhury R, Debnam ES, Srai SK, Rice-Evans C. The small intestine can both absorb and glucuronidate luminal flavonoids. *FEBS Lett*. 1999;458(2):224-230. doi:10.1016/S0014-5793(99)01160-6

87. Santhakumar AB, Battino M, Alvarez-Suarez JM. Dietary polyphenols: Structures, bioavailability and protective effects against atherosclerosis. *Food Chem Toxicol*. 2018;113(February):49-65. doi:10.1016/j.fct.2018.01.022

88. Bohn T. Dietary factors affecting polyphenol bioavailability. *Nutr Rev*. 2014;72(7):429-452. doi:10.1111/nure.12114

89. Zanotti I, Dall'Asta M, Mena P, et al. Atheroprotective effects of (poly)phenols: a focus on cell cholesterol metabolism. *Food Funct*. 2015;6(1):13-31. doi:10.1039/C4FO00670D

90. Day AJ, Cañada FJ, Díaz JC, et al. Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. *FEBS Lett*. 2000;468(2-3):166-170. doi:10.1016/S0014-5793(00)01211-4

91. Gee JM, DuPont MS, Day AJ, Plumb GW, Williamson G, Johnson IT. Intestinal Transport of Quercetin Glycosides in Rats Involves Both Deglycosylation and Interaction with the Hexose Transport Pathway. *J Nutr*. 2000;130(11):2765-2771. doi:10.1093/jn/130.11.2765

92. Saura-Calixto F, Goñi I. Antioxidant capacity of the Spanish Mediterranean diet. *Food Chem*. 2006;94(3):442-447. doi:10.1016/j.foodchem.2004.11.033

93. Pérez-Jiménez J, Díaz-Rubio ME, Saura-Calixto F. Non-extractable polyphenols, a major dietary antioxidant: occurrence, metabolic fate and health effects. *Nutr Res Rev*. 2013;26(2):118-129. doi:10.1017/S0954422413000097

94. Tresserra-Rimbau A, Guasch-Ferré M, Salas-Salvadó J, et al. Intake of Total Polyphenols and Some Classes of Polyphenols Is Inversely Associated with Diabetes in Elderly People at High Cardiovascular Disease Risk. *J Nutr*. 2016;146(4):767-777. doi:10.3945/jn.115.223610

95. Tresserra-Rimbau A, Rimm EB, Medina-Remón A, et al. Polyphenol intake and mortality risk: a re-analysis of the PREDIMED trial. *BMC Med*. 2014;12:77. doi:10.1186/1741-7015-12-77

96. Gutiérrez-Grijalva EP, Ambriz-Pere DL, Leyva-Lopez N, Castillo-Lopez RI, Heiedia JB. Review: dietary phenolic compounds, health benefits and bioaccessibility. *Arch Latinoam Nutr*. 2016;66(2):87-100. http://www.ncbi.nlm.nih.gov/pubmed/29737665.

97. Viskupicŏvá J, Ondrejovič M, Šturdík E. The potential and practical applications of acylated flavonoids. *Pharmazie*. 2009;64(6):355-360. doi:10.1691/ph.2009.9501

98. Walle T. Methylation of Dietary Flavones Increases Their Metabolic Stability and Chemopreventive Effects. *Int J Mol Sci*. 2009;10(11):5002-5019. doi:10.3390/ijms10115002

99. TAMURA M, IWAMI T, HIRAYAMA K, ITOH K. High Fiber Diet Supplemented with Rice Bran Hemicellulose May Reduce Daidzein Absorption in Mice. *Food Sci Technol Res*. 2009;15(2):141-146. doi:10.3136/fstr.15.141

100. Larkin TA, Price WE, Astheimer LB. Increased probiotic yogurt or resistant starch intake does not affect isoflavone bioavailability in subjects consuming a high soy diet. *Nutrition*. 2007;23(10):709-718. doi:10.1016/j.nut.2007.06.010

101. Tamura M, Nakagawa H, Tsushida T, Hirayama K, Itoh K. Effect of Pectin Enhancement on Plasma Quercetin and Fecal Flora in Rutin-Supplemented Mice. *J Food Sci*. 2007;72(9):S648-S651. doi:10.1111/j.1750-3841.2007.00557.x

102. Tomas M, Beekwilder J, Hall RD, Diez Simon C, Sagdic O, Capanoglu E. Effect of dietary fiber (inulin) addition on phenolics and in vitro bioaccessibility of tomato sauce. *Food Res Int*. 2018;106(December 2017):129-135. doi:10.1016/j.foodres.2017.12.050

103. Martínez-Huélamo M, Vallverdú-Queralt A, Di Lecce G, et al. Bioavailability of tomato polyphenols is enhanced by processing and fat addition: Evidence from a randomized feeding trial. *Mol Nutr Food Res*. 2016;60(7):1578-1589. doi:10.1002/mnfr.201500820

104. Tulipani S, Martinez Huelamo M, Rotches Ribalta M, et al. Oil matrix effects on plasma exposure and urinary excretion of phenolic compounds from tomato sauces: Evidence from a human pilot study. *Food Chem*. 2012;130(3):581-590. doi:10.1016/j.foodchem.2011.07.078

105. Martínez-Huélamo M, Tulipani S, Estruch R, et al. The tomato sauce making process affects the bioaccessibility and bioavailability of tomato phenolics: A pharmacokinetic study. *Food Chem*. 2015;173:864-872. doi:10.1016/j.foodchem.2014.09.156

106. Natella F, Belelli F, Ramberti A, Scaccini C. Microwave and Traditional Cooking Methods: Effect of Cooking on Antioxidant Capacity and Phenolic Ccompounds Content of Seven Vegetables. *J Food Biochem*. 2010;34(4):no-no. doi:10.1111/j.1745-4514.2009.00316.x

107. Dolinsky M, Agostinho C, Ribeiro D, et al. Effect of different cooking methods on the polyphenol concentration and antioxidant capacity of selected vegetables. *J Culin Sci Technol*. 2016;14(1):1-12. doi:10.1080/15428052.2015.1058203

108. Colmán-Martínez M, Martínez-Huélamo M, Valderas-Martínez P, et al. trans-Lycopene from tomato juice attenuates inflammatory biomarkers in human plasma samples: an intervention trial. *Mol Nutr Food Res*. 2017;201600993:1-9. doi:10.1002/mnfr.201600993

109. Re R, Bramley PM, Rice-Evans C. Effects of food processing on flavonoids and lycopene status in a Mediterranean tomato variety. *Free Radic Res*. 2002;36(7):803-810. doi:10.1080/10715760290032584

110. Arranz S, Martínez-Huélamo M, Vallverdu-Queralt A, et al. Influence of olive oil on carotenoid absorption from tomato juice and effects on postprandial lipemia. *Food Chem*. 2015;168:203-210. doi:10.1016/j.foodchem.2014.07.053

111. Bohm V, Puspitasari-Nienaber NL, Ferruzzi MG, Schwartz SJ. Trolox equivalent antioxidant capacity of different geometrical isomers of α-carotene, β-carotene, lycopene, and zeaxanthin. *J Agric Food Chem*. 2002;50(1):221-226. doi:10.1021/jf010888q

8. ANNEX

8.1 Other research articles

Publication 9
Authors: Vallverdú-Queralt, Anna; Regueiro, Jorge; Martínez-Huélamo, Miriam; Alvarenga, José Fernando Rinaldi; Leal, Leonel Neto; Lamuela-Raventos, Rosa M.
Title: A comprehensive study on the phenolic profile of widely used culinary herbs and spices: rosemary, thyme, oregano, cinnamon, cumin and bay.
Journal: Food Chemistry
Volume: 154**;** **Pages:** 299-307**;** **Year:** 2014

Publication 10
Authors: Bisconsin-Junior, A.; Alvarenga, J. F. R.; Rosenthal, A.; Monteiro, M.
Title: Effect of high hydrostatic pressure on ascorbic acid, phenolic compounds and antioxidant activity of Pera Rio orange juice.
Journal: Journal of Food Processing & Technology
Volume: 06**;** **Pages:** 416-422**;** **Year:** 2015

Publication 11
Authors: Vallverdú-Queralt, Anna; Regueiro, Jorge; Alvarenga, José Fernando Rinaldi; Martinez-Huelamo, Miriam; Leal, Leonel Neto; Lamuela-Raventos, Rosa Maria
Title: Characterization of the phenolic and antioxidant profiles of selected culinary herbs and spices: caraway, turmeric, dill, marjoram and nutmeg.
Journal: Ciência e Tecnologia de Alimentos
Volume: 35**;** **Pages:** 189-195**;** **Year:** 2015

Publication 12
Authors: Picolini, Vitória; Alvarenga, José; Fila, Juliana; Matroiani, Patrícia
Title: Análise de percentual econômico dos medicamentos adquiridos por via de ações judiciais.
Journal: Jornal Brasileiro de Economia da Saúde
Volume: 8**;** **Pages:** 125-131**;** **Year:** 2016

Publication 13
Authors: Pérez-Areales, Francisco Javier; Betari, Nibal; Viayna, Antonio; Pont, Caterina; Espargar, Alba; Bartolini, Manuela; De Simone, Angela; Rinaldi Alvarenga, José Fernando; Pérez, Belén; Sabate, Raimon; Lamuela-Raventós, Rosa Maria; Andrisano, Vincenza; Luque, Francisco Javier; Muñoz-Torrero, Diego
Title: Design, synthesis and multitarget biological profiling of second-generation anti-Alzheimer rhein-huprine hybrids.
Journal: Future Medicinal Chemistry
Volume: 9**;** **Pages:** 965-981**;** **Year:** 2017

Publication 14
Authors: Hurtado-Barroso, Sara; Quifer-Rada, Paola; Rinaldi De Alvarenga, José; Pérez-Fernández, Silvia; Tresserra-Rimbau, Anna; Lamuela-Raventos, Rosa.
Title: Changing to a Low-Polyphenol Diet Alters Vascular Biomarkers in Healthy Men after Only Two Weeks.
Journal: Nutrients
Volume: 10**;** **Pages:** 1766**;** **Year:** 2018

Publication 15
Authors: Hurtado-Barroso, Sara; Martinez-Huelamo, Miriam; Rinaldi De Alvarenga, José Fernando; Quifer-Rada, Paola; Vallverdú-Queralt, Anna; Perez-Fernandéz, Silvia; Lamuela-Raventós, Rosa M.

Title: Acute effect of a single dose of tomato sofrito on plasmatic inflammatory biomarkers in healthy men.
Journal: Nutrients
Volume: 11**;** **Number: ;** **Pages:** 851**;** **Year:** 2019

8.2 Conference communications

Extended abstracts

Janzantti, N. S.; ALVARENGA, J. F. R.; Monteiro, Magali. Perfil de voláteis do coquinho-azedo. In: XI Brazilian Meeting on Chemistry of Food and Beverages & V Simposio de Engenharia e Ciência de Alimentos, 2016, São José do Rio Preto. **XI Brazilian Meeting on Chemistry of Food and Beverages & V Simpósio de Engenharia e Ciência de Alimentos**, 2016.

ALVARENGA, J. F. R.; Janzantti, N. S.; Monteiro, Magali. Isolamento dos compostos voláteis do coquinho-azedo (Butia capitata) por headspace dinâmico. In: IV Simpósio de Engenharia de Alimentos, 2014, São José do Rio Preto. **IV Simpósio de Engenharia de Alimentos**, 2014.

Monteiro, Magali; ALVARENGA, J. F. R.; Janzantti, N. S. Atividade antioxidante total, compostos fenólicos totais e características físico-químicas do suco de coquinho-azedo. In: XXII Congresso Brasileiro de Fruticultura, 2012, Bento Gonçalves. **XXII Congresso Brasileiro de Fruticultura**, 2012. p. 4Y62-4Y62

Abstracts

ALVARENGA, J. F. R.; QUIFER-RADA, P.; ILLAN, M.; TORRADO, XAVIER; LAMUELA-RAVENTOS, ROSA M. Synergistic effect of cooking and polyphenols improve carotenoids isomerization in mediterranean sofrito. In: XII International Conference Mediterranean Diet, 2018, Barcelona. **XII International Conference Mediterranean Diet**, 2018.

MARHUENDA-MUNOZ, M.; ESCOBAR-AVELLO, D.; ALVARENGA, J. F. R.; VALLVERDÚ-QUERALT, ANNA; QUIFER-RADA, P.; LAMUELA-RAVENTOS, ROSA M. Total polyphenol content of commercial dried fruits. In: XII International Conference Mediterranean Diet, 2018, Barcelona. **XII International Conference Mediterranean Diet**, 2018.

ALVARENGA, J. F. R.; QUIFER-RADA, P.; ILLAN, M.; TORRADO, XAVIER; LAMUELA-RAVENTOS, ROSA M. Metabolomics as tool to study mediterranean culinary practices: cooking and ingredients affect metabolic profile in tomato sofrito. In: XVII Congreso de la Sociedad Española de Nutrición, 2018, Barcelona**. Annals of Nutrition & Metabolism / Abstracts of the XVII Conference of the SEÑ and the X Meeting of the ACCA**. Basel: KARGER, 2018. v. 73. p. 87-87.

MARHUENDA-MUNOZ, M.; ESCOBAR-AVELLO, D.; TRIUS, M.; ALVARENGA, J. F. R.; VALLVERDÚ-QUERALT, ANNA; LAMUELA- RAVENTÓS, ROSA M. Mediterranean dried fruits are a rich source of phenolic compounds. In: XVII Congreso de la Sociedad Española de Nutrición, 2018, Barcelona. **Annals of Nutrition & Metabolism / Abstracts of the XVII Conference of the SEÑ and the X Meeting of the ACCA**. Basel: KARGER, 2018. v. 73. p. 57-58.

RINALDI DE ALVARENGA, JOSE FERNANDO; MAS, P. M.; LAMUELA- RAVENTÓS, ROSA M. Polyphenols content of wines from Binissalem-Mallorca appelation. In: Wine and Health 2017, 2017, Logroño. **Wine and Health 2017**, 2017.

JULIANO, F. F.; RINALDI DE ALVARENGA, JOSE FERNANDO; LAMUELA- RAVENTÓS, ROSA M.; MASSARIOLI, A. P.; LIMA, L. M.; ALENCAR, S. M. Polyphenolic profile of divergent peanut genotype, adapted to drought, by highresolution mass spectrometry. In: Wine and Health 2017, 2017, Logroño. **Wine and Health 2017**, 2017.

HURTADO-BARROSO, SARA; RINALDI DE ALVARENGA, JOSE FERNANDO; TRESSERRA-RIMBAU, A.; JULIANO, F. F.; LAMUELA- RAVENTÓS, ROSA M. Total polyphenols and antioxidant capacity in organic versus conventional white wine from two spanish varieties: Chardonnay and Xarel·lo. In: Wine and Health 2017, 2017, Logroño. **Wine and Health 2017**, 2017.

RINALDI DE ALVARENGA, JOSE FERNANDO; JUNZA, S.; KHYMENETS, O.; GONZALEZ-BENJUMEA, A.; FERNANDEZ-BOLANOS, J. G.; AMERIGO, J. A.; ESTRUCH, RAMON; LAMUELA- RAVENTÓS, ROSA M. Metabolomic insight into oleocanthal metabolism in human body. In: Wine and Health 2017, 2017, Logroño**. Wine and Health 2017**, 2017.

ALVARENGA, J.F.R.; QUIFER-RADA, P.; ILLAN, M.; TORRADO, XAVIER; LAMUELA-RAVENTOS, ROSA M. Olive oil as functional ingredients for mediate thermal isomerization of carotenoids. In: III Workshop Aceite de Oliva, 2017, Santa Coloma de Gramenet**. III Workshop Aceite de Oliva**, 2017. v. 1. p. 10-10.

ROMERO-DEL-CASTILLO-ALBA, J.; ALVARENGA, J.F.R.; JUNZA, S.; GONZALEZ-BENJUMEA, A.; FERNANDEZ-BOLANOS, J. G.; AMERIGO, J. A.; ESTRUCH, RAMON; LAMUELA-RAVENTOS, ROSA M. Comprehensive approach of oleocanthal metabolism in human body. In: III Workshop Aceite de Oliva, 2017, Santa Coloma de Gramenet. **III Workshop Aceite de Oliva**, 2017. v. 1. p. 11-11.

ALVARENGA, J.F.R.; QUIFER-RADA, P.; HURTADO-BARROSO, S.; TORRADO, XAVIER; LAMUELA-RAVENTOS, ROSA M. Synergism effect of home cooking and ingredients enhance tomato polyphenols in Mediterranean sofrito. In: III Workshop anual INSA-UB ?La Salut de la microbiota. Prebiòtics i probiòtics en nutrició animal i humana, 2017, Santa Coloma de Gramanet. **III Workshop anual INSA-UB La Salut de la microbiota. Prebiòtics i probiòtics en nutrició animal i humana**, 2017.

SERRANO-GARCIA, I.; ALVARENGA, J.F.R.; LAMUELA-RAVENTOS, ROSA M. Stability of carotenoids in commercial sofrito during an accelerated storage. In: III Workshop anual INSA-UB ?La Salut de la microbiota. Prebiòtics i probiòtics en nutrició animal i humana, 2017, Santa Coloma de Gramanet. **III Workshop anual INSA-UB La Salut de la microbiota. Prebiòtics i probiòtics en nutrició animal i humana**, 2017.

HURTADO-BARROSO, SARA; TRESSERRA-RIMBAU, A.; ALVARENGA, J.F.R.; LAMUELA-RAVENTOS, ROSA M. Comparison of urinary excretion of total polyphenols after a high antioxidant diet and a low antioxidant diet. In: III Workshop anual INSA-UB ?La Salut de la microbiota. Prebiòtics i probiòtics en nutrició animal i humana, 2017, Santa Coloma de Gramanet. **III Workshop anual INSA-UB La Salut de la microbiota. Prebiòtics i probiòtics en nutrició animal i humana**, 2017.

MAS, P. M.; ALVARENGA, JFR; LAMUELA-RAVENTOS, ROSA M. Capacidad antioxidante de vinos de mallorca. In: XI International Mediterranean Diet Conference, 2016, Barcelona. **XI International Mediterranean Diet Conference**, 2016. v. 1. p. 1-1.

HURTADO-BARROSO, S.; MARTINEZ-HUELAMO, M.; COLMAN-MARTINEZ, M.; ALVARENGA, JFR; LAMUELA-RAVENTOS, ROSA. Biodisponibilidad de los carotenoides del sofrito en varones tras una dieta rica en alimentos con componentes antioxidantes. In: VIII Seminario sobre Alimentación y Estilos de Vida Saludables, 2016, Palma de Mallorca. **VIII Seminario sobre Alimentación y Estilos de Vida Saludables**, 2016.

ALVARENGA, J.F.R.; SCHURACHIO, P.; BONIFACIO, M.T.E.; SYLOS, C.M. Lipophilic antioxidant capacity, color proprieties and carotenoid content of peppers cultivated in Brazil. In: 8th International Congress Pigments in Food, 2016, Cluj-Napoca. **8th International Congress Pigments in Food "Colored Food for Health Benefits"**. Cluj Napoca: Colorama, 2016. p. 92-92.

ALVARENGA, J.F.R.; SCHURACHIO, P.; BONIFACIO, M.T.E.; SYLOS, C.M. Identification and quantification of carotenoids presents in brazilian peppers compared to other varieties. In: 8th Internationl Congress Pigments in Food, 2016, Cluj-Napoca. **8th International Congress Pigments in Food "Colored Food for Health Benefits"**. Cluj Napoca: Colorama, 2016. p. 91-91.

RINALDI DE ALVARENGA, JOSÉ FERNANDO; TRAN, CAMILLA; HURTADO-BARROSO, SARA; MARTÍNEZ-HUÉLAMO, MIRIAM; ILLAN, MONTSERRAT; LAMUELA- RAVENTÓS, ROSA M. Home cooking and ingredients synergism improve lycopene isomers in sofrito. In: II Workshop anual INSA: Cacao y Chocolate: ciencia y gastronomia, 2016, Santa Coloma de Gramenet. **II Workshop anual INSA: Cacao y Chocolate: ciencia y gastronomia**, 2016.

CORDERO-GARCIA, M.; RINALDI DE ALVARENGA, JOSÉ FERNANDO; HURTADO-BARROSO, SARA; LAMUELA- RAVENTÓS, ROSA M. Effect of composition of tomato-based mediterranean sofrito on color changes during accelarated storage. In: II Workshop Anual INSA: Cacao y Chocolate: ciencia y gastronomía, 2016, Santa Coloma de Gramanet. **II Workshop Anual INSA: Cacao y Chocolate: ciencia y gastronomía**, 2016.

HURTADO-BARROSO, S.; ALVARENGA, JFR; QUIFER-RADA, P.; CREUS-CUADROS, A.; LAMUELA-RAVENTOS, ROSA M. Diferencias nutricionales entre una dieta rica en antioxidantes y una dieta baja en antioxidantes. In: XI International Mediterranean Diet Conference, 2016, Barcelona. **XI International Mediterranean Diet Conference**, 2016. v. 1. p. 2-2.

ALVARENGA, J.F.R.; BONIFACIO, M.T.E.; SYLOS, C.M. Perfil de carotenoides e sua correlação com as propriedades de cor de diferentes variedades de pimentas brasileiras (Capsicum sp). In: V Congresso Farmacêutico da UNESP, 2015, Araraquara. **V Congresso Farmacêutico da UNESP**, 2015.

ALVARENGA, J.F.R.; Janzantti, N.S.; MONTEIRO, M. Identification and quantification of jelly palm volatile compounds. In: 29th EFFoST International Conference, 2015, Athens. **29th EFFoST International Conference**, 2015.

ALVARENGA, J.F.R.; BISCOSIN-JUNIOR, A.; Monteiro, Magali. Condições de processamento do suco de laranja por alta pressão hidrostática na resposta da atividade antioxidante. In: IV Congresso Farmacêutico da UNESP, 2014, Araraquara. **IV Congresso Farmacêutico da UNESP.** Araraquara: UNESP, 2014.

ALVARENGA, J.F.R.; MELGAREJO, C.R.V.; FILA, J.B.; ORLANDO, A.B.; PICOLINI, V.M.; SATILIO, D.F.; POLLI, L.M.; CAUNETO, N.M F.; VIEIRA, M.F.; BRITO, M.; FREITAS, E.A.S; RIBEIRO, C.; MASTROIANNI, P.C. Avaliação das boas práticas de armazenamento e distribuição (BPAD) de medicamentos na farmácia de componentes especializados do Departamento Regional de Saúde de Araraquara (DRS-III). In: IV Congresso Farmacêutico da UNESP, 2014, Araraquara. **IV Congresso Farmacêutico da UNESP**. Araraquara: UNESP, 2014.

ORLANDO, A.B.; VIEIRA, M.F.; CAUNETO, N.M. F.; ALVARENGA, J.F.R.; FREITAS, E.A.S.; FILA, J.B.; MASTROIANNI, P.C. Percepção dos gerentes municipais da assistência farmacêutica municipal sobre a logistica dos medicamentos dos componentes especializados. In: IV Congresso Farmacêutico da UNESP, 2014, Araraquara. **IV Congresso Farmacêutico da UNESP**. Araraquara: UNESP, 2014.

POLLI, L.M.; FILA, J.B.; MELGAREJO, C.R.V.; ALVARENGA, J.F.R.; ORLANDO, A.B.; SANTOS, E.R.; PICOLINI, V.M.; SATILIO, D.F.; CAUNETO, N.M. F.; VIEIRA, M.F.; BRITO, M.; FREITAS, E.A.S.; RIBEIRO, C.; MASTROIANNI, P.C. Percepção sobre o programa de medicamentos dos componentes especializados por usuários da Diretoria Regional de Saúde (DRS III). In: IV Congresso Farmacêutico da UNESP, 2014, Araraquara. **IV Congresso Farmacêutico da UNESP**. Araraquara: UNESP, 2014.

ALVARENGA, J.F.R.; BISCOSIN-JUNIOR, A.; Monteiro, Magali. Influência do processamento de alta pressão hidrostática no conteúdo de ácido ascórbico do suco de laranja. In: 10 Simpósio Latino Americano de Ciência de Alimentos, 2013, Campinas. 10 **Simpósio Latino Americano de Ciência de Alimentos**, 2013.

ALVARENGA, J.F.R.; VALLVERDÚ-QUERALT, ANNA; MARTINEZ-HUELAMO, M.; LAMUELA-RAVENTOS, ROSA M. Carotenoid profile of organic and conventional

ketchups and tomato juices. In: 10 Simpósio Latino Americano de Ciência de Alimentos, 2013, Campinas. 10 **Simpósio Latino Americano de Ciência de Alimentos**, 2013.

ALVARENGA, J. F. R.; Monteiro, Magali. Determinação da atividade antioxidante de bebida de fruta. In: XXIII Congresso Brasileiro de Ciência e Tecnologia de Alimentos, 2012, Campinas. **XXIII Congresso Brasileiro de Ciência e Tecnologia de Alimentos**, 2012.

BISCOSIN-JUNIOR, A.; ALVARENGA, J.F.R. ROSENTHAL, A.; Monteiro, Magali. Impacto da alta pressão hidrostática e da temperatura sobre enzima PME e o ácido ascórbico do suco de laranja. In**: IV Simpósio em Ciência e Tecnologia de Alimentos**, 2012, João Pessoa. IV SCTA CD-ROM, 2012.

FRABRICIO, L.F.; ALVARENGA, J.F.R.; BONIFACIO, M.T.E.; Monteiro, Magali. Atividade antioxidante de seriguela, tamarindo e umbu. In: 11º Congresso Nacional da Sociedade Brasileira de Alimentação e Nutrição, 2011, Fortaleza. **11º Congresso Nacional da Sociedade Brasileira de Alimentação e Nutrição**, 2011. p. 29-29.

ALVARENGA, J.F.R.; Monteiro, Magali. Caracterização físico-química de bebida funcional desenvolvida para o público idoso. In: Congresso Farmacêutico da UNESP, 2011, Araraquara. Revista de Ciências Farmacêuticas Básica e Aplicada. Araraquara, 2011. v. 32. p. 22-22.

BISCOSIN-JUNIOR, A.; ALVARENGA, J.F.R.; BONIFACIO, M.T.E.; Monteiro, Magali. Atividade antioxidante de néctar e suco de laranja pronto para beber orgânico e convêncional. In: Simpósio Latino Americano de Ciência de Alimentos, 2011, Campinas. **9 Simpósio Latino Americano de Ciências dos Alimentos**, 2011.

ALVARENGA, J.F.R.; Monteiro, Magali. Atividade antioxidante de bebida funcional para o público idoso. In: Simpósio Latino Americano de Ciência de Alimentos, 2011, Campinas. **9 Simpósio Latino Americano de Ciências dos Alimentos**, 2011.

ALVARENGA, J.F.R.; Janzantti, N.S.; Monteiro, Magali. Atividade antioxidade total e compostos fenólicos totais do suco de laranja e de acerola. In: 57a Jornada Farmacêutica da UNESP, 2010, Ararquara**. 57a Jornada Farmacêutica UNESP** - Anais 2010, 2010. p. 25-26.

ALVARENGA, J.F.R.; Monteiro, Magali. Total antioxidant activity and total phenolic compounds of orange and acerola juice. In: VIII Brazilian Meeting on Chemistry of Food and Beverages, 2010, São Carlos. Book of Abstracts **VIII Brazilian Meeting on Chemistry of Food and Beverage**, 2010. p. 43-43.

ALVARENGA, J.F.R.; Monteiro, Magali. Avaliação do suco de uva light pronto para beber. In: 56a Jornada Farmacêutica da UNESP, 2009, Araraquara. Anais da **56a Jornada Farmacêutica da UNESP**, 2009. v. 1.