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PROGRAMA DE DOCTORAT EN ALIMENTACIÓ I NUTRICIÓ

REVALORITZACIÓ DE LES LIES DEL CAVA COM A NOVA ESTRATÈGIA DE FORMULACIÓ PER A ALIMENTS FERMENTATS

Memòria presentada per Alba Martín Garcia per optar al títol de doctor per la Universitat de Barcelona

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RESUM

La indústria alimentària genera una gran quantitat de residus durant la producció d'aliments, entre els que s'hi troba el vi escumós, com és el Cava. De cada 100 kg de raïm que entra en un celler, es generen 25 kg de residu, on el 25% són lies. Les lies són els llevats naturalment plasmolitzats durant la criança en ampolla del Cava que, tot i ser rics en polisacàrids, fibra i proteïnes, no tenen cap ús, pel que necessiten esser gestionats, el que comporta un cost econòmic i mediambiental. Per aquest motiu, la tendència dels últims anys és la de re-introduir-los en la mesura del possible, a la cadena de producció, passant d'una economia lineal a una de circular. En aquest sentit, i d'acord amb els Objectius de Desenvolupament Sostenible (ODS), la indústria i la comunitat científica estan desenvolupant estratègies de revalorització per a aquest tipus de subproductes.

Per tot això, l'objectiu principal d'aquesta tesi era la valorització de les lies del Cava com a ingredient per afavorir el procés de fermentació de la massa mare i el pa, i avaluar-ne la capacitat prebiòtica. En concret, s'han avaluat tres àmbits relacionats amb les lies del Cava: I) la valoració *in vivo* de la seguretat alimentària i de les lies i el seu efecte sobre la microbiota intestinal en model animal; II) la caracterització de les lies des d'un punt de vista organolèptic; i III) l'avaluació de l'efecte sobre la fermentació i la fracció volàtil de la massa mare i el pa.

Primerament, en avaluar la seguretat alimentària i la capacitat prebiòtica de les lies del Cava, no es van trobar diferències significatives entre els grups (control i amb suplementació de lies) respecte els paràmetres d'observació de les rates de l'estudi *in vivo* (pes, ingesta d'aliments o aigua), hematologia, proves bioquímiques, histopatologia, immunogenicitat o necrosi, pel que s'infereix la seguretat alimentària de l'ingredient. Quan a la capacitat prebiòtica, es va observar un augment significatiu de l'abundància relativa de bacteris potencialment probiòtics, com *Limosilactobacillus fermentum*, *Lactiplantibacillus plantarum* o *Ligilactobacillus ruminis*, pertanyents a la família *Lactobacillaceae*; així com també bacteris de l'espècie *Blautia hansenii*, *Ruminococcus obeum* i *Roseburia intestinalis*. Per tot això, les lies del Cava presenten un potencial efecte prebiòtic sobre la

microbiota intestinal tot i que s'haurien de realitzar més estudis per confirmar aquests resultats preliminars.

Seguidament, les lies es van caracteritzar, mostrant un alt contingut d'èsters, tenint un perfil molt similar al del Cava del que provenien. A més, en augmentar el temps de criança amb les lies, presentaven un perfil de volàtils més ric i complex. Això suposaria que les lies retindrien diversos compostos a la seva superfície, i podrien acabar tenint un efecte sobre l'aliment on s'utilitzin.

En efecte, quan es van afegir les lies a la formulació tant de massa mare com de pa, aquestes van modificar el corresponent perfil volàtil. Per una banda van augmentar la concentració dels compostos característics dels productes del pa però alhora també van aportar volàtils propis del Cava. De fet, aquest increment dels volàtils podria relacionar-se amb l'augment de la viabilitat dels microorganismes fermentadors de la massa mare i del pa formulat amb lies. Tot això aportaria arguments positius cap a la revalorització de les lies com a nou ingredient en aliments fermentats, com la massa mare i el pa.

En conclusió, l'ús a la indústria alimentària de les lies del Cava com a ingredient seria una bona estratègia de revalorització d'aquest subproducte, contribuint a una economia circular, amb un menor impacte sobre el medi ambient i potenciant el compliment dels ODS fixats per les Nacions Unides.

ABSTRACT

The food industry generates a large amount of waste during food production, including sparkling wine, such as Cava. For every 100 kg of grapes that come into the winery, 25 kg of waste are generated, and 25% of such residue are lees. Lees are naturally plasmolyzed yeast during bottle ageing of Cava that, despite being rich in polysaccharides, fiber, and proteins, they are not used, so lees need to be managed, resulting in economic and environmental costs. For that, there is a trend to re-introduce residues in the production chain, changing from a linear economy to a circular one. In this sense, and in accordance with the Sustainable Development Goals (SDG), the food industry and the scientific community are developing new strategies to revalorize these types of by-products.

For that, the main objective of this thesis was the valorization of Cava lees as an ingredient to favor the fermentation process of sourdough and bread and evaluate its potentially prebiotic capacity. Specifically, the present thesis was divided in three major areas: I) the *in vivo* evaluation of the food safety and effect of lees on the intestinal microbiota in an animal model; II) the characterization of Cava lees from an organoleptic point of view; and III) the evaluation of the effect on fermentation and the volatile fraction of sourdough and bread.

Firstly, when evaluating the food safety and prebiotic capacity of the Cava lees, no significant differences were found between the groups (control and with lees supplementation) regarding the observational parameters of the studied animals (weight, food or water intake), hematology, biochemical tests, histopathology, immunogenicity or necrosis, by which the food safety of the ingredient is inferred. Regarding the prebiotic capacity, a significant increase was observed in the relative abundance of potentially probiotic bacteria, such as *Limosilactobacillus fermentum*, *Lactiplantibacillus plantarum* or *Ligilactobacillus ruminis*, belonging to the *Lactobacillaceae* family; as well as bacteria of the species *Blautia hansenii*, *Ruminococcus obeum* and *Roseburia intestinalis*. Therefore, Cava lees show a potential prebiotic effect on the intestinal microbiota, although more studies should be performed to confirm these preliminary results.

Then, Cava lees were characterized, showing a high content of esters, having a very similar profile to the Cava from which they came. In addition, by increasing the ageing time with lees, they presented a richer and more complex volatile profile. Therefore, lees can retain several compounds on their surface, which could end up influencing the formulated food.

Indeed, when lees were added to sourdough and bread formulations, they modified the corresponding volatile profile. On the one hand, they increased the concentration of the characteristic compounds of bakery products, but at the same time they also contributed with volatiles specific to Cava. In fact, that increase in volatiles could be related to an incremented viability of the fermenting microorganisms in sourdough and bread with lees. All that would provide positive arguments towards the revalorization of lees as a new ingredient in fermented foods such as sourdough and bread.

In conclusion, the use of Cava lees as an ingredient in the food industry would be a good strategy to revalorize this by-product, contributing to a circular economy, with a lower impact on the environment and promoting the fulfillment of the SDG set by the United Nations.

ABREVIACIONS

AGCC	Àcids grassos de cadena curta
AGCM	Àcids grassos de cadena mitja
BAL	Bacteris de l'àcid làctic
DOP	Denominació d'Origen Protegida
EFSA	Autoritat Europea de Seguretat Alimentària (*European Food Safety Authority*)
EPS	Exopolisacàrids
FODMAPs	Oligo-, Di-, Mono-sacàrids i poliols fermentables (*Fermentable Oligo-, Di-, Mono-saccharides and Polyols*)
GC-MS	Cromatografia de gasos – espectrometria de masses (*Gas Chromatography ¡– Mass Spectrometry*)
HS-SPME	Microextracció en fase sòlida de l'espai de cap (*Head Space Solid Phase MicroExtraction*)
IC	Intensitat de color
IFT	Índex fenòlic total
ODS	Objectius de Desenvolupament Sostenible
QF	Quocient de Fermentació
TC	Tonalitat de color
TPA	Anàlisi del perfil de textura (*Texture Profile Analysis*)
VEQPRD	Vi escumós de qualitat produït en una regió determinada

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CAPÍTOL 1. INTRODUCCIÓ

Durant l'any 2020 la indústria alimentària ha generat 878 milers de tones de residus a Catalunya (25% del total de residus per sectors) [1], suposant un important impacte mediambiental així com econòmic, derivat de la seva manipulació, tractament i eliminació. Així doncs, la situació actual demana un canvi de paradigma en referència al model de producció industrial. De fet, en els últims anys s'ha intentat passar d'una economia lineal, on l'objectiu es transformar una matèria prima en un producte acabat desestimant els residus; cap a un a economia circular, en la que s'atorga un valor afegit als residus i es re-introdueixen al cicle industrial com a nous ingredients. És més, des de les Nacions Unides s'ha posat èmfasi en la sostenibilitat com a mesura per combatre el canvi climàtic mitjançant els Objectiu de Desenvolupament Sostenible (ODS). En aquest sentit, la re-valorització de subproductes s'engloba en l'objectiu 12 de producció i consum responsables. Es per això que la comunitat científica, juntament amb la indústria, investiga i desenvolupa noves estratègies de re-utilització i re-valorització de subproductes alimentaris.

De fet, la generació de residu alimentari per part de la indústria vitivinícola és especialment preocupant, estimant que per cada 100 kg de raïm es produeixen uns 25 kg de residus. En efecte, entre els principals residus produïts per la indústria del vi s'hi troben la brisa de raïm (60%) i les lies (25%) [2]. Però per la seva composició rica en polisacàrids solubles, aquestes últimes es podrien re-aprofitar com a ingredients amb propietats tecnològiques per desenvolupar nous productes alimentaris o optimitzar productes ja existents.

Per tant, a la introducció d'aquesta tesi doctoral s'examinarà el procés d'obtenció de les lies del Cava (vi escumós), així com la seva composició i possibles aplicacions a la indústria alimentària. D'altra banda, es farà una breu introducció als prebiòtics i probiòtics que es troben en els aliments fermentats i a la microbiota intestinal. Finalment, es revisarà el procés d'elaboració de la massa mare i quins beneficis té el seu ús a la formulació del pa.

2.1. El Cava

El Cava és un vi escumós de qualitat produït en una regió determinada (VEQPRD) que requereix de dues fermentacions. Aquest tipus de vi escumós s'inclou dins la Denominació d'Origen Protegida (DOP) Cava, i engloba bodegues de diferents regions espanyoles (Figura 1), essent Catalunya la principal productora (aproximadament el 95% del volum total). De fet, la seva producció està regulada per la DOP [3] i pel Consell Regulador del Cava [4].

Figura 1. Regions productores de la DOP Cava. Extreta de [5].

Aquesta regulació inclou les varietats de raïm permeses per la producció de Cava, així com la descripció dels paràmetres de qualitat que han de mantenir tant el vi base com el cava final per tal d'incloure's dins la DOP.

2.1.1. Elaboració del Cava

Com s'ha mencionat anteriorment, l'elaboració del Cava segueix el mètode tradicional, també anomenat *champenoise*, i consta de dues etapes diferenciades (Figura 2). La primera és la preparació del vi base o *cuvée*; mentre la darrera es tracta d'una segona fermentació en ampolla amb envelliment *sur lie*.

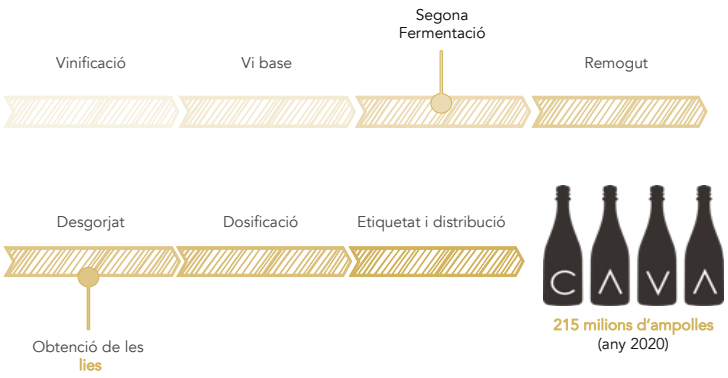

Figura 2. Procés d'elaboració del Cava.

La primera etapa, amb la que s'obté el vi base, segueix el procés de producció dels vins blancs i rosats, començant pel premsat del raïm obtenint el most, seguit de la fermentació alcohòlica. De fet, en formar part d'una DOP, les varietats de raïm utilitzades per tal d'elaborar el Cava estan regulades [4], i aquestes poden ser de raïm blanc (*blanc de blancs*) o de raïm negre (*blanc de noirs*). D'una banda, les varietats blanques permeses són: Macabeu, Xarel·lo, Parellada, Chardonnay i Malvasia (també denominada Subirat Parent). D'altra banda, les varietats negres autoritzades són: Garnatxa negra, Trepat, Pinot Noir i Monastrell. Les varietats negres només es poden utilitzar per la producció de caves rosats, excepte la Pinot Noir que des del 2007 també es pot fer servir per l'elaboració de *blanc de noirs*. Tot i això, les varietats més habituals són les autòctones Macabeu, Xarel·lo i Parellada.

Una vegada s'ha premsat el raïm, s'afegeixen els llevats al most per tal de començar la primera fermentació alcohòlica. De fet, el llevat responsable d'aquesta vinificació és un factor que contribueix substancialment a les característiques del vi base obtingut. És per aquest motiu que les soques de llevat utilitzades actualment són seleccionades per tal d'assegurar el control de la fermentació, estandarditzant el producte i reduint el risc d'aparició de defectes en el vi base. Els llevats més utilitzats per la fermentació alcohòlica del vi base pertanyen a l'espècie *Saccharomyces cerevisiae*. Aquesta fermentació es produeix a una temperatura controlada d'entre 16°C i 18°C, durant aproximadament 12 – 15 dies.

A continuació, el vi base ha de passar per un procés d'estabilització tartàrica per tal d'evitar una posterior precipitació de bitartrat potàssic a l'ampolla, provocant terbolesa en el vi. Per aquest fi es manté el vi en fred (-4°C aproximadament) entre 5 i 6 dies, ocasionant la formació dels cristalls, que s'eliminaran per filtració.

La segona etapa, la més característica de l'elaboració del vi escumós, comença amb el tiratge i l'embotellament. Aquest procés consisteix en l'addició del sucre i els llevats necessaris per tal de realitzar la segona fermentació. En aquest cas, els llevats utilitzats (habitualment soques de *S. cerevisiae* i *S. bayanus*) han de presentar certes característiques, entre les quals s'hi troben la resistència a l'etanol (9 – 10% v/v) i a la pressió de CO_2 (5 – 6 atm), així com la capacitat de fermentar a baixes temperatures i flocular amb facilitat [6].

Després del tiratge, les ampolles tancades hermèticament es col·loquen en posició horitzontal a les caves (rima) i comença la segona fermentació. Aquest procés té lloc a baixa temperatura (10 – 15°C). La fermentació dura, aproximadament, entre 1 i 3 mesos, en els que la viabilitat dels llevats disminueix en un 99%. Passats 6 mesos, la fermentació ja s'ha completat i no s'hi troben cèl·lules viables [6,7].

Seguidament, i sense eliminar els llevats autolisats, hi ha un període d'envelliment *sur lie* (en contacte amb lies) de mínim 9 mesos [8]. Les lies, o mares del Cava, es defineixen com les restes sòlides formades després de la fermentació i durant l'emmagatzematge, i consisteixen, bàsicament, en microorganismes plasmolitzats (generalment *S. cerevisiae*), àcid tartàric i altres compostos adsorbits [9]. Segons el temps d'envelliment, la DOP classifica el Cava en diferents categories (Taula 1).

Taula 1. Classificació dels tipus de Cava en funció del temps d'envelliment en contacte amb lies.

Categoria	Classificació	Temps d'envelliment
Cava de Guarda	Cava	9 mesos
Cava de Guarda Superior	Cava Reserva	18 mesos
	Cava Gran Reserva	30 mesos
	Cava de Paratge Qualificat	36 mesos

Una vegada finalitzat el procés d'envelliment s'han d'eliminar les restes sòlides (entre elles, les lies), realitzant el desgorjat. Per a aquest fi, les ampolles es col·loquen en posició vertical, aconseguint tenir tots els sediments al coll de l'ampolla. Llavors es congela el coll d'ampolla i es treu el tap. Per diferència de pressió entre l'interior i l'exterior de l'ampolla, les restes sòlides acumulades surten a l'exterior, alhora que es perden uns mil·lilitres de cava. És per això que abans de tapar l'ampolla amb el tap de suro final s'afegeix l'anomenat licor d'expedició (dosatge), que consisteix en una mescla de vi base i sucre. La DO Cava també estableix uns paràmetres de concentració de sucre pel qual es classifiquen els caves: Brut Nature (< 3 g/L), Extra Brut (< 6 g/L), Brut (< 12 g/L), Extra Sec (12 – 17 g/L), Sec (17 – 32 g/L), Semi Sec (32 – 50 g/L) i Dolç (> 50 g/L) [8].

2.1.2. Llevats i lies del Cava

Els llevats responsables de la segona fermentació en l'elaboració de Cava majoritàriament pertanyen al gènere *Saccharomyces*, habitualment essent soques de *S. cerevisiae* o *S. bayanus*. Generalment, les soques fermentadores són seleccionades en funció de la seva habilitat per fermentar i la seva capacitat per tolerar les condicions d'estrès pròpies dels vins escumosos com les baixes temperatures, altes pressions, altes concentracions d'etanol i les poques fonts de nutrients [6,10]. Aquests llevats són fongs unicel·lulars, anaerobis facultatius, del grup dels ascomicets. L'estructura cel·lular dels llevats es composa de paret cel·lular i membrana plasmàtica, que rodegen el citoplasma, format pel citosol i els orgànuls (**Figura 3**).

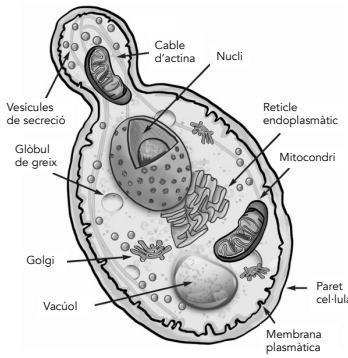

Figura 3. Estructura cel·lular de *S. cerevisiae*. Adaptat de [11].

2.1.2.1. La paret cel·lular

La paret cel·lular de *S. cerevisiae* (Figura 4) suposa entre un 20% i 50% del pes sec de la cèl·lula, i està formada, principalment, per manoproteïnes (exterior) i cadenes de ß-glucans i quitina (interior), representant el 85-90% de la paret cel·lular [10,12,13].

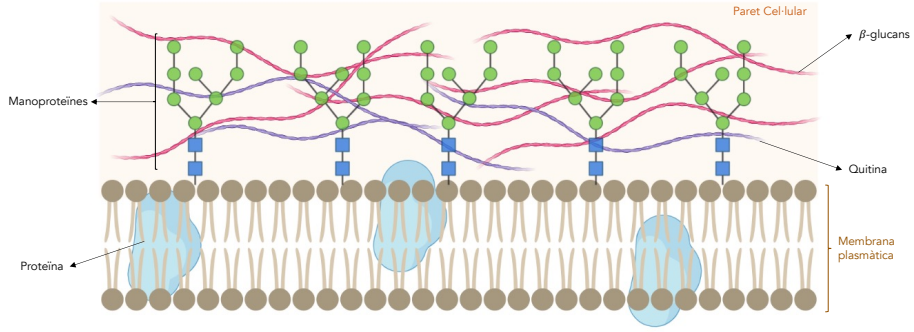

Figura 4. Representació esquemàtica de la paret cel·lular i la membrana plasmàtica de *S. cerevisiae*. Adaptat de [14].

Les manoproteïnes són proteoglicans (90% sucres, principalment manoses; 10% proteïnes) situats a la part més externa de les cèl·lules de llevats que actuen com a components estructurals. Aquestes molècules s'associen positivament amb la qualitat i les propietats tecnològiques del vi: redueixen la terbolesa proteica, prevenen la precipitació d'àcid tartàric, contribueixen al *mouthfeel*, estimulen la fermentació malolàctica, influencien la intensitat aromàtica del vi i poden interaccionar amb els compostos fenòlics, millorant l'estabilitat de color i reduint l'astringència del vi. A més a més, diferents estudis han demostrat les propietats emulsionants i estabilitzants de les manoproteïnes degut a la seva estructura amfifílica [15–17]. Aquestes propietats funcionals estan relacionades principalment

amb les interaccions fisicoquímiques que es desenvolupen entre les manoproteïnes i els constituents del vi. Tanmateix, aquests efectes positius són variables i depenen de la composició del vi, però, sobretot, de l'origen de les manoproteïnes estudiades. Diversos estudis indiquen que les propietats funcionals de les manoproteïnes depenen de la seva massa molar i de la relació entre els fragments de polisacàrids i proteïnes. Tanmateix, encara no estan clarament establerts ni els vincles entre les estructures del polisacàrid i les parts proteiques (composició, grau de ramificació, fosforilació, etc.) i les propietats de les manoproteïnes, ni les seves respectives contribucions a aquestes últimes [17].

Els ß-glucans són polímers solubles de glucosa majoritàriament units per enllaços ß-1,3 i, en menor proporció, ramificats amb enllaços ß-1,6. Als ß-glucans se'ls confereixen diferents propietats potencials, com per exemple, antiinflamatoris o antioxidants o reguladors de glucosa en sang [18]. A més a més, tenen diferents característiques funcionals que poden ser utilitzats a la indústria alimentària, ja que poden actuar com a estabilitzadors, espessidors i emulsionants [19]. De fet, s'ha autoritzat l'ús dels ß-glucans dels llevats com a nous ingredients alimentaris [20] i la Autoritat Europea de Seguretat Alimentària (EFSA) ha aprovat algunes de les declaracions de salut derivades de la ingesta de ß-glucans que poden indicar-se a l'etiquetat, presentació i publicitat dels aliments [21].

Finalment, la quitina és el component minoritari de la paret cel·lular que es relaciona amb el procés de gemmació, ja que es troba sobretot al voltant i dins les cicatrius de la gemmació del llevat. Consisteix en cadenes de polímers de N-acetilglucosamina amb enllaços ß-1,4. Les cadenes de quitina solen estar enllaçades amb els extrems no reductors dels ß-glucans mitjançant enllaços ß-1,4 [22].

2.1.2.2. Autòlisi i envelliment *sur lie*

L'autòlisi dels llevats és un procés irreversible catalitzat pels enzims hidrolítics intracel·lulars que actuen per alliberar compostos del citoplasma i la paret cel·lular al vi. Aquest procés generalment es dóna al final de la fase estacionària de creixement dels llevats, i normalment s'associa a la mort cel·lular [10,23].

La Figura 5 mostra una representació esquemàtica dels canvis morfològics i bioquímics de la cèl·lula de llevat durant l'autòlisi i criança biològica en ampolla. Un cop acabada la segona fermentació, i per inanició, la cèl·lula importa els amino àcids del medi a l'interior. Durant les primeres etapes de l'envelliment (3 – 6 mesos), es presenten els primers signes de degradació de la paret cel·lular i la membrana plasmàtica. Les manoproteïnes s'alliberen, probablement per la ruptura dels enllaços entre manoproteïnes, glucans i quitina. Com a resultat, la paret es torna porosa i els diferents components (amino àcids, proteïnes i pèptids, i polisacàrids) són alliberats al medi. Passats els 9 – 12 mesos, i després de la mort cel·lular, la membrana plasmàtica s'ha degradat completament i s'alliberen nucleòtids, lípids, polisacàrids, proteïnes i amino àcids al medi [10,15,16].

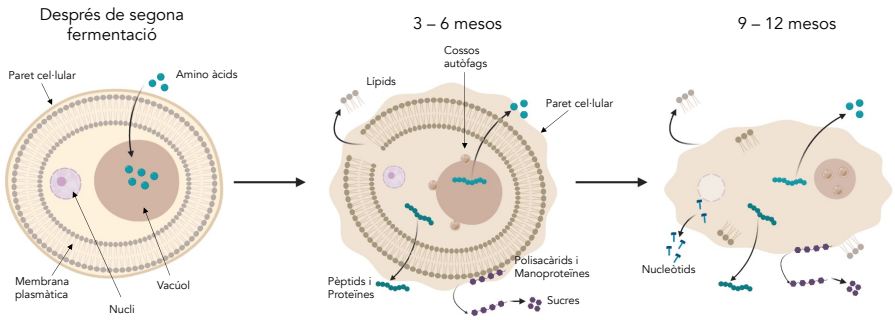

Figura 5. Representació esquemàtica dels canvis morfològics i bioquímics de la cèl·lula de llevat durant l'autòlisi. Adaptat de [10].

A nivell morfològic també es produeixen canvis a la cèl·lula. Durant el primer mes de fermentació en ampolla les cèl·lules assoleixen la fase estacionària, tot i que morfològicament s'assemblen a les de principi de fermentació. Passats 9 mesos d'envelliment la majoria de llevats estan plasmolitzats i la membrana plasmàtica queda separada de la paret cel·lular. A partir dels 18 mesos d'envelliment, la paret cel·lular consisteix en una capa fibrosa i difusa, tot i que no arriba a trencar-se en cap moment (fins a 48 mesos d'envelliment). En Caves de criança llarga (Gran Reserva i de Paratge Qualificat), les capes interna i externa de la paret cel·lular formen una estructura de fibres enredades que podria està composta per ß-1,3-glucans associats a proteïnes [23].

D'altra banda, l'autòlisi dels llevats i l'envelliment en contacte amb les lies del Cava modifiquen la composició d'aquest. Aquests canvis vénen donats per l'alliberament de compostos de la cèl·lula al Cava durant l'autòlisi de *S. cerevisiae*, i poden tenir efecte sobre les propietats organolèptiques del Cava, com la capacitat escumant i l'aroma, millorant-ne la complexitat [7,10]. A més a més, també s'ha observat una capacitat antioxidant de les lies degut als components de la paret cel·lular i als polifenols adsorbits [9].

2.1.2.3. Les lies com a subproducte

Durant l'any 2020, la indústria del Cava va produir 215 milions d'ampolles. Al llarg d'aquesta producció es generen certs residus o subproductes, com la brisa de raïm (60% del total de subproductes), que es composa de pell de raïm (50%), tiges (25%) i llavors (25%); així com les lies (25% del total) [2]. Per tant, s'estima que aquesta indústria genera, aproximadament, unes 200 tones de lies anualment.

Tot i que diversos estudis han observat que aquests subproductes contenen una àmplia gamma de compostos potencialment valuosos (rics en fibra, proteïnes i polifenols) [2,24–28], actualment es destil·len per recuperar alcohol o elaborar begudes destil·lades, essent realment tasques de gestió dels residus generats sense atorgar-hi cap valor afegit. En la literatura revisada es poden trobar alguns exemples per tal de reaprofitar aquests subproductes. De fet, Mildner et al. (2013) [25] van estudiar l'ús de la brisa del raïm en l'elaboració de galetes com a font alternativa de fibra alimentària i compostos fenòlics. A més a més, Hernández-Macias et al. (2021) [29] han avaluat *in vitro* l'efecte de les lies del Cava sobre diferents soques de bacteris de l'àcid làctic (BAL) obtenint un major creixement i supervivència en certes soques amb una addició del 5% de lies al medi de cultiu. D'altra banda, també s'ha observat com l'ús de lies del Cava en la formulació d'embotits fermentats millora la seguretat microbiològica disminuint el creixement de bacteris patògens (*Salmonella* spp. i *Listeria monocytogenes*) [28].

Les lies del Cava es composen, principalment, de cèl·lules naturalment plasmolitzades d'una sola espècie de llevat, juntament amb coadjuvants tecnològics que ajuden a la floculació i eliminació de les lies al final del procés d'envelliment [10]. En aquesta etapa (Figura 5), les cèl·lules de llevat bàsicament es composen de la paret cel·lular, formada per polisacàrids (ß-glucans, manoproteïnes i quitina), és a dir, fibra soluble [10,23]; a més a més dels diferents compostos que hagin pogut adsorbir a la superfície (com els compostos fenòlics i volàtils) durant el procés [30].

Diversos estudis han demostrat que tant els ß-glucans com els mananoligosacàrids (MOS) tenen un efecte promotor del creixement de bacteris de l'àcid làctic (BAL) [31–34]. També cal destacar un creixent interès en l'efecte de l'addició de fibra a aliments fermentats per tal d'estimular el creixement dels microorganismes i accelerar-ne l'acidificació [35–38], millorant la seguretat alimentària dels productes en qüestió. A més a més, s'ha atribuït un efecte prebiòtic a la fibra, essent fermentada per la microbiota intestinal [39–41] i produint àcids grassos de cadena curta (acetat, butirat i propionat) que actuen com a nutrients de les cèl·lules epitelials i la microbiota del còlon. Per tant, per la seva composició, les lies del Cava podrien tenir un possible efecte prebiòtic beneficiós sobre la microbiota intestinal.

Com s'ha comentat anteriorment, les lies poden adsorbir diversos compostos, com són els compostos fenòlics (Figura 6). Aquests compostos provenen del raïm però la seva composició en el vi, i conseqüentment, a les lies, es veu afectada pel procés de vinificació [24,42]. De fet, Martínez-Lapuente et al. (2013) [43] van observar una disminució dels compostos fenòlics durant l'envelliment del vi escumós en contacte amb lies, essent adsorbits a la superfície d'aquestes.

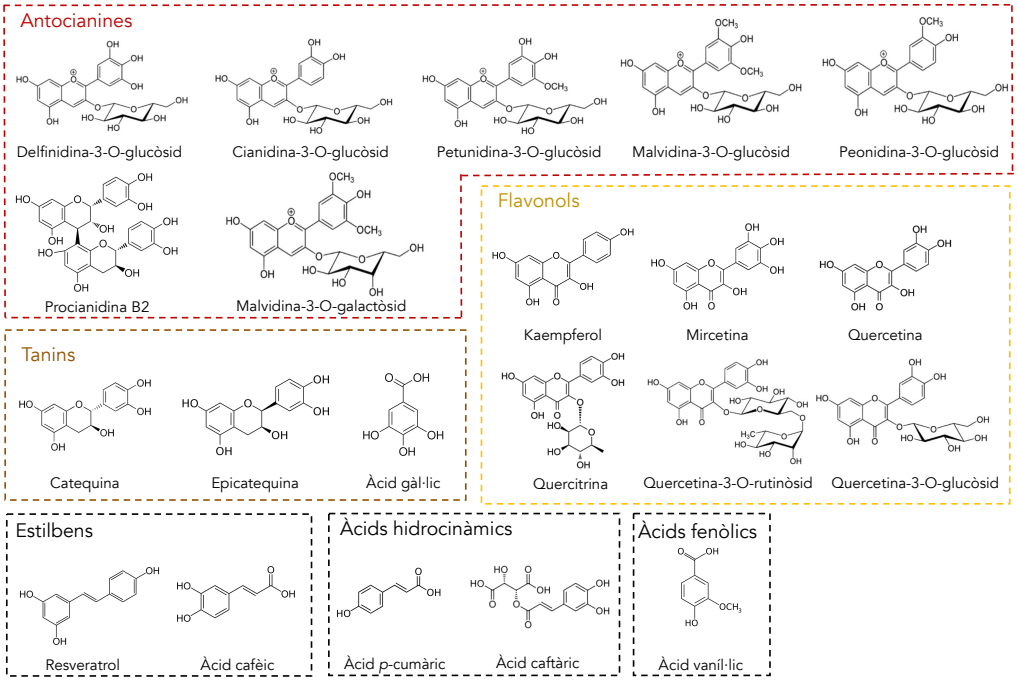

Figura 6. Estructures químiques dels principals compostos fenòlics identificats a les lies. Adaptat de [42].

En els subproductes de la indústria vínica, l'activitat antioxidant es relaciona directament amb la concentració total de polifenols. La presència d'aquest compostos fenòlics a la superfície de les lies, juntament amb les manoproteïnes i els glucans, els hi atribueixen activitat antioxidant [9,42].

2.2. Els aliments fermentats: Prebiòtics i Probiòtics

En els últims anys la població ha augmentat la tendència cap al consum d'aliments més saludables, entre els que destaquen aliments fermentats de producció ancestral (com el quefir, el garum, el kombutxa i el kimchi, entre d'altres) i que actualment es consumeixen de forma creixent en associar-se a un bon estat de salut. És per això que tant la indústria com la comunitat científica treballen pel desenvolupament d'aliments més nutritius, però que alhora aportin beneficis saludables extres. Aquest tipus d'aliments es coneixen com a aliments funcionals [41]. En aquest sentit, els prebiòtics i els probiòtics entren dins d'aquesta categoria, aportant millores tecnològiques (volum, textura, aroma, etc.), a part de les seves propietats nutricionals inherents [44]. Els productes de panificació s'inclourien en aquesta tipologia de productes alimentaris tradicionals que tornen a prendre protagonisme, tal i com es desprèn de les dades mostrades a la taula 3, on s'hi presenten el número de publicacions científiques dels últims 20 anys relacionades amb els prebiòtics i probiòtics, la massa mare i el pa.

Taula 2. Comparativa de les publicacions sobre prebiòtics i probiòtics relacionats amb la massa mare i el pa, en el període de 2000 a 2022, segons cerca realitzada a través de la base de dades Scopus (Cerca actualitzada el mes d'agost de 2022).

	Criteri de cerca			
Període (anys)	Prebiotic & food	Prebiotic & sourdough or bread	Probiotic & food	Probiotic & sourdough or bread
2000-05	395	15	1341	12
2006-10	788	26	2371	22
2011-15	1514	68	3964	69
2016-22	3917	97	9920	169

Els prebiòtics són ingredients alimentaris no digeribles que suposen beneficis per la salut de l'hoste mitjançant l'estimulació del creixement o l'activitat metabòlica dels bacteris de la microbiota intestinal [41], suposant una millora per la salut de l'hoste. Els principals compostos prebiòtics són els galacto-oligosacàrids (GOS), els xilo-oligosacàrids (XOS), els fructo-oligosacàrids (FOS), la inulina, els isomalto-oligosacàrids (IMO), la polidextrosa, la lactulosa i el midó resistent [41,45,46]. El midó resistent és la part del midó que pot resistir la digestió a l'intestí

per part de l'amilasa pancreàtica arribant al colon on podrà esser fermentat per la microbiota intestinal [44].

De fet, el pa conté carbohidrats, vitamina B, minerals i proteïnes, a més a més d'una font de fibra per la microbiota intestinal [47,48]. Addicionalment, l'activitat enzimàtica dels bacteris fermentadors de la massa mare pot contribuir a incrementar la quantitat de fibra soluble del pa [49]. Cal destacar que la fibra amb més efecte sobre la microbiota i els seus productes de fermentació és la fibra soluble [48]. Aquesta fibra, així com altres polisacàrids complexos, poden ser metabolitzats pels bacteris de l'intestí gros, produint àcids grassos de cadena curta (AGCC) (Figura 7) [50].

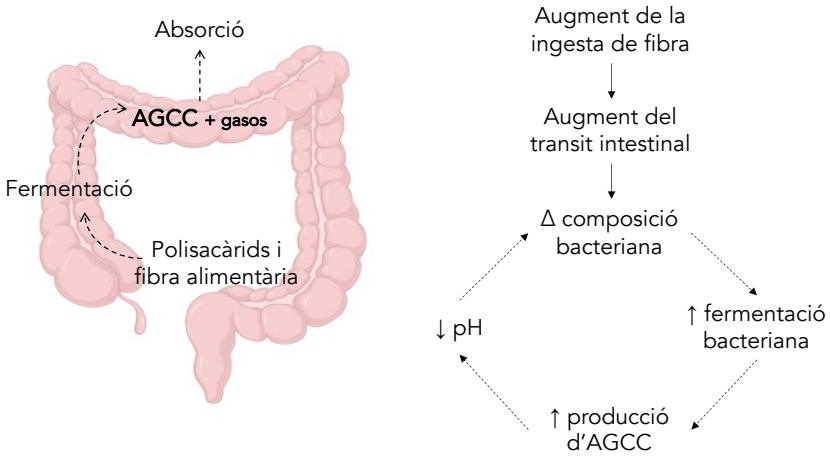

Figura 7. Diagrama esquemàtic que il·lustra el destí de la fibra alimentaria i els polisacàrids complexos a l'intestí gros, i el possible impacte de l'augment de la ingesta de fibra en el trànsit intestinal i altres paràmetres. Les línies sòlides indiquen un efecte directe; les línies de punts indiquen efectes interconnectats. Adaptat de [50].

A més a més, els BAL de la massa mare poden produir exopolisacàrids (EPS) que, a més de millorar la textura del pa, se'ls ha atribuït potencial prebiòtic [51]. Així mateix, la massa mare presenta un índex glicèmic més baix (Taula 2), el que contribueix en un augment del midó resistent de lenta digestió que arriba al colon [48,52]. Alguns estudis indiquen que el pa elaborat amb massa mare pot actuar com a prebiòtic i tenir un impacte positiu sobre la microbiota intestinal, la resposta immunitària intestinal i la inflamació sistemàtica de l'intestí (malaltia inflamatòria de l'intestí, MII) [47,48,53]. Lluansí et al. (2021) [48] van estudiar *in vitro* l'efecte

prebiòtic del pa sobre el microbioma de la MII. Per aquest fi van elaborar diferents pans seguint diferents processos: amb i sense massa mare, i amb diferents temps de fermentació. En aquest estudi van observar una tendència cap a la millora de la microbiota intestinal, similar a la de pacients sans, indicant un potencial efecte prebiòtic del pa en pacients amb MII. A més a més, també van reportar un increment en microorganismes productors d'AGCC, com l'acètic, el butíric i el propiònic que tenen propietats antiinflamatòries [48,54].

D'altra banda, els probiòtics són microorganismes vius que aporten beneficis saludables a l'hoste quan s'administren en quantitats adequades [55]. Alguns aliments fermentats confereixen aquests beneficis, ja que poden contenir probiòtics, a més a més de prebiòtics, proteïnes i pèptids beneficiosos per l'hoste [56]. En general, aquest tipus d'aliments són fermentats amb bacteris de la família *Lactobacillaceae*, molts dels quals es consideren probiòtics. A la Taula 3 hi ha descrits aliments que inclouen bacteris probiòtics i els beneficis saludables que comporten a l'hoste.

A més, s'ha observat que la ingesta d'alguns probiòtics del gènere *Bifidobacterium* redueix els símptomes de la MII i millora en la regularitat del còlon després de la ingesta de productes làctics fermentats [57]. A més a més, aquests probiòtics també poden prevenir infeccions gastrointestinals per exclusió competitiva de patògens [57,58]. A més dels efectes positius sobre el tracte digestiu, els probiòtics també poden produir compostos bioactius com els antioxidants, compostos fenòlics, EPS i AGCC que també milloren la qualitat i els valors nutricionals dels productes fermentats [59,60]. En el cas de la massa mare, s'han descrit que algunes soques dels microorganismes fermentadors d'aquesta tenen potencial probiòtic [59,61–64]. Entre aquests microorganismes s'hi troben soques de les espècies *Enterococcus mundtii* [64], *E. faecium* [59], *Lactiplantibacillus plantarum* [61–63], *Leuconostoc citreum* [59] i *Pediococcus pentosaceus* [59,63].

Taula 3. Aliments fermentats amb bacteris probiòtics i beneficis que aporten a l'hoste.

Taxonomia		Aliment	Benefici	Ref.
Família	Espècie			
Lactobacillaceae	*Lactobacillus acidophilus*	Iogurt	Millora de la intolerància a la lactosa.	[65]
	Levilactobacillus brevis *Lactiplantibacillus plantarum* *Limosilactobacillus fermentum*	Formatge fumat sec Escabetx vegetal Chhang (cervesa a base d'ordi)	Disminució de colesterol, producció de substàncies antimicrobianes, producció dEPS[1], activitat ß-galactosidasa	[66]
	,*Levilsctobacillus brevis* *Lactiplantibacillus plantarum* i *Pediococcus pentosaceus*	Massa mare	Efecte antifúngic i antibacterià. Reducció de colesterol, millora de la inflamació i modulació la microbiota intestinal. Capacitat de degradació de fitats, capacitat antioxidant, producció d'EPS.	[59,61–63,67]
	Lactiplantibacillus plantarum	Xucrut, tofu i kefir	Capacitat antioxidant	[68]
	Lactiplantibacillus plantarum	Fesol amarg (*Parkia speciosa*) fermentat	Activitat BSH[2] i activitat antimicrobiana contra bacteris patògens.	[69]
	Lactiplantibacillus plantarum i *Limosilactobacillus fermentum*	Cacau natural fermentat	Activitat antimicrobiana contra bacteris patògens.	[70]
	Lactoccous lactis	Kèfir	Producció d'ALC[3] i canvis sobre el perfil d'àcids grassos.	[60]
	Lacticaseibacillus rhamnosus	Productes fermentats grecs (olives, formatge i salmorra)	Manteniment de l'homeòstasi intestinal i agent antidiabètic per alleujar-ne els símptomes.	[71]
	Weissella cibaria	Tejuino (beguda fermentada de blat de moro)	Activitat antimicrobiana contra bacteris patògens.	[72]

Taula 4. *Continuació.*

Taxonomia		Aliment	Benefici	Ref.
Família	Espècie			
Bifidobacteriaceae	*Bifidobacterium* spp.	Llet fermentada i iogurt	Protecció de l'hoste contra patògens, modulació del sistema immunitari i descomposició dels carbohidrats no digeribles. Millora de la intolerància a la lactosa.	[57,65,73]
Enterococcaceae	*Enterococcus faecalis, Enterococcus faecium* i *Enterococcus durans*	Embotit de peix	Activitat antimicrobiana contra patògens, activitat antioxidant.	[74]
	Enterococcus faecium	Massa mare	Capacitat de degradació de fitats, capacitat antioxidant, producció d'EPS.	[59]
		Kimchi	Producció de bacteriocines inhibidores de *Listeria* i *Enterococcus* spp. (bacteris patògens). Producció de compostos antimicrobians.	[75]
Leuconostocaceae	*Leuconostoc citreum*	Massa mare	Capacitat de degradació de fitats, capacitat antioxidant, producció d'EPS.	[59]
		Tejuino (beguda fermentada de blat de moro)	Activitat antimicrobiana contra patògens, producció d'àcids grassos de cadena curta.	[72]

[1] EPS: Exopolisacàrids. [2] BSH: Hidrolasa de sals biliars. Influencia el metabolisme del colesterol. [3] ALC: Àcid linoleic conjugat

En el disseny de productes de pa funcionals amb probiòtics s'ha de tenir en compte la temperatura del fornejat, ja que molts d'aquests microorganismes s'inactiven o eliminen durant aquest procés [44]. Tot i això, aquests bacteris inactius (no viables), anomenats paraprobiòtics, també poden conferir beneficis a l'hoste en concentracions adequades [76].

Una alternativa és l'ús de la massa mare, ja que com s'ha comentat anteriorment, els bacteris probiòtics poden produir compostos bioactius que es queden a la massa i el posterior pa. Aquests compostos s'anomenen postbiòtics [44,76]. És més, tant els paraprobiòtics com els postbiòtics poden arribar a aportar els mateixos beneficis que els probiòtics sense haver d'administrar microorganismes vius que podrien causar una reacció immunitària [44].Finalment, degut a la possible sinèrgia entre els prebiòtics i els probiòtics, els aliments en que s'hi troben combinats s'anomenen simbiòtics [41]. Actualment hi ha un creixent interès pel desenvolupament de simbiòtics per tal d'aconseguir les correctes proporcions i quantitats de pre- i probiòtics que aportin més beneficis saludables al consumidor que consumint-los per separat [44].

2.3. El pa i la massa mare

El pa és un aliment consumit en grans quantitats arreu del món, representant entre un 12% (domèstic) i un 28% (fora de casa) del consum d'aliments total a Espanya. Així doncs, el consum mitjà de pa de l'any 2020 ha estat de 32,8 kg per persona, incrementant un 5,5% respecte l'any anterior [77].

De fet, l'any 2019 va entrar en vigor a l'Estat Espanyol la nova normativa (RD 308/2019) sobre la qualitat del pa [78]. Amb aquesta legislació es garanteix als consumidors l'adquisició de productes de qualitat, caracteritzats i correctament etiquetats. Entre els punts específics, s'hi troba la definició i regulació d'elaboració de pa amb massa mare (fins el moment no legislat), així com la reducció de la quantitat de sal permesa en l'elaboració del pa comú.

L'ús de la massa mare per la fermentació del pa és una pràctica bastant estesa ja que millora la qualitat del pa final, així com l'estabilitat microbiològica i la vida útil del producte [79]. A més a més, hi ha una tendència creixent cap al consum de productes artesans, més sans i seguint la preferència dels consumidors per les "etiquetes netes" o *clean-label*. Per tant, l'addició de massa mare a la formulació del pa comporta beneficis relacionats amb la seguretat alimentària, així com acceptació entre els consumidors.

2.3.1. Elaboració i característiques de la massa mare

La massa mare és una barreja de farina i aigua resultant d'un procés de fermentació dut a terme per bacteris de l'àcid làctic (BAL) i llevats. Aquesta fermentació pot ser espontània o producte de la inoculació de cultius iniciadors, i pot patir refrescos o no. Així doncs, l'activitat metabòlica dels microorganismes fermentadors resulta en l'acidificació de la massa i la formació dels compostos volàtils responsables de l'aroma (Publicació 1). Tal com s'ha mencionat, el procés pot donar-se de manera espontània, essent la microbiota pròpia de la farina i demés ingredients utilitzats els responsables de la fermentació, o inocular els microorganismes desitjats. Tot i així, les poblacions microbianes poden modificar-se en funció dels paràmetres tecnològics aplicats, el que resulta en diferents característiques organolèptiques de la massa mare i el pa final.

2.3.2. Beneficis de l'ús de massa mare

Com s'ha comentat anteriorment, l'addició de massa mare a la preparació de pa suposa una millora en la qualitat, el sabor i l'aroma del producte final. Aquestes diferències són, principalment, conseqüència de la fermentació de la massa per part dels BAL.

D'una banda, aquest bacteris provoquen una disminució del pH més ràpida, acidificant la massa degut a la producció d'àcids orgànics (com l'àcid acètic). Això provoca, principalment, una estabilització microbiològica (seguretat alimentària) del producte allargant-ne la vida útil, així com una modificació dels atributs sensorials [80,81]. A més a més, els BAL (sobretot els heterofermentatius) donen lloc a una major producció de compostos volàtils (èsters, aldehids i cetones), incrementant la complexitat de l'aroma del pa [82].

D'altra banda, s'ha observat que l'ús de massa mare comporta beneficis nutricionals (Taula 4): contribueix a la degradació de l'àcid fític (anti-nutrient); millora la digestibilitat; i pot suposar una disminució de l'ús de sal en la formulació del pa.

Taula 4. Contribució de la massa mare a la millora de les propietats nutricionals del pa.

Característiques nutricionals		Referències
Carbohidrats	↓ Índex glicèmic	[83,84]
	↓ FODMAPs [1]	[85]
Proteïnes	↓ Contingut de gluten	[85][86,87] [2]
	↑ Alliberament de pèptids bioactius	[88–91]
Minerals	↑ Biodisponibilitat	[92]
Anti-nutrients	↓ Àcid fític	[91,92]
Activitat antioxidant	↑ compostos fenòlics	[91]
Altres ingredients	↓ Sal	[88]

[1] FODMAPs: Oligo-, Di-, Mono-sacàrids i poliols fermentables.

[2] BAL aïllats de massa mare.

2.3.2.1. Millora de les propietats organolèptiques

L'aroma i el gust són els principals factors determinants en quant a l'acceptació dels aliments per part dels consumidors. Considerant que la massa mare té una fermentació més llarga en la que hi participen bacteris i llevats, hi ha una major formació de compostos relacionats amb el sabor, un aroma més complexa, aconseguint major puntuació en tests sensorials [81,93]. Aquesta millora de l'aroma i la textura del pa és conseqüència del procés d'hidròlisi enzimàtica produïda pels BAL i la generació de compostos de reacció de Maillard durant la cocció del pa [81].

En general, hi ha tres rutes per les que es formen els compostos volàtils en el pa: a) fermentació; b) oxidació lipídica; i c) reacció de Maillard producte del procés de cocció. El procés fermentatiu és la principal ruta de formació d'aquests compostos, produint majoritàriament àcids, alcohols, aldehids, cetones i èsters [82,94]. En canvi, la oxidació lipídica condueix a la formació d'aldehids, cetones, alcohols i èsters (depenent de la concentració inicial d'àcids grassos) [82,95].

Els compostos volàtils de la massa mare es divideixen en diferents famílies químiques: alcohols, aldehids, cetones, èsters i compostos sulfurats. La majoria d'aquests compostos provenen del metabolisme dels microorganismes fermentadors. A més a més, tant els llevats com els BAL produeixen precursors de l'aroma, com els aminoàcids lliures, que condueixen a la formació d'aldehids o dels alcohols corresponents, mitjançant la via Ehrlich [81]. Així doncs, existeixen diversos factors que influencien l'activitat microbiana de la massa mare, podent-se dividir en factors endògens (farina, aigua i altres ingredients) i exògens (temperatura i temps de fermentació, rendiment de la massa i condicions d'oxigen) (Publicació 1). Per tant, modificant la microbiota o la matèria prima, no només es modifiquen els compostos volàtils resultants de la fermentació, sinó també els precursors de l'oxidació lipídica i la reacció de Maillard [82].

Tot i que el perfil volàtil del pa ha estat molt estudiat en comparació amb el de la massa mare (Figura 8), s'han identificat un total de 169 compostos en massa mare de blat i sègol. Entre els volàtils identificats, els més citats són: hexanal, nonanal, 1-pentanol, 1,4-butanediol, 6-methyl-5-hepten-2-ona i acetat d'octil [82].

D'altra banda, en pa elaborat amb massa mare de blat i sègol s'han trobat un total de 150 compostos volàtils, on els majoritaris han estat: 3-metilbutanal, 1-pentanol i 1,4-butanediol [82].

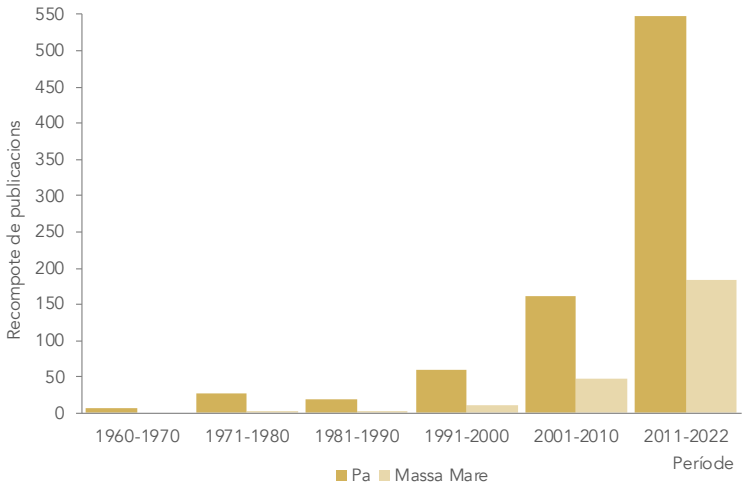

Figura 8. Recompte de publicacions científiques que contenen les paraules clau *bread volatile compounds* o *sourdough volatile compounds* segons cerca realitzada a través de la base de dades Scopus (Cerca actualitzada el mes d'agost de 2022).

La influència dels diferents paràmetres de procés sobre la microbiota i les propietats fisicoquímiques i sensorials de la massa mare es troben resumides a la revisió bibliogràfica publicada a la revista *Food Reviews International*, que s'adjunta a continuació.

PUBLICACIÓ 1

Influence of Process Parameters on Sourdough Microbiota, Physical Properties and Sensory Profile.

Alba Martín-Garcia, Montserrat Riu-Aumatell, Elvira López-Tamames.
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Influence of Process Parameters on Sourdough Microbiota, Physical Properties and Sensory Profile

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ABSTRACT

Sourdough is the result of a fermentation process involving mainly LAB and yeasts. The present microbiota increase acidification, leavening and flavour. Moreover, consumers demand for more nutritious, healthy and clean-label products, has increased the use of sourdough in bread-making. Generally, LAB rapidly acidify the sourdough medium due to organic acids production (lactic and acetic acid, mostly). Nevertheless, different process parameters may influence sourdough microbiota, inducing changes in both sourdough and the resulting bread. Finally, sourdough fermentation involving probiotics can provide extra health benefits to its consumers. Also, long fermentations can reduce FODMAPs and phytate content, besides lowering the glycemic index.

KEYWORDS

Sourdough; lactic acid bacteria; yeasts; volatile compounds

Introduction

Bread is consumed in large quantities all over the world. During bread making, sourdough has traditionally been used as a leaving agent, influencing bread quality.[1,2] Sourdough is a mixture of cereal flour and water, fermented by either homo- or hetero-fermentative lactic acid bacteria (LAB). This process can happen either by the addition of a starter culture or by spontaneous fermentation, involving backslopping, or not.[3]

The metabolic activity of the present microbiota results in acidification, leavening and flavour formation.[4,5] These changes lead to an increased microbiologic stability and shelf life (delaying the spoilage of bread other by microorganisms), and an improvement of nutritional and sensory characteristics of bread.[6] Sourdough can be produced from a numerous sources of flour, which grant different characteristics to sourdough bread, like flavour, organoleptic properties or nutritional value. Furthermore, chemical composition and quality of the flour influence the microbial community dynamics and metabolite kinetics of the sourdough fermentation process.[7–9]

Nowadays, clean-label, healthier and artisan products are trending, which has increased the use of sourdough in bread-making. For that reason, some breads have already received the Protected Designation of Origin (PDO) or the Protected Geographical Indication (PGI). Those breads, such as Pane di Altamura and Pagnotta del Dittaino (PDO, produced in Italy) or Pa de Pagès Català, Pan de Cruz de Ciudad Real and Pan de Cea (PGI, produced in Spain) and Pane di Matera, Pane Casareccio di Genzano and Coppia Ferrarese (PGI, produced in Italy), are protected by European regulations and must use sourdough as a leavening agent.[10] Additionally, UNESCO officially added the German bread culture to its Intangible Cultural Heritage list in 2015.

Even though there is no European specific regulation to produce sourdough, France ("pain au levain"; Décret nº93-1074), Germany ("Sauerteig"; Leitsätze für Brot und Kleingebäck vom

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19.10.1993) and Spain ("masa madre"; Real Decreto 308/2019) do have their own regulations regarding sourdough, including its definition and usage. Moreover, there is a "Real Bread" Campaign in the UK that has presented a Code of Practice for the labelling of sourdough bread and rolls to the Department for Environment, Food and Rural Affairs (DEFRA). The new legislation is intended to include a clear and legal definition of sourdough bread, in order to clarify the term and prevent misinformation to the consumers.

Therefore, the aim of this review will focus on summarizing the latest knowledge about the quality of sourdough, highlighting the microbiota, physical and sensory properties.

Types of sourdough

Sourdoughs can be classified into three different types (Fig. 1): (i) type I, traditional sourdoughs, they are restarted using a part of the previous fermentation; (ii) type II, industrial sourdoughs that use adapted strains to start the fermentation; (iii) type III, dried sourdoughs, and (iv) type IV, a combination between types I and II.[2,11]

Type I, or traditional sourdoughs, are initiated spontaneously and regularly propagated. This type of sourdough involves a cyclic reinoculation, using a part of the already fermenting dough (5–20%, m/v) and a new mixture of flour and water. This process is usually performed at room temperature (20–30°C) with a long fermentation process (5 to 7 days to get a stable microbiota and chemical system), obtaining firm sourdoughs with a low DY (<200) [DY = ratio between the dough obtained and the flour used].[3] Traditional sourdoughs are characterized by daily propagation ("backslopping"), that results in selection and dominance of the best adapted strains, keeping them metabolically active.[12,13]

On the other hand, types II and III are inoculated with a starter culture to perform sourdough fermentation. Type II sourdoughs are described as liquid industrial doughs, generally produced in a one-step fermentation of shorter duration (24 h aproximately) and high temperatures (30–37°C) with a high DY (>200).[3] This process allows a faster and higher acidification than type I sourdoughs, and can inhibit the growth of other microorganisms such as flour yeasts .[13]

Type III is defined as type II dried sourdoughs. In order to obtain type III sourdoughs, spray drying, and drum drying are the most commonly used techniques. Spray drying consists in pulverizing the liquid sourdough in a hot air stream. The water content (approx. 90%) is evaporated, while dried particles can be collected from the dryer. Maillard products formation is limited because of

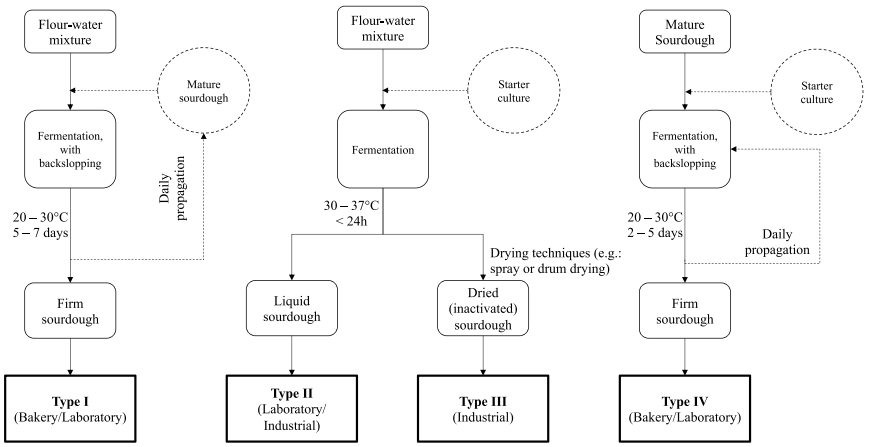

Figure 1. Types of sourdough fermentation according to the technology applied (Adapted from[3]).

evaporative cooling. As for drum drying technology, steel cylinders are heated with steam. Then, a thin film of the pasty or liquid sourdough is spread over the cylinder and water and volatile compounds evaporate immediately. During drum drying Maillard and other flavour reactions take place producing malty and roasty-flavours. Depending on the temperature and time combination, the final sourdough product can be more or less toasted and caramelised.[14]

Types II and III are often used in industrial bakeries, because they are easy to use. Furthermore, the use of industrial sourdough allows a greater variety in flavour and taste of the resulting bread.[14] Both are used as dough acidifiers and flavour ingredients or carriers,[11] although type III sourdoughs are used as non-living supplements.[3] In that case, because the microbiota is inactivated, it is necessary a backslopping prior to use.[3] In contrast to type I sourdoughs, types II and III often require the addition of baker's yeast for leavening, since the acidic environment inhibits yeasts spontaneous growth.[15]

Lastly, type IV is a mix between types I and II, usually used in laboratories and some bakeries. This type of sourdough consists of inoculating a starter culture to a mature sourdough. Then, the sourdough follows the same process as type I (in every other aspect, they are very similar), although the microbial populations can change during backslopping.[13]

Sourdough microbiota

Cereal flours and other sourdough ingredients are not sterile, therefore its microbiota is quite complex, especially when it is fermented spontaneously.[16] Although LAB and yeasts dominate sourdough fermentation, other microorganisms like acetic acid bacteria, can be found, especially in the beginning of the fermentation process.[17,18] Moreover, the sourdough ecosystem is characterized to be a stressful environment, in which the microbiota has to adapt to the variable carbohydrate and nutrient contents and a low pH.[3]

Sourdough lactic acid bacteria

In general, LAB can rapidly acidify the raw material due to their organic acids production in addition to exopolysaccharides and several enzymes. This results in an enhanced shelf life and microbial safety, improving texture and the sensory profile of the final bread.[19]

LAB can be classified into two groups according to their fermentation products: homofermentative, which produce only lactic acid as the main product of glucose fermentation; and heterofermentative, that produce acetic acid, CO_2 and ethanol besides lactic acid.[20] While homofermentative bacteria produce more lactic acid, consequently lowering pH and increasing the total titratable acidity (TTA)[6]; heterofermentative lactobacilli slightly contribute in leavening due to the CO_2 production besides producing further variety of flavour compounds.[21] In addition, heterofermentative bacteria can be classified into two subgroups: facultatively heterofermentative and obligately heterofermentative .[2,3]

The sourdough fermentation process is usually dominated by LAB, generally heterofermentative (either facultative or obligately), in particular species of *Lactobacillus*.[4,8,9,22,23] However, other bacterial species, such as *Pediococcus, Enterococcus, Lactococcus* and *Weissella*, are also present.[17,24,25] Furthermore, the number of bacteria species in sourdough is higher than yeasts, usually following a 100:1 ratio.[6,26] Among the LAB present in sourdough (Table 1), the most prevalent species are *Lb. sanfranciscensis, Lb. plantarum, Lb. brevis, P. pentosaceus, Lb. paralimentarius* and *Lb. fermentum*.[3]

Obligately heterofermentative LAB prevail within the sourdough ecosystem due to their adapted carbohydrate metabolism, amino acid conversion mechanisms and response mechanisms to acidic stress.[3] Additionally, a few lactobacilli can produce antimicrobial compounds, for example, some *Lb. reuteri* strains can generate reutericyclin, a low-molecular mass antibiotic with bacteriostatic and bactericidal activity against Gram-positive bacteria that cause ropy spoilage of bread.[46]

As previously stated, the ingredients used to elaborate sourdough are not sterile, nor is the bakery environment. Therefore, LAB originate from the flour used and the space where it is prepared. *Lb.*

Table 1. Overview of the lactic acid bacteria (LAB) diversity found in sourdough elaborated with different flours either in an artisan bakery or laboratory environment.

Cereal flour	Microorganisms reported	Sourdough type	Period of backslopping	Ref.
Barley	*Lb. fermentum*[b], *Lb. plantarum*[c], *Lb. brevis*[b], *W. confusa*[b], *P. pentosaceus*[c]	L	10 days	[15]
	L. citreum[b], *L. mesenteroides*[b], *W. confusa*[b], *W. cibaria*[b]	B	10 days	[15]
Buckwheat	*Lb. plantarum*[c], *Lb. graminis*[c], *Lb. sakei*[c], *W. cibaria*[b], *P. pentosaceus*[c], *Lc. holzapfelii*[c]	L	10 days	[9]
	Lb. plantarum[c], *Lb. fermentum*[b], *Lb. vaginalis*[b], *Lb. crispatus*[a], *Lb. gallinarum*[a]	L	12 days	[9]
Chestnut	*Lb. plantarum*[c], *P. pentosaceus*[c], *W. cibaria*[b], *P. lolii/stilesii*[b], *W. paramesenteroides*[b], *Lb. farciminis*[a]	L	12 days	[7]
Oat	*Lb. coryniformis*[b], *L. argentinum*[b], *P. pentosaceus*[c], *W. cibaria*[b]	L	7–8 days	[8]
Rye	*Lb. fermentum*[b], *Lb. plantarum*[c], *Lb. brevis*[b], *P. pentosaceus*[c], *Lb. sakei*[c], *Weissella spp*[b]	L	10–11 days	[27,28]
	Lb. amylovorus[a], *Lb. panis*[b], *Lb. reuteri*[b]	B	6–13 h	[29]
	Lb. helveticus[a], *Lb. panis*[b], *Lb. pontis*[b], *Lb. vaginalis*[b], *Lb. casei*[c], *Lb. paracasei*[c], *Lb. amylovorus*[a], *Lb. frumenti*[b]	B	3–6 years	[22]
	Lb. pontis[b], *Lb. casei*[c], *Lb. paracasei*[c], *Lb. pantarum*[c], *Lb. rhamnosus*[c], *Lb. zymae*[b], *Lb. fermentum*[b], *P. acidilactici*[c], *L. lactis*[b]	B	1 year (freeze dried)	[22]
	Lb. helveticus[a], *Lb. pontis*[b], *Lb. zymae*[b]	B	30 years	[22]
	Lb. brevis[b], *Lb. crustorum*[b], *Lb. paralimentarius*[c], *Lb. plantarum*[c]	L	56 days	[30]
	Lb. sanfranciscensis[b], *Lb. plantarum*[c], *Lb. diolivorans*[b], *Lb. hammesii*[b], *Lb. xiangfangensis*[c], *Lb. koreensis*[b], *Lb. farraginis*[b], *Lb. hilgardii*[b]	B	1–5 h	[23]
	Lb. sanfranciscensis[b], *Lb. pontis*[b]	B	1 h	[23,31]
Rye-wheat	*Lb. brevis*[b], *Lb. plantarum*[c], *Lb. zymae*[b], *Lb. pentosus*[c], *Lb. sanfranciscensis*[b]	B	2 h	[23,32]
	Lb. brevis[b], *Lb. alimentarius*[c], *Lb. pentosus*[c]	B	8 years	[24]
	Lb. sakei[c], *Lb. sanfranciscensis*[b]	B	4 years	[24]
Teff	*P. pentosaceus*[c], *Lc. holzapfelii*[c]	L	10 days	[9]
	Lb. fermentum[b], *Lb. vaginalis*[b], *Lb. gallinarum*[a], *Lb. pontis*[b]	L	13 days	[9]
Wheat	*Lb. plantarum/pentosus/paraplantarum*[c], *Lb. fermentum*[b]	L	10 days	[4]
	Lb. sanfranciscensis[b], *Lb. fermentum*[b], *W. cibaria*[b]	B	3–7 months	[25]
	Lb. sanfranciscensis[b], *W. cibaria*[b], *Lb. fermentum*[b], *Lb. plantarum*[c], *Lb. pontis*[b]	B	10–20 days	[25]
	Lb. sanfranciscensis[b]	B	2 years	[25]
	W. cibaria[b], *W. confusa*[b], *L. citreum*[b], *Lb. sanfranciscensis*[b], *Lb. plantarum*[c], *L. mesenteroides*[b], *Lb. sakei*[c], *P. pentosaceus*[c], *Lb. paralimentarius*[c], *Lb. gallinarum*[a], *Lc. lactis*[c], *Lb. brevis*[b], *P. inopinatus*[c], *Lb. casei*[c], *P. argentinicus*[c], *Lb. rossiae*[b], *W. paramesenteroides*[b], *Lb. spicheri*[b], *Lb. namurensis*[b], *E. durans*[a], *Lb. curvatus*[b], *Lb. hammesii*[b], *Lb. lindneri*[b]	B	3–24 h	[1,10]
	Lb. plantarum[c], *Lb. sanfranciscensis*[b], *Lb. graminis*[c], *Lb. rossiae*[b]	L	3 days	[18]
	Lb. sanfranciscensis[b], *P. pentosaceus*[c], *Lb. brevis*[b], *L. holzapfelii*[b], *Lb. sakei*[c], *Lb. rossiae*[b], *Lb. plantarum*[c], *W. cibaria*[b], *Lb. spicheri*[b], *Lb. graminis*[c]	B	3 days	[18]
	Lb. sanfranciscensis[b], *L. citreum*[b], *Lb. sakei*[c], *L. mesenteroides*[b], *W. cibaria*[b], *P. pentosaceus*[c]	B	1 year	[33]
	Lb. sanfranciscensis[b], *Lb. brevis*[b], *Lb. plantarum*[c], *Lb. zymae*[b], *Lb. pentosus*[c], *Lb. paralimentarius*[c], *Lb. sakei*[c], *Lb. paracasei*[c], *Lb. paraplantarum*[c], *Lb. rossiae*[b], *W. cibaria*[b], *P. pentosaceus*[c], *L. pseudomesenteroides*[b], *L. mesenteroides*[b], *L. citreum*[b], *P. parvulus*[c], *S. salivarus*[a], *Lb. namurensis*[b], *Lb. graminis*[c], *Lb. curvatus*[b], *E. durans*[a], *E. faecium*[a], *Lb. acetotolerans*[a], *Lb. casei*[c], *Lb. farciminis*[a], *Lb. mindensis*[a], *Lb. spicheri*[b], *W. confusa*[b], *P. acidilactici*[c], *E. casseliflavus*[a], *Lb. cellobiosus*[b], *Lb. diolivorans*[b], *Lb. heilongjiangensis*[b], *Lb. koreensis*[b], *Lb. parabuchneri*[b], *Lb. kimchii*[c]	B	-	[31,32,34–41]
	Lb. sakei[c], *Lb. plantarum*[c], *Lb. zeae*[c], *P. pentosaceus*[c], *Leuconostoc spp.*[b], *Weissella spp.*[b], *Lc. lactis*[c], *Lb. brevis*[b], *Lb. curvatus*[b], *Lb. fermentum*[b], *L. mesenteroides*[b]	L	11 days	[28,42,43]
	Lb. brevis[b], *Lb. vaccinostercus*[b]	B	9 years	[24]

(*Continued*)

Table 1. (Continued).

Cereal flour	Microorganisms reported	Sourdough type	Period of backslopping	Ref.
Wheat-Legumes (chickpea; lentile; bean)	*Lb. plantarum*[c], *W. cibaria*[b], *L. mesenteroides*[b], *Lb. parabuchneri*[b], *Lb. paraplantarum*[c], *Lb. coryneformis*[c], *Lb. fermentum*[b], *Lb. brevis*[b], *Lb. pentosus*[c], *Lb. sanfranciscensis*[b], *Lb. rossiae*[b]	L	10 days	[44]
Wheat-Grape	*P. pentosaceus*[c], *W. cibaria*[b], *Lb. brevis*[b], *Lb. sakei*[c], *Lb. plantarum*[c]	L	5–10 days	[45]
Wheat-Pear; Wheat-Navel Orange	*Lb. brevis*[b], *Lb. plantarum*[c], *Lb. rossiae*[b]	L	-	[43]
Wheat-Yogurt	*P. pentosaceus*[c], *Lb. sakei*[c]	L	5–10 days	[45]

Types of sourdough reported (B: bakery; L: Laboratory): i: dough; ii: liquid
LAB species reported (a: obligately homofermentative; b: obligately heterofermentative; c: facultatively heterofermentative): E., *Enterococcus*; L., *Leuconostoc*; Lb., *Lactobacillus*; Lc., *Lactococcus*; P., *Pediococcus*; W., *Weissella*

Table 2. Overview of yeast diversity found in sourdough elaborated with different flours either in an artisan bakery or laboratory environment.

Cereal flour type	Microorganisms reported	Period of backslopping	Ref.
Barley	*S. cerevisiae*	10 days	[15]
Buckwheat	*K. barnettii*	10 days	[9]
Rye	*S. cerevisiae*	6–20 h	[23,29]
	W. anomalus, C. glabrata, S. cerevisiae	2 years	[49]
	C. humilis, K. telluris	1 year – 30 years	[22]
	C. glabrata, K. unispora, P. kudriavzevii, S. cerevisiae	56 days	[30]
Rye-wheat	*S. cerevisiae, T. delbrueckii, W. anomalus, C. humilis*	1 month – 25 years	[23,24,49]
	S. cerevisiae, S. bayanus, C. humilis, W. Anomalus	11 days	[28]
Spelt	*W. anomalus, C. glabrata, S. cerevisiae*	1,5–2 years	[49]
Teff	*C. glabrata, S. cerevisiae*	10–13 days	[9]
Wheat	*S. cerevisiae, C. humilis, W. anomalus*	10 days – 2 years	[25]
	S. cerevisiae, K. bulderi, K. barnettii, K. saulgeensis, K. unispora, K. humilis, C. carpophila, T. delbrueckii, H. pseudoburtonii, R. mucilaginosa, W. anomalus, M. guilliermondii, C. parapsilosis, C. pararugosa, S. barnettii	-	[26,34,35,37,39,41,50]
	S. cerevisiae, C. humilis, K. barnettii, K. exigua, K. servazzii	1–24 h	[10,23]
	S. cerevisiae, K. barnettii, K. unispora, T. delbrueckii, W. anomalus, C. glabrata	1–12 years	[24,49]
	S. cerevisiae, W. anomalus, S. barnettii	3 days	[18]
	K. exigua/barnettii/bulderi, C. glabrata	10 days	[4]
Wheat-Pear; Wheat-Navel Orange	*S. cerevisiae*	-	[43]
Wheat-Apple	*C. oleophila, C. pararugosa, Cy. misumaiensis, R. pinicola, M. guilliermondii, S. cerevisiae*	5–10 days	[51]
Wheat-Grape	*H. uvarum, S. cerevisiae*	5–10 days	[51]
Wheat-Rye-Spelt	*S. cerevisiae*	4 years	[49]
Wheat-Yogurt	*M. guilliermondii, S. cerevisiae, W. anomalus*	5–10 days	[51]

Yeast species reported: C., *Candida*; H., *Hanseniaspora*; K., *Kazachstania*; Kl., *Kluyveromyces*; M., *Meyerozyma*; P., *Pichia*; R., *Rhodotorula*; S., *Saccharomyces*; T., *Torulaspora*; W., *Wicherhamomyces*.

sanfranciscensis is reported as the main bacteria found in type I sourdough[23] and, recently, it has been identified as a predominant species in frass released by insects infesting stored grains, pointing them as a potential source for *Lb. sanfranciscensis* prevalence in sourdough ecology.[25] Although in most cases heterofermentative bacteria dominate the sourdough fermentation, homofermentative LAB have been reported in rye sourdoughs, such as *Lb. helveticus* and *Lb. amylovorus*, as the predominant bacteria.[22,29] Other frequent species found in type I sourdoughs include *Lb. brevis, Lb. plantarum, Lb. sakei, P. pentosaceus, Leuconostoc* spp. and *Weissella* spp.; while type II sourdoughs are dominated

by *Lb. plantarum, Lb. fermentum* and *Lb. panis*, even though it depends on the starter culture and the concentration in which it has been inoculated. Finally, model sourdoughs started and propagated in a laboratory environment, in which the flour is the only source of microorganisms, *Lb. plantarum* and *Lb. fermentum* are the most frequently reported bacteria isolated.[47]

Sourdough yeasts

Sourdough is a complex biological system, where yeasts are commonly associated with LAB.[5,6,26] While LAB contribute to acidification and the production of volatile and other metabolic compounds of bread, yeasts mainly act as leaving agents as well as aroma compound formation of sourdough bread.[48]

More than 30 yeast species are found in the sourdough environment (Table 2), but the dominant yeast species belong to *Saccharomyces* and *Candida* genes,[52] although *Wickerhamomyces anomalus* can be found in large quantities in Asian countries.[53] Other common yeasts species found in sourdough are *Torulaspora delbrueckii, Kazachstania exigua* and *Pichia kudriavzevii*.[3,16,52]

Yeast species found in sourdough are a result of their adaptation to the stressful environment, created by LAB and characterized by low pH, high carbohydrate concentration and high cell densities.[26] Besides, yeasts themselves contribute to that specific environment, hindering the growth of other microorganisms. For example, in spontaneously fermented sourdoughs, the presence of non-fermentative microorganisms, like wheat pathogens, may be reduced by the secondary metabolites produced by fermenting yeasts such as ethanol, acids and carbon dioxide during the fermentation process.[50]

Yeast-bacteria interactions

As previously stated, the sourdough ecosystem is formed by LAB and yeasts. In that environment, there can be some interactions between both microorganisms. These interactions can be determined by the effect of one species on another, and regarded as positive (synergistic), when one microorganism population tends to increase in the presence of another species; negative (antagonistic), when it tends to decrease; or neutral, when there is no effect. Those types of interaction (Fig. 2) include competition for resources (fermentable carbohydrates consumption, nitrogen sources, etc.), cross-

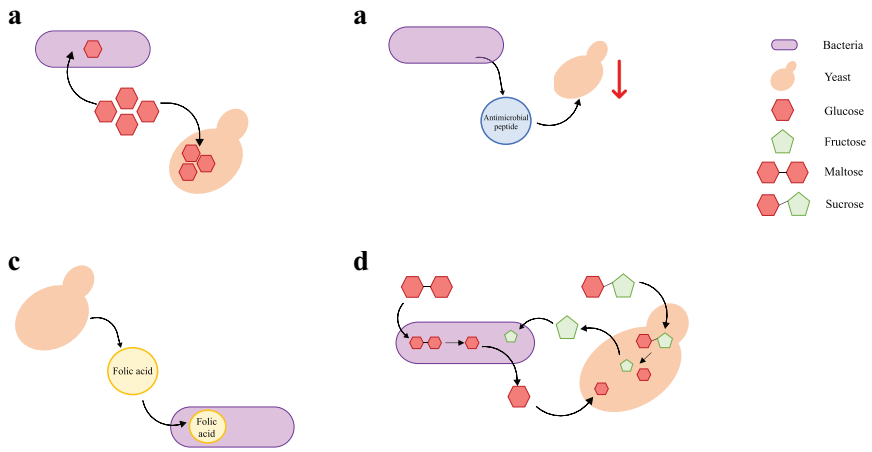

Figure 2. Main interactions that occur in the sourdough environment. A) Competition for nutrients (glucose); B) Amensalism (BAL produce antimicrobial peptides that inhibit yeast growth); C) Commensalism; and D) Mutualism (cross-feeding).

feeding (maltose degradation or amino acid production), and the production of secondary metabolites (antimicrobial compounds, medium acidification, etc.).[11,54,55]

Regarding stimulating interactions, cross-feeding (Fig. 2D) involves one species producing and essential nutrient that enables another microorganism (auxotroph) to survive.[55] For example, *Lb. sanfranciscensis* can metabolise maltose, releasing and accumulating glucose in the medium. Then, maltose-negative yeasts, such as *C. milleri* or *S. exiguus*, have a fermentable carbohydrate source, therefore preventing their population to be affected by glucose exhaustion and nutrient depletion.[56] Even more, the same occurs with sucrose, which yeasts can hydrolyse providing other carbon sources for LAB and stimulating their growth.[54] Therefore, cross-feeding reduces competition between microorganisms and enhances their growth.

In addition, it has been reported that CO_2 produced by yeasts as a result of carbon metabolization can also increase LAB growth, such as *Lb. sanfranciscensis* and *Lb. plantarum*, since it provides of an anaerobic environment. Moreover, *S. cerevisiae* is able to consume lactic acid, which results in a deacidification of the sourdough environment allowing more microbial growth.[54]

However, during fermentation, LAB and yeasts can produce certain metabolites that can modify the medium and can affect directly another species growth (Fig. 2B). For instance, lactic acid and acetic acid produced by LAB, as well as other minor organic acids (such as isovaleric, pentanoic and hexanoic acids) exhibit antimicrobial activity, are effective against figrope spoilage and may act synergistically protecting the sourdough bread against other microorganisms.[57] In addition, some lactobacilli strains are able to produce antimicrobial peptides, like *Lb. reuteri* that produces antifungal compounds.[58] In fact, Axel et al.[58] observed that some of the antifungal compounds found in sourdough came from amino acid metabolism due to LAB fermentation, reporting that higher proteolysis in sourdough fermentation result in more potential antifungal compounds.

All those interaction mechanisms can modify microbial populations, which can have a significant impact on the sourdough flavour and sensory characteristics. Consequently, is of importance the use of a stable microbiota for sourdough production, in order to obtain the desired final product.

Influence of process parameters on sourdough microbiota

As previously described (**Section 2**), sourdoughs can be classified in three types according to the process technology applied. Moreover, different parameters can affect microbial growth, following to changes in the sourdough and the sourdough bread (Fig. 3). These parameters can be endogenous or not fully controllable (e.g. nutrient composition) and, exogenous or technological (e.g. fermentation temperature). All of these parameters and their combination are responsible for the selection of the sourdough microbiota,[59] which can, therefore, influence the volatile composition of the sourdough and the bread produced with it. Thus, using the same technological parameters leads to the dominance of particular microbial strains, which are the best adapted to those conditions.

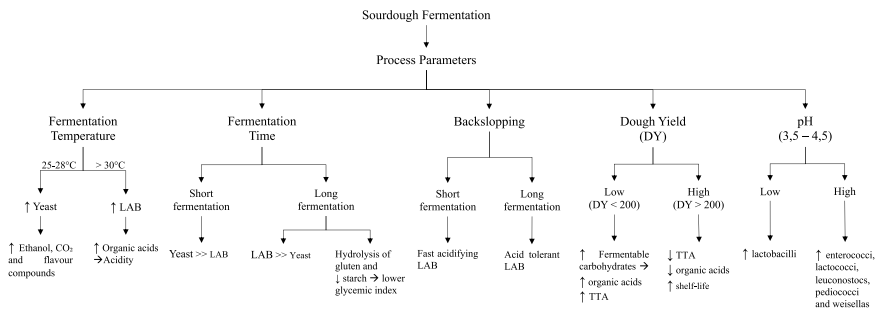

Figure 3. Influence of process parameters on sourdough microbiota and sourdough bread.

Temperature

Temperature is one of the main factors influencing the microbial communities, since each species have different optimal growth temperatures.[5] For instance, G. Vrancken et al.[59] observed that *Leuconostoc citreum* is more adapted to low temperatures (prevails and dominates fermentations at 23°C), while *Lb. fermentum* or *Lb. plantarum* dominate the sourdough fermentation at higher temperatures (30°C – 37°C and 30°C, respectively). The same happened with yeasts, while *C. humilis* or *S. cerevisiae* have an optimal growth temperature of 27–28°C; *P. kudriavzevii* or *T. delbrueckii* are found in sourdoughs fermented at higher temperatures (35°C or higher). Furthermore, yeast have relatively low counting at high temperatures, probably as a consequence of growth limitation, which may increase LAB competitiveness.[59] Therefore, seasonal temperature fluctuations can affect the microbial community dynamics of the sourdough, being responsible for microorganism selection.[3]

As a result, different fermentation temperatures might influence the secondary metabolites, organic acids and aromatic compounds formation by microorganisms, as well as other enzymatic reactions.[5,24,60] For instance, lower temperatures (25–28°C) have a positive effect on yeast growth, which results in the increased production of ethanol, CO_2 and flavour components[24,61]; while high fermentation temperatures (≥ 30°C) enhance LAB metabolism, influencing the fermentation quotient (i.e. the ratio between lactic and acetic acid) and increasing the acidification of the sourdough,[3] consequently increasing the organic acids biosynthesis due to a better usage of carbohydrates.[5] Additionally, enzymatic reactions, such as lipid oxidation, and polysaccharide breakdown into maltose or glucose, are also promoted at higher temperatures.[60]

Fermentation time and backslopping

Another exogenous parameter influencing microbial communities in sourdough is fermentation time. It can modify the balance between different stress-resistant LAB, and it is inversely related to fermentation temperature.[62]

Italian, Belgian and San Francisco sourdoughs are characterized by long fermentation times, at low temperatures.[59] In that case, *Lb. sanfranciscensis* is the predominant microorganism performing sourdough fermentation, due to its preferred long fermentation times at relatively low temperature, characteristics that prevail during type I sourdough preparation.[25] On the other hand, *S. cerevisiae* is predominant in short fermentation sourdoughs, because of its metabolism and growth rate, being more rapid than those of LAB.[24] Moreover, longer fermentation times result in higher values of lactic and acetic acids, lowering starch digestion and obtaining breads with lower glycemic index (GI).[63]

Besides fermentation time, it must also be considered the backslopping steps performed during sourdough preparation. When the number of backsloppings increases, the environment conditions become more selective, resulting in the dominance of certain species, generally selecting for hetero-fermentative LAB, such as *Lb. sanfranciscensis*.[12] Moreover, the frequency and duration of the backslopping steps modify the microbial community dynamics and stability, influencing the growth and acidification rates.[3] Short refreshment times select for rapidly growing and fast-acidify LAB species; while long backslopping times or fermentations without backslopping appear to benefit acid-tolerant LAB species that are highly adapted to a nutrient-poor and hostile environment. Additionally, resting time between refreshments/backsloppings can also influence the microbiota and its metabolite kinetics, by selecting strains that are able to survive periods of stress and starvation.[3]

Dough yield and pH

The Dough yield (DY) is the ratio between dough (flour, water and other ingredients) and flour weight, referencing the sourdough consistency. Usually, flour and water are the main ingredients of

the dough, thus, DY is mainly related to water content. Therefore, higher DY values are a consequence of the amount of water.[3]

DY, alone, affects directly the a_w and the acidity of sourdoughs.[5] Lower values of DY (firm sourdoughs), with a fixed temperature and fermentation time, result in more fermentable carbohydrates for the sourdough microbiota to metabolise, and also amplify the flour buffering capacity, which lowers the acidification rate despite higher levels of organic acids (lactic and, especially, acetic acids), resulting in higher TTA values.[2]

On the other hand, high DY values produce liquid sourdoughs with lower TTA values, and lower concentrations of organic acids such as lactic and acetic acid. Thereby, the higher the DY, the relative acidification is faster and stronger, even though the acetic acid production is negatively affected.[2] This may happen due to a higher population of obligately heterofermentative LAB and yeasts found in liquid sourdough, that resulted in a greater production of ethanol than acetic acid, therefore causing lower TTA values.[2] Moreover, high water content increases shelf-life and delays starch retrogradation.[64]

Hence, the DY influences the LAB-yeast ratio, as well as the ratio between homo- and heterofermentative LAB.[62] For example, liquid sourdoughs (with higher DY values) favour LAB growth over yeasts, [3,62] although yeast growth should be greater and more stable, due to higher concentrations of free amino acids, in comparison with firm sourdoughs.[2] But, because LAB lower the pH and increase the TTA, yeasts stop growing and only certain strains adapted to higher concentration of organic acids, such as *C. humilis* or *K. exigua* prevail.[4] On the other hand, lower DY (< 160) sourdoughs have a higher population of yeasts (demand a lower a_w than bacteria) but present a more selective environment for LAB.[62]

In addition, DY combined with other controllable technological parameters of the sourdough production, have a greater impact on the microbiota. Sourdoughs with long fermentation time and high DY value promote LAB growth over yeasts, while high DY and temperature select for heterofermentative LAB.[62]

Traditional sourdoughs have a pH range of 3,5–4,5. Among LAB, lactobacilli dominate the sourdough fermentation due to their adaptation to low pH, although higher pH values allow the prevalence of other bacterial species, such as enterococci, lactococci, leuconostocs, pediococci and weissellas.[59] For example, *Lb. sanfranciscensis* has an optimal pH for growth of 5,0 and it is mainly associated with low-DY sourdoughs; yet it shows adaptation to acidic conditions, even though it is outcompeted in low-pH sourdoughs (values below 3,8)[3] since it is unable to grow under these conditions. In contrast, *Lb. fermentum* is often found in liquid, high-DY, acidic sourdoughs because of its acidic stress tolerance.[59]

Physical and organoleptic properties of sourdough bread

In recent years, the use of sourdough has gained popularity due to the reported improvement of quality and flavour of bread. The addition of sourdough to the dough fermentation influences final bread quality and many of its inherent properties depend on sourdough resident microbiota and its metabolic activity (e.g. lactic fermentation, proteolysis, synthesis of volatile compounds and its precursors, and other antimicrobial compounds), producing non-volatile and volatile compounds that affect bread flavour.[20,24,25,34,65–68]

One of the main differences between regular bread and sourdough bread is the drop in pH and, consequently, the acidification or souring, resulting in lower pH values when sourdough is used. This pH decrease due to LAB metabolic activity, producing organic acids, cause many changes in the dough, influencing the final characteristics of bread, mainly texture and sensory attributes.[69] Regarding organic acids production, the fermentation quotient (FQ) is an important factor that affects the aroma of the final product, and, as previously stated, it depends on the microorganisms fermenting the sourdough and the fermentation temperature.[3] For example, heterofermentative LAB are responsible for accumulation of acetic acid, although yeasts can also contribute to its

production.[34] Moreover, higher concentrations of organic acids may be responsible for extending shelf-life of sourdough bread, since they exhibit antimicrobial activity, especially lactic and acetic acids, being effective against rope spoilage. Furthermore, other organic acids, such as isovaleric, pentanoic and hexanoic acids, may also present an antimicrobial effect in sourdough bread, acting in a synergistic way.[57] Also, it has been observed that certain LAB strains can produce phenyllactic acid (PLA) that also contribute to the antifungal activity in sourdough bread.[58] Therefore, the use of sourdough in bread-making can promote a clean-label by avoiding the use of chemical preservative additives.

Additionally, some studies reported that certain LAB strains are able to produce exopolysaccharides (EPS). The presence of these EPS can contribute improving flavour, texture and shelf-life of sourdough bread by promoting additional metabolic activity, enhancing the production of lactate, acetate and ethanol.[64] Torrieri et al.[64] observed that using EPS producing strains (EPS+) to ferment, a higher amount of sourdough can be added to bread without having a negative effect on its sensory characteristics. They concluded that a 30% of sourdough obtained using EPS+ strains of *L. lactis* and *Lb. curvatus* had a positive effect on bread volume and crumb texture. Besides, the synthesis of EPS by LAB during sourdough fermentation also contributes to consumers demands for a clean label, since it does not require labelling and can substitute additives (e.g. xanthan), although it is suggested that EPS synthesis depends on environmental conditions.[70]

Although both homo- and heterofermentative LAB are responsible for lowering the pH, while the former exclusively promote the pH decrease and the TTA increase[6]; the latter also produce acetic acid, CO_2 and ethanol, generating more flavour compounds.[21] Actually, microbiological fermentation is the most relevant path for the production of volatile compounds in sourdough.[25] For instance, heterofermentative LAB generate more esters like ethyl acetate, hexyl acetate, ethyl hexanoate and isopentyl acetate, whereas homofermentative fermentations produce 2,3-butanedione (ketone), acetaldehyde (aldehyde) and 2-methylbutanol (alcohol).[66]

As mentioned above, heterofermentative LAB contribute to the leavening of the dough.[16] In fact, dough leavening is, mainly, due to the alcoholic fermentation performed by yeasts, that, as a result, produce CO_2 and ethanol.[71] But, even if the primary function of yeasts is to act as a leavening agent,[20] they play a role in the structure formation of the gluten network[67] and produce other by-products resulting from the alcoholic fermentation, such as glycerol, aldehydes, ketones and esters among other substances, may increase the flavour of bread.[48] All that improves and changes the characteristics of the final bread product.[52] In fact, it has been observed that the addition of bakers' yeast increases acetaldehyde and decanal (aldehydes), ketones like acetoin and alcohols (especially ethanol and phenethyl alcohol).[34] Also, there are some volatile compounds that have only been reported in yeast fermentations, such as 3-methyl-1-butanol,[20] even though it has been found that some LAB may also produce it depending on species and strain.[66,68] Furthermore, sourdoughs that start with a microbial association produce a larger range of volatiles.[20]

However, the age of the sourdough must be considered since it has been reported that traditional sourdoughs have a less complex volatile profile despite their rich microbiota, compared to *ex-novo* sourdoughs, that may have a more active microbiota regarding the synthesis of volatiles.[20] In fact, Ripari et al.[20] propose tridecane and ethyl nonanoate to discriminate between *ex-novo* and traditional sourdoughs, since the first one is always present in traditional samples and absent in *ex-novo* samples whilst the second is the opposite.

The flour used must be taken into account, because it may determine the volatile profile according to their role as a substrate for the microorganisms fermenting the sourdough.[65] But other parameters, such as fermentation time and temperature also affect the volatile fraction. For instance, the longer the fermentation, more volatile compounds are formed as a result of higher amino acid degradation by Ehrlich pathway, leading to aldehydes and their corresponding alcohols odour-active compounds.[20] Moreover, peptides and free amino acids take part in Maillard reactions, which generate compounds that influence the aroma of bread.[24]

Finally, the type of sourdough (firm, liquid or dried) has an impact on the volatile profile too, particularly in dried sourdoughs. For instance, during drying (type III sourdoughs), water and volatile compounds evaporate, and Maillard reaction takes place, besides other flavour reactions, and produce roasty and malty flavours.[14] But firm and liquid sourdoughs also influence flavour. It has been reported that liquid sourdoughs have higher levels of alcohols (derived from free amino acid metabolization), while firm sourdoughs mainly contain ethyl-acetate, acetic acid and terpenes, that markedly affect the flavour of baked goods.[2]

Future trends

In recent years, consumers have become more aware of their health and its relationship to food consumption, demanding more nutritious and safe food. As a result, industry and scientist have developed new products that can provide extra health benefits to its consumer, known as functional foods.[63] Under these characteristics fall prebiotics and probiotics, providing technological enhancements (e.g. volume, flavour and aroma) to bakery products, besides their inherent health properties.[63]

During the design of functional bakery products including probiotics, the temperatures reached during baking must be considered, since most of these microorganisms would be inactivated or eliminated throughout the baking process. However, these inactivated (non-viable) probiotics, also known as paraprobiotics, might confer benefits to the consumer when administrated in sufficient amounts.[72] In addition, the probiotics benefits might be able to reach consumers by supplying bioactive compounds during sourdough fermentation, there being postbiotics.[63,72] In fact, both paraprobiotics and postbiotics can imitate the health benefits conferred to probiotics while avoiding the administration of live microorganisms, that can cause an immune reaction.[72]

Nevertheless, it has been reported that prolonged fermentation processes in breadmaking, such as sourdough, can reduce the Fermentable Oligo-, Di-, Mono-saccharides and Polyols (FODMAPs) content by up to a 74%, since sourdough fermentation activates proteolytic and fructosidase enzymes decreasing the amount of proteins and fructans.[73] Moreover, sourdough fermentation lowers the glycemic index (GI) of the resulting bread, due to a higher amount of acetic and lactic acids, therefore reducing starch digestibility. Besides, it also reduces to 50% or less the phytate content in whole wheat bread, due to the lower pH favours the phytase activity, decreasing the quantity of antinutritional components and potentially improving mineral absorption.[63] Additionally, Laatikainen et al.[73] reported a lower content in α-amylase/trypsin inhibitors (ATIs), gluten and additives in sourdough breads.

Finally, it has been observed that the use of dietary fibers and ingredients rich in fiber, such as a mixture of wheat and legume (chickpea, lentil and bean) flours, in sourdough fermentation increased the concentration of functional compounds.[44] In fact, legume proteins are rich in lysine but lack of sulfur amino acids; whereas cereal proteins are rich in sulfur amino acids and lack lysine. Thus, the combination of wheat (cereal) with legume flours result in an optimal balance of essential amino acids. Furthermore, Rizzello et al.[44] observed a higher antioxidant and acidification activity in legume sourdough, related to a high content of phenolic compounds found in legume flours. The study concluded that wheat-legume sourdough maximized the nutritional, sensory and functional properties of bread.

Conclusions

Sourdough is the result of a fermentation process, that can be spontaneous or not, with or without backslopping, involving mainly LAB and, also, yeasts, following a 100:1 ratio. The present microbiota increases acidification, leavening and flavour. In fact, the use of sourdough results in bread with an improved shelf life, nutritional value and sensory attributes. Process parameters, such as fermentation

time or temperature, may influence sourdough microbiota and its secondary metabolites production, which can induce changes in both sourdough and the resulting bread, especially the volatile profile of the product. While LAB generate more esters, yeasts increase the aldehydes, ketones and alcohols content. Moreover, consumers are demanding for more nutritious and healthy food, like functional foods containing probiotics and/or prebiotics. In that regard, sourdough might be elaborated with probiotic microorganisms, which can release bioactive compounds. Although when bread is baked microorganisms are inactivated, those bioactive compounds remain in the product as postbiotics and can reach consumers, providing health properties. Also, sourdough fermentation can lower the phytate content, FODMAPs and GI of bread, reducing antinutritional components and improving mineral absorption. Finally, the use of non-conventional flours, such as legume flours, in sourdough fermentation increase the concentration of functional compounds, maximizing the nutritional, sensory and functional properties of bread.

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CAPÍTOL 2. OBJECTIUS

En els darrers anys, donada la crisi climàtica actual, ha augmentat l'interès de la societat per canviar d'un model d'economia lineal cap a un model circular. D'aquesta manera, i en línia amb els ODS els residus es re-introdueixen a la indústria de manera que se'ls hi confereix un valor afegit i es re-aprofiten. La indústria del Cava produeix una gran quantitat de residus que per la seva composició podrien ser objecte d'aquest tipus de valorització, entre ells, les lies del Cava.

A més, els hàbits alimentaris de la població tendeixen cap al consum d'aliments més saludables, entre els que s'hi inclouen els fermentats tradicionals (quefir, kombutxa, kimchi, etc.) i els fortificats amb fibra. Per aquest motiu, la indústria alimentària i la comunitat científica busquen la recuperació d'aliments tradicionals reforçant-ne l'aspecte més nutritiu. En aquest sentit, els probiòtics i prebiòtics aportarien millores tecnològiques als aliments, tenint també un component de salut i nutrició. De fet, els productes de panificació entrarien dins aquesta categoria, essent aliments que tornen a prendre protagonisme en la dieta tant per la vessant tradicional com nutritiva, sobretot en elaborar-los amb massa mare. A més a més, introduint les lies en la seva formulació també seria una estratègia per augmentar el consum de fibra alimentària en la dieta habitual de la població actual.

En aquest context, aquesta tesi doctoral planteja com a objectiu general la **valorització de les lies del Cava com a ingredient per afavorir el procés fermentatiu de la massa mare del pa, així com avaluar-ne la capacitat prebiòtica**.

Per tal d'assolir aquest objectiu general es plantegen tres àmbits d'estudi amb els següents objectius específics:

ÀMBIT 1: Potencial prebiòtic de les lies del Cava.

Objectiu 1. Valorar *in vivo* les característiques prebiòtiques de les lies del Cava per promoure el creixement de probiòtics a la microbiota intestinal (efecte simbiòtic)

ÀMBIT 2: Caracterització de les lies del Cava.

Objectiu 2. Caracterització de la fracció volàtil de les lies del Cava i avaluació de la seva capacitat de retenció d'aromes

ÀMBIT 3: Les lies del Cava com a ingredient.

Objectiu 3. Estimar l'efecte de les lies del Cava com a promotores de la fermentació de la massa mare del pa

Objectiu 4. Avaluar les característiques volàtils i sensorials de la massa mare i del pa amb l'addició de mares del Cava

CAPÍTOL 3. DISSENY EXPERIMENTAL

Els diferents àmbits relacionats amb els objectius específics de la tesi van requerir el desenvolupament de diferents mètodes i tècniques analítiques. En aquest capítol es presenta esquemàticament el disseny experimental de la tesi doctoral (Figura 9). Les metodologies específiques es troben recollides a les Publicacions 2, 3, 4, 5 i 6.

Primerament, el **Capítol 4** engloba l'àmbit relacionat amb la seguretat alimentària i el potencial prebiòtic de les lies del Cava. Per aquest objectiu, es va administrar una dosi diària de lies a rates Wistar Han. A partir de l'ADN microbià extret de les femtes dels animals, es va determinar mitjançant *shot-gun* sequencing la composició de la microbiota intestinal a l'inici i final del període d'estudi (Publicació 2).

El **Capítol 5** se centra en la caracterització de les lies del Cava des d'un punt de vista organolèptic, englobant el segon àmbit d'estudi de la tesi. Per aquest objectiu, es van desgorjar ampolles de Cava amb diferents característiques (temps d'envelliment o tipus de raïm vinificat) per avaluar la capacitat de retenció d'aromes i compostos fenòlics de les lies. Per això, es van analitzar els mateixos paràmetres tant en el Cava com en les lies i es van comparar els perfils resultats (Publicació 3). Una vegada recuperades les lies, aquestes es van liofilitzar, obtenint un percentatge de recuperació del 60%, i es van emmagatzemar en tubs hermètics i protegits de la llum i la humitat. Els paràmetres analitzats van ser els següents:

- Número de cèl·lules de llevat per gram de lies
- pH
- Índex fenòlic total (IFT)
- Intensitat de color (IC)
- Tonalitat de color (TC)
- Compostos volàtils

Finalment, el tercer àmbit (**Capítol 6**) se centra en l'efecte de les lies del Cava sobre la massa mare i el pa. Per adreçar els objectius específics 2 i 3, primer es van formular diferents masses mare amb lies del Cava i se'n va monitoritzar la fermentació i la producció de compostos volàtils (Publicacions 4 i 5). Una vegada determinat el percentatge de lies més adient, es va aplicar a la massa mare (que

posteriorment es va utilitzar per a fer pa) així com a la formulació del pa directament (Publicació 6).

Addicionalment, els pans formulats amb i sense massa mare i amb i sense lies (Publicació 5) es van sotmetre a un anàlisi de textura (TPA, *Texture Profile Analysis*) per determinar possibles diferències dels diferents paràmetres que engloben la textura d'un aliment (duresa, elasticitat, cohesió, masticació i resistència). Les mostres es van tallar en llesques de 25 mm de gruix. Els tests de compressió es van realitzar mitjançant un texturòmetre TA-XT2 (Stable Micro Systems Ltd., Surrey, Regne Unit) utilitzant un pistó pla d'alumini de 75 mm de diàmtre. El procediment de mesura va consistir en un pre-assaig de 5 mm/s, un assaig a velocitat constant de 1 mm/s, i un post-assaig de 5 mm/s, amb un temps d'espera entre compressions de 5 s i una deformació del 90%. Cada mostra es va col·locar a la base del texturòmetre i es va esprémer dues vegades amb la sonda. El TPA de les mostres es va dur a terme mitjançant el software proporcionat amb l'equip (Exponent v.6.1).

A més a més, es va realitzar un anàlisi sensorial dels pans elaborats amb les diferents concentracions de lies. Per això, el panel estava format per 30 voluntaris semi-entrenats (homes i dones, d'entre 18 i 45 anys) del personal de laboratori i estudiants universitaris. Abans del test sensorial, els pans es van tallar a rodanxes i es van identificar mitjançant codis numèrics únics. Cada panelista va avaluar 5 llesques de pa corresponents a les concentracions de lies (control, 0,5%, 1%, 2% i 5%). Es va utilitzar un anàlisi hedònic per avaluar el color, la molla, la crosta, l'olor i el gust, així com l'acceptació general del pa. La puntuació de cada atribut anava d'1 (més baix) a 10 (més alt).

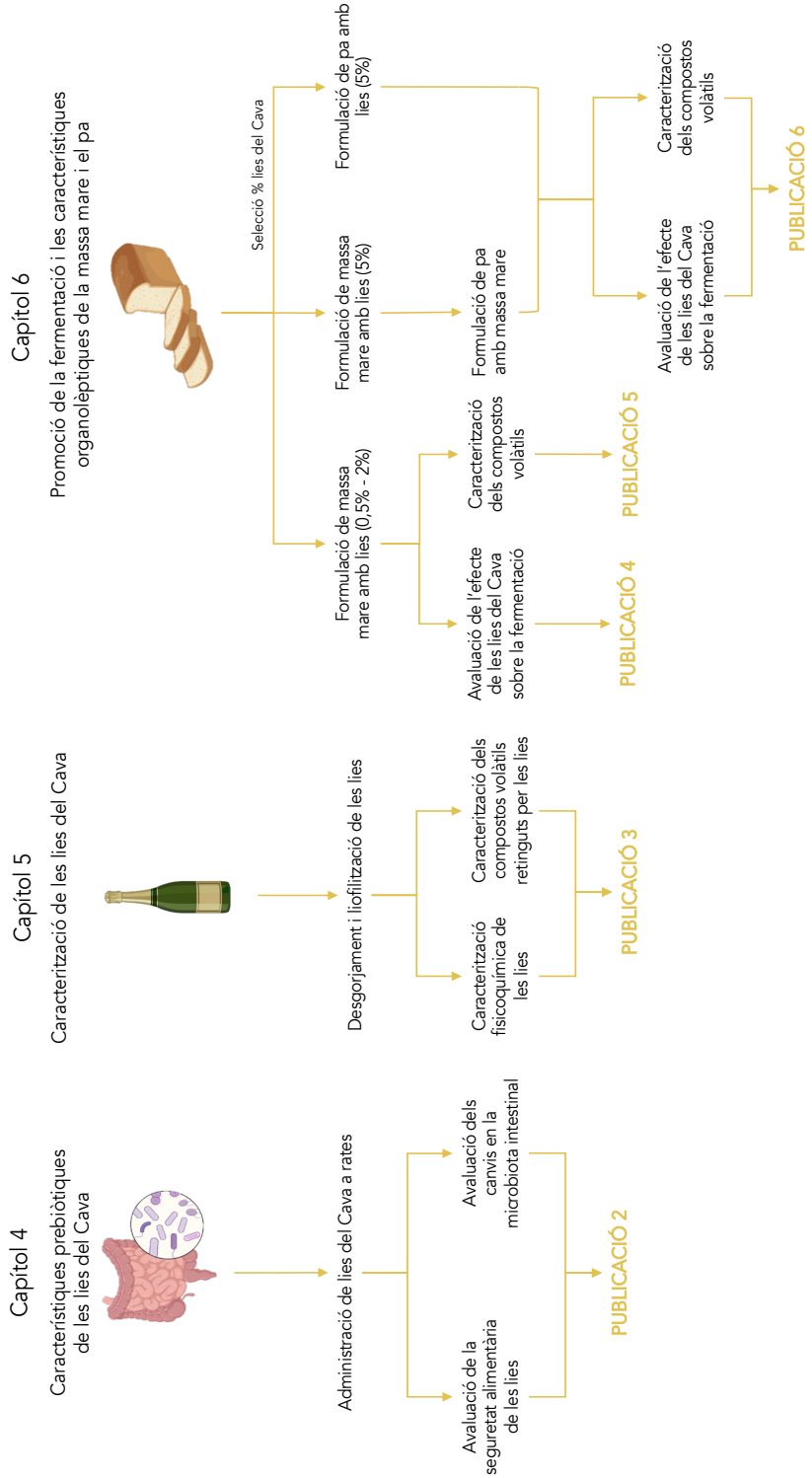

Figura 9. Disseny experimental de la tesi doctoral englobant els tres àmbits estudiats.

CAPÍTOL 4. POTENCIAL PREBIÒTIC DE LES LIES DEL CAVA

4.1. Avaluació *in vivo* de la seguretat alimentària i les característiques prebiòtiques de les lies del Cava sobre la microbiota intestinal

Les lies del Cava tenen un alt contingut de fibra i altres polisacàrids complexes (ß-glucans i manoproteïnes). Aquests poden tenir un efecte promotor sobre el creixement dels microorganismes presents a l'intestí [96,97]. Degut a aquesta composició, es podria atorgar característiques prebiòtiques a les lies del Cava.

Per això, el tercer àmbit de la tesi es va centrar en l'avaluació del potencial prebiòtic de les lies. En aquest cas, es va utilitzar un model animal (rates Wistar Han) a les que es va dividir en dos grups: un grup control i un grup amb l'administració de 3 x 10^6 cèl·lules de lies/Kg pes corporal/dia. Diàriament, durant 14 dies, se'n van recollir les femtes per poder fer un estudi de la composició de la microbiota intestinal. Se'n va extreure DNA i es va fer una seqüenciació metagenòmica de *shotgun*. Una vegada es van tenir les dades, aquestes es van processar mitjançant eines de bioinformàtica (softwares QIIME 2 v. 2022.2 i MG-RAST v. 4.04).

La suplementació amb lies del Cava no va suposar cap diferència respecte el control en quant a pes dels animals, ingesta d'aliments i aigua. Es va observar un augment en l'abundància relativa de bacteris de la família *Lactobacillaceae* així com de *Blautia hansenii*, *Roseburia intestinallis* i *Ruminococcus obeum*, tots ells amb caràcter probiòtic. Per tant, les lies del Cava van mostrar un potencial prebiòtic.

4.2. Publicacions científiques

PUBLICACIÓ 2

Potential Prebiotic Effect of Cava Lees: Changes in Gut Microbiota

Alba Martín-Garcia, Montserrat Riu-Aumatell, Elvira López-Tamames.
Resultats enviats per la seva publicació a *Fermentation*, Agost **2022**.

LES IDEES CLAU

La ingesta de lies del Cava va augmentar l'abundància de bacteris amb potencial probiòtic pertanyents a la família *Lactobacillaceae* i als gèneres *Blautia*, *Roseburia* i *Ruminococcus*. Per tant, les lies del Cava mostren un potencial caràcter prebiòtic.

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Article

Potential Prebiotic Effect of Cava Lees: Changes in Gut Microbiota

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Abstract: Lees are a winery by-product with a fiber-rich composition that could have a potential prebiotic effect on gut microbiota. Prebiotics cannot be digested by humans but can be used by bacteria found in the large intestine. To evaluate the potential prebiotic effect of lees, those were administered to Wistar Rats for 14 days. Feces were collected daily, and DNA was extracted and analyzed by shot gun sequencing. The supplementation with lees did not affect weight, food intake or water consumption of the studied rats. It was found that lees promoted the increase of relative abundance of probiotic bacteria belonging to *Lactobacillaceae* family, as well as other potentially probiotic species such as *Blautia hansenii*, *Roseburia intestinalis* and *Ruminococcus obeum*. In conclusion, lees can improve the presence of beneficial bacteria in the gastrointestinal tract and can be re-valorized as a new ingredient in food formulation.

Keywords: gut microbiota; prebiotic; cava lees; dietary fiber; by-product

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1. Introduction

Prebiotics are non-digestible ingredients for humans, such as dietary fiber, that can stimulate the growth and/or metabolic activity of a healthy gut microbiota [1–3]. In fact, the large intestine is one of the human body organs with greater microbial diversity, with over a 1,000 bacterial species with a concentration of $10^{11} – 10^{12}$ UFC/g [4]. In that regard, when dietary soluble fiber arrives to the colon, it is fermented by the gut microbiota, producing short chain fatty acids (SCFA), such as acetic, butyric and propionic acids [5]. The SCFA are absorbed to the blood stream, getting to different organs and tissues like the brain, muscles, or liver, where they can induce several positive effects. For instance, butyrate is the preferred energy source for the intestinal mucosa, while propionate contributes to gluconeogenesis in the liver [4,6]. Moreover, dietary fiber also contains bioactive compounds that can increase its antioxidant activity [7].

Since dietary fiber has a positive effect on human health, the European Food Safety Authority (EFSA) recommends an intake of 25 g of fiber per day [8]. Among the health benefits of fiber are the prevention of cardiovascular diseases, hypertension, diabetes and obesity [9,10]. Furthermore, it contributes to improve the activity of the gastrointestinal tract, satiety and the modulation of the immune response of the intestinal mucosa [10,11]. Finally, dietary fiber can reduce the glycemic response and the blood cholesterol levels, that being a risk factor in cardiovascular diseases [9,12].

Dietary fiber comes from numerous sources (cereals, fruits and vegetables), but due to the eating habits of the population it is hard to achieve EFSA's goal [8]. In fact, most European countries do not reach the minimum of 25 g/day of dietary fiber intake, the mean being of 19.6 g/day [13]. This results in the need to find alternative sources of fiber [9]. Recently, there has been a growing interest in the composition of several by-products and their possible revalorization [5,14–17]. In that regard, wine lees have been described as a source of fiber and antioxidant compounds [5].

Cava lees are a sparkling wine by-product obtained after the second fermentation of wine. Lees are rich in fiber, as well as proteins and polyphenols [5,9], but are considered a waste with no added value. In fact, the consumption of sparkling wine in 2021 was around 11.1 mhL worldwide [18], where each bottle contains approximately 1g of lees, being a total of 300 tons per year of such by-product (25 % of the total waste generated by the wine industry) [19]. Such volume of waste must be managed, which not only represents and economic impact for the industry but also an important effect on the environment. Therefore, there is an increasing tendency to reduce waste production by valorizing by-products and re-introducing them into the production cycle.

In that regard, the valorization of lees has been studied by different research groups. They have been tested *in vitro* to improve the viability of certain lactic acid bacteria (LAB) with probiotic characteristics [20] and have been described as being active against food pathogens (*Listeria monocytogenes* and *Salmonella* spp.) [21]. Also, wine lees have been proposed as an alternative source of antioxidants for meat [22] and as an emulsion stabilizer [23]. Moreover, our research group has studied a potential revalorization of Cava lees as a new ingredient in sourdough and bread, improving the growth and survival of the fermenting microbiota [24,25].

Therefore, if Cava lees are included in food formulation, given their composition they can contribute to the fiber intake, reaching the daily intake values recommended. To that end, the aim of this study was to evaluate the food safety and the potential prebiotic effect of Cava lees on the intestinal microbiota in an animal model.

2. Materials and Methods

2.1. Ethical Approval

Ethical approval for this study was provided by the Bioethics Committee of the University of Barcelona (IRB00003099).

2.2. Study Design

The study was carried out with 24 Wistar Han rats (Table 1). A daily dose of 3×10^6 lees cells/kg body weight was administered by gavage (Lees diet) for 14 days. The test item (Cava lees) was weighed and prepared daily, in 0.5% aqueous solution of carboxymethyl cellulose (CMC) (w/v) (CAS Num.: 9004-32-4; Ref.: 144441.1209, Panreac), before administration. Animals with a Control diet were administered only the aqueous solution of CMC.

Throughout the entire study, animal weight was controlled, as well as food and water intake and any clinic symptoms. During the dietary intervention, feces samples were collected daily to evaluate the prebiotic effect of Cava lees. Animals were subjected to hematological, chemical, and immunological blood analyzes at the beginning and end of the treatment (14 days). At the end of the study, the animals were sacrificed to perform a necropsy, a macroscopic examination and the collection of organs and tissues.

Table 1. Experimental design describing the two tested diets (control and lees diet).

Group	Label	Lees Dose (mg/kg/day)	Administered volume (mL/kg/day)	Animal number	
				Male	Female
1	Control	0	10	1 – 6	7 – 12
2	Lees	2,000 [1]	10	13 – 18	19 – 24

[1] Safety factor equivalent to 15 times more the recommended dose.

2.3. Intestinal Microbiota Extraction and Analysis

Bacterial DNA was isolated from feces samples using a kit QIAamp PowerFecal DNA Kit (Ref.: 12830-50, QIAGEN, Germantown, MD, EEUU), following the manufacturer's instructions. DNA concentration was measured by BioDrop µLite (Biotech, Madrid, Spain). To analyze the microbial composition, shot gun sequencing was performed on the Illumina MiSeq platform by the Genomic and Bioinformatic Service of the Universitat Autònoma de Barcelona. Then, bioinformatics analysis of the microbial composition was performed using the software MG-RAST version 4.04 [26] and QIIME 2 version 2022.2 [27].

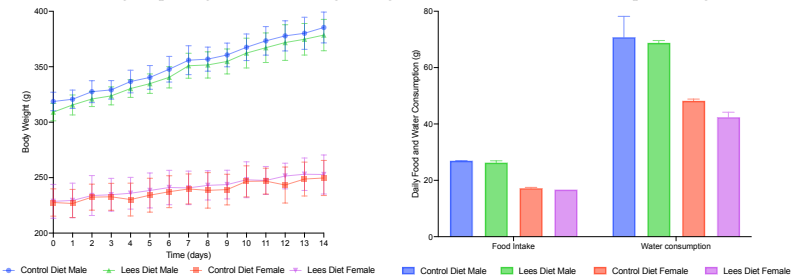
2.4. Statistic Analysis

Statistical analysis was performed using the Prism 9 v.9.1.2 (225) (GraphPad Software, LLC., San Diego, CA, EEUU). Differences in the microbiota composition between groups were analyzed by the Kruskal-Wallis test for non-parametric data. Alpha diversity was measured by the Shannon index and evenness, and, for beta diversity, a Bray-Curtis dissimilarity analysis was performed and visualized using a principal coordinates analysis (PCoA). Differences were considered significant with a p-value < 0.05.

3. Results and Discussion

3.1. Effect of Cava Lees on Body Weght, Food Intake and Organs of Rats

Throughout the study (14 days), weight and food and water intake data were registered daily (Figure 1). There were no significant differences of weight gain between the two groups (Figure 1A), nor regarding food intake or water consumption (Figure 1B).



(**a**) (**b**)

Figure 1. Evolution of body weight and food and water intake during the 14-day dietary intervention: (**a**) Body weight of rats according to sex (male and female) and the dietary intervention (control or lees diet); (**b**) Food and water consumption by rats according to sex (male and female) and the dietary intervention (control or lees diet).

Once the study was finished, animals were sacrificed, and their organs were removed. In general, there were no statistically significant differences regarding the organ (thymus, liver, spleen, and kidneys) weight of the two animal groups (Table 2). Nevertheless, male rats with a lees diet presented a significant increase of spleen weight. The spleen is a peripheric lymphoid organ that plays a key role in the immune response of a healthy

body [28,29]. Therefore, changes in its volume and structure could have a direct impact on the immunity and resistance of the rat [28]. Even though an increase in spleen weight has been related to obesity [29], data obtained in this study regarding spleen values of both control and lees diet rats match those of healthy rats [28,29].

Table 2. Weight (g) of different organs (thymus, liver, spleen, and kidneys) of rats according to sex (male and female) and diet (control or lees). Values are mean ± standard deviation.

Organ	Males			Females		
	Control Diet	**Lees Diet**	**p-value**	**Control Diet**	**Lees Diet**	**p-value**
Thymus	0.80 ± 0.06	0.72 ± 0.12	0.500	0.66 ± 0.18	0.64 ± 0.07	0.970
Liver	15.40 ± 0.74	14.46 ± 2.07	0.333	8.42 ± 0.71	7.96 ± 0.48	0.762
Spleen	0.97 ± 0.07	1.15 ± 0.10	0.004	0.66 ± 0.05	0.73 ± 0.12	0.291
Kidneys	2.61 ± 0.21	2.47 ± 0.16	0.489	1.61 ± 0.35	1.65 ± 0.08	0.960

Finally, no signs of necrosis nor other adverse effects were seen in the study of acute toxicity between the rats fed with lees and the controls. The observational parameters/general measurements (weight, food, and water intake) hematology, biochemistry, histopathology, necropsy and immunogenicity did not reflect significant differences between the control groups and the mixed trials.

3.2. Effect of Cava Lees on Gut Microbiota Composition

Gut microbial composition of animals with both control and lees diet was analyzed and compared at phylum, family, genus and species levels. At the beginning of the study the gut microbiota of both groups had a similar profile ($p > 0.05$). Overall, 5 phylum, 20 families, 88 genus and 204 species were found with a relative abundance greater than 1%.

At a phylum level, Bacteroidetes (26 %) and Firmicutes (68 %) were the dominant bacteria in both groups. After the 14-day diet intervention, those were still the dominant phylums, although there was a shift in rats with a lees diet, where Bacteroidetes represented a 17 % and Firmicutes a 75 % (23 % and 66 % respectively in control rats), being significant changes respect the control group ($p < 0.05$). In general, bacteria belonging the phylum Firmicutes produce more butyrate, while the ones corresponding to Bacteroidetes phylum mainly produce acetate and propionate [30–32]. In that regard, butyrate is considered a health promoter since it can increase insulin sensitivity, has anti-inflammatory activity and regulates the energetic metabolism [30].

On the other hand, propionate and acetate act in different organs and tissues. For instance, the former is stimulates GLP-1 and PYY release by L-entero-endocrine cells, which results in the inhibition of appetite (colon) and participates in hepatic gluconeogenesis lowering the expression of enzymes related to the synthesis of fatty acids and cholesterol (liver) [30,31]. Whereas the latter, acetate, stimulates the synthesis of lipids contributing to dyslipidemia (liver), activates the parasympathetic nervous system (brain) by promoting insulin and gastric mucosa secretions (pancreas) [30,32].

Firmicutes includes the families *Lachnospiraceae, Lactobacillaceae* and *Ruminococcaceae*, among others. Table 3 shows the differences in the relative abundance of these families found between control and lees group.

Table 3. Differences regarding relative abundance (%) of bacteria from the phylum Firmicutes between rats with control and lees diet. Values are mean ± standard deviation.

Family	Control Diet	Lees Diet	p-value
Lachnospiraceae	10,87 ± 1,91	15,22 ± 1,87	0,02
Lactobacillaceae	9,71 ± 0,83	11,71 ± 1,09	0,03
Ruminococcaceae	8,55 ± 1,53	11,00 ± 1,02	0,02

Bacteria classified as *Lachnospiraceae*, *Lactobacillaceae* and *Ruminococcaceae* are able to hydrolyze starch and other polysaccharides (e.g. inulin), and produce SCFA such as butyrate [33,34]. In the present study, the relative abundance of such bacteria increased significantly in the animals with a lees diet. In fact, Zhang et al. (2017) [35] reported that mice fed with a pomegranate extract (rich in polyphenols) the abundance of *Ruminococcaceae* increased and *Clostridiaceae* decreased. It has been reported that wine lees are rich in bioactive compounds such as polyphenols [9,23]. Moreover, *Ruminococcaceae* bacteria are responsible for the degradation of several polysaccharides and fibers, and are related to the prevention of hepatitis (alcoholic and non-alcoholic) and hepatic encephalopathy, and to an increase in intestinal permeability [36].

Lachnospiraceae bacteria found in the human intestine mainly belong to the genus *Blautia*, *Coprococcus*, *Dorea*, *Lachnospira*, *Oribacterium* and *Roseburia* [33]. It was found that *Blautia hansenii*, *Ruminococcus obeum* and *Roseburia intestinalis* increased significantly with the intake of Cava lees (Figure 2). In this sense, Guo et al. (2017) [37] studied the possible prebiotic effect of polyphenols from green tea to reduce induced obesity in mice with a high-fat diet. They focused on the gut microbiota composition as well as the bacterial metabolic products, finding a positive correlation between an increase of *Roseburia* and the production of butyrate in mice fed with polyphenols from green tea. That increase in SCFA production could be related to the prevention of opportunistic pathogens and colon diseases by favoring the growth of commensal bacteria [37,38]. Furthermore, it has been observed that certain species of *Blautia* present antimicrobial activity against pathogens such as *Clostridium perfringens*, which makes them potential probiotics that benefit the host [39].

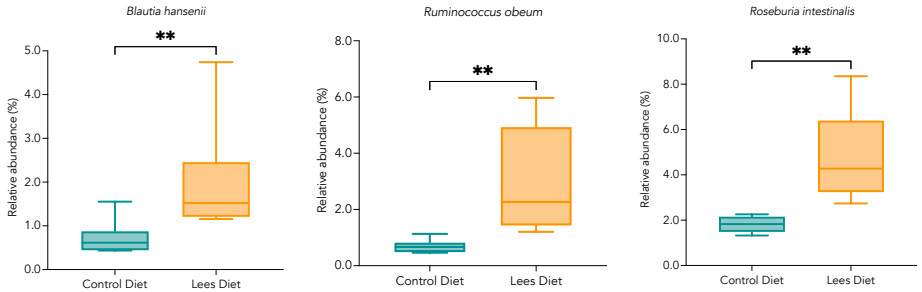


Figure 2. Differences of relative abundance (%) of potentially probiotic bacteria between animals with control and lees diet. Significant differences between groups are indicated with an asterisk (*): (**a**) *Blautia hansenii*; (**b**) *Ruminococcus obeum*; (**c**) *Roseburia intestinalis*.

Nonetheless, Oliver et al. (2021) [40] reported a negative correlation between the relative abundance of *Roseburia* and *Ruminococcus*, and *Bifidobacterium*, suggesting a negative interaction between species from these taxa. Indeed, they observed a decrease of *Bifidobacterium* in animals with lees intake (2.7 %) versus control (4.7 %), while there was an increase of *Roseburia* (8.3 % - lees; 3.9 % control) and *Ruminoccocus* (8.9 % - lees; 5.8 % - control). As a matter of fact, *Bifidobacterium* participate of cross-feeding with other bacteria that produce butyrate and release oligo- and mono-saccharides from more complex substrates [34]. As a matter of fact, we observed lower abundance of *Bifidobacterium* in rats with lees supplementation, although it was not significant.

Finally, various species of the family *Lactobacillaceae* are classified as probiotics. In general, we found that rats with lees supplementation presented higher relative abundance of potentially probiotic bacteria (Table 3). These bacteria can modulate the immune response of the host, provide extra energy *via* SCFA production and influence the

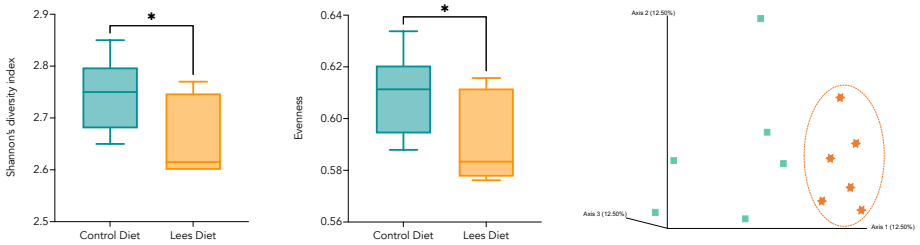
structure, function and integrity of the intestine [41]. Moreover, different strains of *Lactobacillus*, isolated from gut microbiota, have shown antimicrobial activity against pathogens resulting from the production of lactic acid, bacteriocins and other bactericidal compounds [41–43]. In addition, several studies report that the intake of dietary fiber increases the relative abundance of *Lactobacillus* [44]. Table 4 shows the species of *Lactobacillaceae* bacteria that presented significant differences between control and lees groups.

Table 4. Differences regarding relative abundance (%) of bacteria from the family *Lactobacillaceae* between rats with control and lees diet. Values are mean ± standard deviation.

Family	Control Diet	Lees Diet	p-value
Lactobacillus brevis	240 ± 124	671 ± 443	0,02
Limosilactobacillus fermentum	327 ± 226	1144 ± 905	0,02
Lacticaseibacillus paracasei	115 ± 68	370 ± 290	0,04
Lactiplantibacillus plantarum	636 ± 422	2072 ± 967	0,04
Ligilactobacillus ruminis	1109 ± 775	5224 ± 1628	0,02
Latilactobacillus sakei	166 ± 105	509 ± 286	0,04
Ligilactobacillus salivarius	1721 ± 1143	7684 ± 2820	0,02

3.2.1. Bacterial Diversity

Bacterial diversity was assessed by alpha (Figure 3A and 3B) and beta diversity (Figure 3C). Alpha diversity is a measure of the number of different bacteria found within a sample, taking into account the evenness and distribution of such bacteria. The higher the value of the Shannon index, the more diversity there will be in the sample studied.



(**a**) (**b**) (**c**)

Figure 3. Alpha and beta diversity between animals with control (green) and lees (orange) diet. Significant differences between groups are indicated with an asterisk (*): (**a**) Shannon diversity index (alpha diversity); (**b**) Evenness (alpha diversity); (**c**) Beta diversity determined by the Bray–Curtis index and principal coordinates analysis (PCoA).

Regarding alpha diversity, it was observed that rats from the control group had a significantly higher diversity that lees group. That could be a result of an increase of bacteria that produce antimicrobial compounds (e.g. *L. brevis*, *L. plantarum* or *L. sakei*) and can reduce the relative abundance of other microorganisms.

Finally, beta diversity is related to the differences between individuals regarding the distribution of genera and species. Figure 3C showed that the animals with lees supplementation clustered together, while controls were more dispersed. This can translate to a greater homogeneity in the gut microbiota of animals with lees supplementation.

4. Conclusions

Due to the rich fiber composition of Cava lees, this by-product can present potential prebiotic characteristics. The supplementation with lees did not result in a weight increase nor a modification of the food and water intake. In addition, it did not have a negative impact on the studied organs. As for their effect on gut microbiota, lees promoted an increase in the relative abundance of potential probiotic bacteria (*B. hansenii*, *R. intestinalis* and *R. obeum*). Additionally, the abundance of certain species of bacteria from the family *Lactobacillaceae* also increased significantly. All that could result in an increase of SCFA production (acetate, butyrate, and propionate). Therefore, it could be interesting to study the evolution of SFCA with a prolonged supplementation of Cava lees to confirm these preliminary results. Hence, the supplementation or formulation of food with Cava lees could be a new strategy for the revalorization of such by-product, while promoting a higher daily intake of fiber closer to the recommended values.

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CAPÍTOL 5. CARACTERITZACIÓ DE LES LIES DEL CAVA

5.1. Caracterització de les lies del Cava: Capacitat de retenció d'aromes

El primer objectiu de la tesi fa referència a la caracterització de les lies del Cava a nivell de perfil volàtil, degut a la capacitat de retenció d'aromes del Cava a la seva superfície [30,98]. Aquesta habilitat de les lies per captar els aromes del Cava podria afectar el perfil volàtil dels productes on s'incorporin per la seva valorització.

Per poder avaluar quins compostos acaben essent adsorbits a la superfície de les lies, es van analitzar ampolles de diferents Caves en funció del seu temps de criança (Reserva i Gran Reserva), així com del raïm utilitzat (varietats blanques o negres) per la vinificació. Les ampolles es van desgorjar al laboratori i se'n van extreure les restes de lies, que es van liofilitzar. A continuació, es van extreure els compostos volàtils per microextracció en fase sòlida de l'espai de cap (*Head-Space Solid Phase Microextraction*, HS-SPME) i es van analitzar per cromatografia de gasos acoblada a espectrometria de masses (GC-MS) tant els Caves com les lies obtingudes. A més a més, també es van analitzar mostres de lies recollides directament del celler, representant el residu que generen les Caves. Paral·lelament, també es van avaluar diferents paràmetres fisicoquímics com el pH, l'índex de polifenols totals (IPT) i la intensitat (IC) i tonalitat de color (TC) tant dels Caves com de les lies. A més, també es va fer un recompte de cèl·lules de llevat en les mostres de lies.

De manera general, els valors dels paràmetres d'IPT i IC van ser més alts en les lies que en els Caves. Aquest fet pot estar relacionat amb una capacitat de les lies d'adsorbir polifenols a la seva superfície, disminuint-ne el contingut en el Cava final un cop desgorjat. D'altra banda, es va identificar un total de 45 compostos, dels quals 19 només es van trobar a les mostres de lies. La majoria d'aquestes diferències es van observar a les mostres L-CV1, que en ser el residu del celler era una barreja de totes les lies dels diferents Caves que produeixen sense distingir entre temps d'envelliment ni varietats de raïm. Això podria suposar una font d'aromes per la indústria alimentària, de manera que es podria donar un nous ús a aquest subproducte com a nou ingredient dins la formulació d'aliments.

5.2. Publicacions científiques

PUBLICACIÓ 3

Characterization of white and rosé sparkling wine lees surface volatiles

Alba Martín-Garcia, Montserrat Riu-Aumatell, Elvira López-Tamames.

BIO Web of Conferences, **2022**.

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Capítol 11. Annex 1

LES IDEES CLAU

Les lies del Cava són capaces de retenir compostos fenòlics i volàtils a la seva superfície, incrementant amb el temps de criança, pel que podrien ser de gran interès per la indústria d'aromes i alimentària com a nou ingredient.

FER GRÀFIC ABSTRACT PER POSAR AMB LA IDEA RELLEVANT.

Characterization of white and rosé sparkling wine lees surface volatiles

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Abstract. Cava is a sparkling wine that requires a second fermentation in the bottle. Its volatile fraction is conditioned by different parameters (grape, vinification process, fermentative yeast, and aging time). During the autolysis process, yeasts release compounds into the wine, but lees can adsorb certain compounds on their surface. Therefore, the aim of this work was to characterize different white and rosé Cavas, and their lees. For this, white Cavas (CGR1: 40 months; CR1: 16 months) and rosé Cavas (CRR1: multivarietal coupage; CRR2: monovarietal; both 20 months) were studied. Once disgorged, lees were freeze-dried (L-CGR1, L-CR1, L-CRR1 and L-CRR2). In addition, lees waste from the winery were collected. pH, total polyphenol index (TPI) and colour intensity (CI) of Cavas and lees were determined. The volatile fraction was analysed by Head-Space Solid Phase Microextraction followed by gas chromatography coupled to mass spectrometry. Lees showed higher values than their respective Cavas for TPI and CI, especially in the case of the L-CGR1. Most of the volatiles were identified both in Cavas and their lees, esters being the main compounds. Therefore, lees can retain phenolic and volatile compounds on their surface, which could be of interest as a new ingredient in the food industry.

1. Introduction

Cava is a Quality wine produced in specified regions (QWPSR) that requires a second fermentation in the bottle, with a minimum time of 9 months of biological ageing *sur lie* [1]. During the ageing period, fermenting yeasts undergo the autolysis process, in which they can release different compounds (lipids, carbohydrates, amino acids, peptides and volatile compounds) to the wine [2]. Once the ageing process ends, lees are removed from the bottle (disgorgement) and become waste [3]. In fact, the Cava industry generates a great amount of organic waste, such as skins, stems and seeds from the grapes, as well as lees from the alcoholic fermentation. Indeed, lees represent a 25 % of the total waste of these cellars, which is about 300 tons per year [4].

Lees consist of naturally plasmolyzed yeast cells, tartaric acid and other adsorbed compounds [5]. Actually, the cell wall of yeasts is constantly in contact with Cava during ageing [3]. It mainly consists of mannoproteins (exterior) and branched glucans (interior). It is this structure that gives the lees the properties to interact with the compounds of Cava [3,6]. In fact, different studies have focused on the lees ability to adsorb compounds such as polyphenols and other volatile compounds that contribute to wine aroma [3,7,8].

Recently, since lees are rich in fiber and antioxidant compounds [5,9], they have been used as an ingredient in several food matrices in order to re-valorize such by-product [10–12]. Since the addition of lees to food formulation may change its organoleptic properties, Cava lees should be characterized regarding volatile compounds and other physicochemical parameters such as pH and color. Therefore, the aim of this study was to characterize Cava lees regarding different parameters related to sparkling wine quality as well as to evaluate their ability to adsorb volatile compounds from Cava.

2. Materials and methods

2.1. Cava lees recovery

Four types of Cava were selected from the winery Freixenet, S.A. (Sant Sadurní d'Anoia, Spain) produced with different grapes (Macabeu, Xarel·lo and Parellada for white sparkling wines; Garnatxa and Trepat for rosé sparkling wines) as well as different ageing time (Table 1). Moreover, samples of the cellar lees waste were also

obtained (L-CV1). All bottles were disgorged at the same time and wet lees were extracted. Cava samples were stored at -20 °C until the analysis. Then, wet lees were frozen (-80 °C, 15 min) and freeze-dried (Cryodos-50, Telstar, Terrasa, Spain). Lyophilized lees were stored in sealed tubes protected from light and humidity.

Table 1. Studied Cava and their respective lees.

Sample ID	Lees Sample ID	Grape [1]	Biological ageing (months)	Category [2]
CGR1	L-CGR1	M-X-P	40	Gran Reserva
CR1	L-CR1	M-X-P	16	Reserva
CRR1	L-CRR1	GA-TR	20	Reserva
CRR2	L-CRR2	TR	20	Reserva

[1] M: Macabeu; X: Xarel·lo; P: Parellada; GA: Garnatxa; TR: Trepat. [2] Categories according to PDO Cava [1].

2.2. Determination of physicochemical parameters

The plasmolyzed cells were determined by cell counting with a Neubauer chamber (Ref.: 640110, Paul Marienfeld GmbH & Co., Germany). pH was determined in both Cava and lees using a pH meter XS PH60 Violab (XS Instruments, Carpi, MO, Italy).

The optical density (OD) for the analysis of the total phenolic index (TPI), color intensity (CI) and color hue (CH) was determined using a UV-3600 UV-Vis-NIR Spectrophotometer (Shimadzu Scientific Instruments, Inc., MD, USA). Samples were placed in quartz cuvettes with a length of 10 mm. OD was measured at a wavelength range between 280 nm and 620 nm. Ultrapure water was used as blank. For TPI it was necessary to dilute the samples with ultrapure water to obtain OD values between 0.1 and 0.9 according to OIV official analysis regulations [13].

2.3. Analysis of volatile compounds in Cava and lees

The extraction of volatile compounds in Cava and lees was performed by head-space solid phase microextraction (HS-SPME). It was carried out using a 2 cm long Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber supplied by Supelco (Bellefonte, PA, USA). Samples of 5 mL (Cava) or 25 mg (lees) were placed in 10 mL vials. After 15 min of equilibration at 50 °C under continuous agitation (250 rpm), the fiber was exposed to the headspace for 40 min.

Chromatographic analysis was carried out in a 6890N Network GC system (Agilent, Palo Alto, CA, USA) coupled to MS Agilent technologies 5973 Network selective detector (Agilent, Palo Alto, CA, USA). Helium was used as a carrier gas. Separations were accomplished in a DB Wax USN 125-7031 column (30 m x 0.25 mm x 0.25 μm) (Agilent, Palo Alto, CA, USA). A splitless injector suitable for SPME was used. After extraction, the fibre was removed from the headspace vial and inserted directly into the injection port of the GC. The SPME fibre was thermally desorbed for 2.5 min at 260°C.

The initial temperature of the column was 40°C for 10 min, and this was subsequently increased at 4°C/min up to 75°C, then temperature was increased at 2°C/min up to 260°C and hold for 5 min using splitless injection mode. GC-MS detection was performed in complete scanning mode (SCAN) in the 40–350 amu mass range with two scans per second. Electron impact mass spectra were recorded at an ionization voltage of 70 eV and ion source of 280°C. The results reported were calculated by dividing the peak area of the compounds of interest by the total area, obtaining the relative abundance of each compound. The relative response factor was considered to be 1. Identification was performed by comparison of their mass spectra with those of the mass spectra library database Wiley 6.0., and their retention times with those of pure standards when they were available.

2.4. Statistical Analysis

All assays were performed in triplicate and in a randomized run order. The statistical analysis was performed using Prism 9 version 9.1.2 (225) (GraphPad Software, LLC., California, USA) statistical package. The results are reported as the means ± standard error (SE) for parametric data. A one-way ANOVA and comparison of the means were conducted using Tukey's test, with a confidence interval of 95% and significant results with a p-value of ≤ 0.05. Principal component analysis (PCA) was also performed to determine differences between Cavas and lees.

3. Results and discussion

For this study, four different types of Cava, as well as their lees, were analyzed. In addition, wine lees from the cellar waste were also collected and analyzed, since the wineries do not separate the lees according to the type of Cava, but deposit them all together to manage them as waste.

3.1. Determination of physicochemical parameters

Cava and lees pH values and cell counts are shown in Figure 2. In general, pH values of Cava are within the requirements established in the legislation (minimum 2.8 and maximum 3.4) [1]. It should be noted that the pH of Caves with Reserva category (CR1, CRR1 and CRR2) had a pH close to 3.1, regardless of the grape variety, while Cava Gran Reserva (CGR1) obtained a lower pH (2.94 ± 0.05) ($p < 0.05$). As for the lees, the pH values showed no significant differences except for the L-CV1 (cellar residue) samples, which obtained the lowest value (3.02 ± 0.02) ($p < 0.05$). However, the pH range obtained by lees was lower than that reported by other studies, in which the values ranged from 3.6 to 7.2 [4].

Table 2. UV-Vis spectrometry results regarding total phenolic index (TPI), color intensity (CI) and color hue (CH) expressed as absorbance units.

	Sample ID	TPI [1]	$OD_{320\ nm}$	CI [2]	CH [3]
CAVA	**CGR1**	6.47 ± 0.25	3.72 ± 0.62 [ab]	0.604 ± 0.062 [a]	1.612 ± 0.162 [a]
	CR1	5.43 ± 0.57	3.11 ± 0.04 [ac]	0.370 ± 0.097 [b]	1.111 ± 0.097 [b]
	CRR1	6.96 ± 0.70	4.21 ± 0.32 [b]	1.145 ± 0.064 [c]	1.490 ± 0.064 [a]
	CRR2	5.59 ± 0.88	2.65 ± 0.06 [c]	0.540 ± 0.013 [ab]	1.247 ± 0.109 [b]
LEES	**L-CGR1**	9.68 ± 0.48 [a]	4.35 ± 0.71	1.320 ± 0.035 [a]	1.140 ± 0.031 [a]
	L-CR1	9.64 ± 0.36 [a]	4.40 ± 0.47	1.412 ± 0.038 [a]	1.023 ± 0.010 [b]
	L-CRR1	8.07 ± 0.64 [b]	4.66 ± 0.19	0.832 ± 0.087 [b]	1.025 ± 0.002 [b]
	L-CRR2	7.68 ± 0.52 [b]	5.01 ± 0.18	1.836 ± 0.052 [c]	0.999 ± 0.016 [b]
	L-CV1	6.36 ± 0.21 [c]	4.24 ± 0.12	0.766 ± 0.045 [d]	0.942 ± 0.002 [c]

[1] TPI: Total Polyphenols Index, $OD_{280\ nm}$ x 10. [2] CI: Color Intensity, $OD_{420\ nm} + OD_{520\ nm} + OD_{620\ nm}$. [3] CH: Color Hue, $OD_{420\ nm} / OD_{520\ nm}$. Results are expressed as mean ± standard deviation of triplicates. Different letters denote statistically significant differences ($p < 0.05$) between the samples of Cava and between the samples of lees for each parameter.

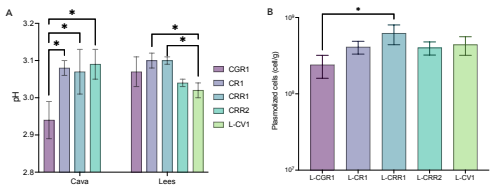

Figure 1. pH (A) and cell counts (B) of the different types of Cava and lees.

Plasmolyzed cells were then counted in lees samples using the Neubauer chamber (Figure 1B). The number of cells was found to be between 2.4 x 10^8 ± 8.0 x 10^7 cell/g (L-CGR1) and 6.2 x 10^8 ± 1.8 x 10^8 cell/g (L-CRR1). The Reserva Caves (both white and rosé) had a similar concentration of cells. Also, the residue from the winery (L-CV1) had a concentration of 4.4 x 10^8 cells/g.

The different types of polyphenols found in a wine have absorbance depending on the wavelength: at 280 nm the absorption is related to the benzene ring common to all phenolic compounds; at 320 nm are flavones and non-flavonoid compounds (hydrocinnamic acids, stilbenes, and hydrobenzoic acids); and finally, 520 nm is related to the presence of anthocyanins, which provide a reddish or purple pigment [15,16]. Therefore, the white Cavas will mainly show absorbance in the 280 nm and 320 nm region, while in the pink cava samples there will be an extra absorption region at 520 nm. On the other hand, the color intensity (CI) represents the amount of color, varies depending on the wine and the grape variety used during vinification, and is in the range of 0.3 to 1.8 units. In addition, the color tone (TC) shows the development of orange tones with the aging of the wine, obtaining values between 0.5 and 0.7 for young wines and increasing up to 1.2 - 1.3 for aged wines [14]. The values obtained by UV-Vis spectrometry are found in Table 2.

Recent studies indicate a first increase and a subsequent decrease in the values of TPI, CI and CH as a result of the absorption of polyphenols by lees, as well as their polymerization and precipitation during the aging of Cava [15]. In fact, a higher TPI was observed in the samples of white lees compared to their respective Cava, obtaining the highest values in L-CGR1 and L-CR1 (white Cava). In rosé samples the same tendency of increase of the TPI in the lees was observed, obtaining a difference of 1.11 (CRR1 and L-CRR1) and 2.09 (CRR2 and L-CRR2) between the Cava and its lees. The sample of the cellar waste (L-CV1) showed the lowest TPI values (6.36 ± 0.21).

Regarding the CI, among Cavas of white varieties CGR1 had a higher intensity than CR1 ($p < 0.05$). However, the CI of L-CR1 was slightly higher than that of L-CGR1. Therefore, color intensity of Cava increases with the biological ageing. In contrast, for rosé samples, CRR1 had a higher CI than CRR2 ($p < 0.05$). In fact, single-variety rosé Cava (CRR2) had a very pale coloration, so adding the Garnatxa variety to Trepat (CRR1) significantly increased the CI. With respect to the lees obtained from these Cavas, L-CRR1 had a lower CI, as opposed to L-CRR2, that significantly increased its CI ($p < 0.05$). Similarly to TPI, the CI of L-CV1 was the lowest.

Finally, CH results were as expected for Cava with biological ageing, obtaining values between 1.111 ± 0.097 (CR1) and 1.612 ± 0.162 (CGR1); and 0.942 ± 0.002 (L-CV1) and 1.140 ± 0.031 (L-CGR1) for lees. In fact, the highest CH values were those of Cava with the highest aging.

3.2. Analysis of the volatile compounds of Cava and lees

The volatile fraction, or aroma, of Cava is one of the most relevant quality factors of such product. Aroma is influenced by different parameters, such as grape variety, the vinification process, the fermenting yeasts, and biological ageing in contact with lees [7,15,16]. In fact, the second fermentation and biological ageing have a great impact on the volatile compounds of Cava. For instance, during autolysis, yeasts release compounds to the wine [6]. Nevertheless, yeast lees are able to adsorb certain compounds in their surface [3]. Therefore, ageing time can

determine the volatile profile of a product such Cava [15,17].

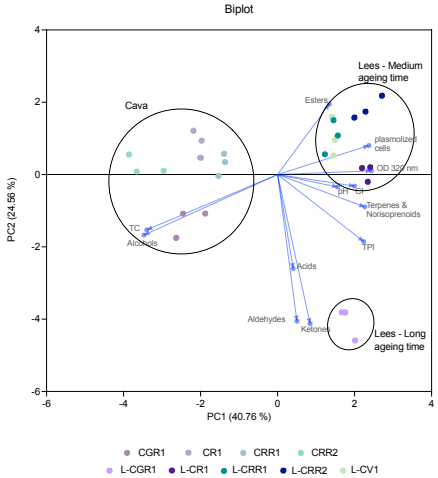

Figure 2. Principal component analysis (PCA) biplot of Cava and lees.

In this study, a total of 68 different compounds were identified in the samples of Cava and lees. The results obtained were subjected to a PCA (Figure 2). Generally, Cava with more time of biological ageing (CGR1) and their lees (L-CGR1) were the samples with a greater variety of identified compounds. On the other hand, Cavas with the same biological ageing time (CR1, CRR1 and CRR2) showed differences regarding the grapes variety used for vinification, being significant between CR1 and rosé Cavas, but not between CRR1 and CRR2 for most of the compounds.

Both Cava and lees presented a similar volatile profile (Figure 3). In both matrices esters were the major volatile compounds (45% - 79%), highlighting ethyl hexanoate and ethyl octanoate in Cava, and ethyl octanoate and ethyl decanoate in lees. It can be observed that Cava lees presented more variability regarding the relative abundance of each family compound, although their general profile was very similar to Cava.

Acids are a product of long chain fatty acids catabolism and, depending on their concentration, they are related to a decrease in wine quality [18,19]. It has been reported that when acids between C6 and C10 are above 20 mg/L have a negative impact on wine organoleptic quality, while below that concentration, acids contribute with pleasant aromas [18]. In the present study, acids accounted for 12% - 17% of the total volatile compounds identified, increasing with biological ageing. Octanoic acid was the major acid, in accordance with other studies [15,18,20]. Moreover, Mendes de Souza Nascimento et al. (2018) [18] reported that higher values of chromatographic areas of acids can be related to the double fermentation that take place in sparkling wine vinification following the traditional method, as it is the case of Cava production. Regarding lees, acids showed a greater area for L-CV1 (34.37 ± 10.91), L-CGR1 (31.95 ± 9.34) and L-CR1 (16.85 ± 2.10), being a 26%, 22% and 17%, respectively. Lees from rosé Cava presented lower values with a relative abundance of 10.27 ± 0.61 (9%, L-CRR1) and 7.01 ± 1.00 (7%, L-CRR2).

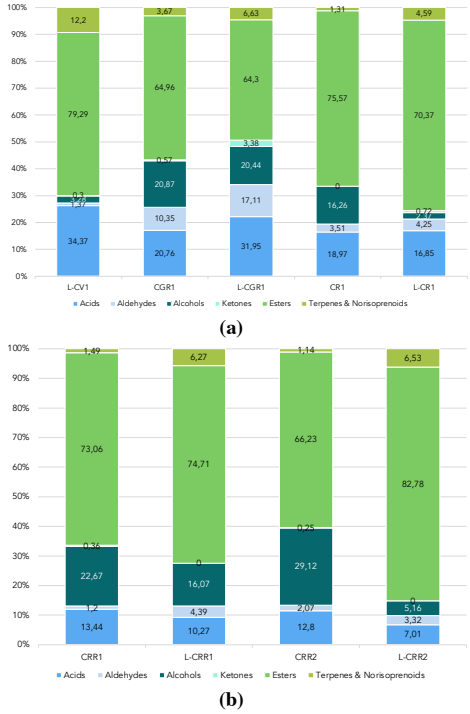

Figure 3. Relative abundance of the volatile compounds identified in Cava and lees A) White Cava and lees; B) Rosé Cava and lees.

Aldehydes are the result of carbohydrate and lignin degradation and are responsible for toasty notes in wine [18,21]. Even though aldehydes can be reduced during ageing to form their respective alcohols [21], in this study, both Cava and lees increased the total abundance of aldehydes with the ageing time. Furthermore, it has been reported that aldehydes are easily adsorbed by lees [3,21]. For instance, a few aldehydes were identified in CR1 (furfural, benzaldehyde and 2-methylbenzaldehyde), CRR1 (furfural and benzaldehyde) and CRR2 (furfural and 3-methylbenzaldehyde), but the aldehydes found in their lees presented a higher diversity.

During the fermentation process, higher alcohols are produced from sugars and amino acids. They are an important fraction of the sparkling wine volatile profile, even though alcohols may have a positive or a negative impact on wine aroma [18]. In the present study, isoamyl alcohol and 2-phenylethanol, both major products of alcoholic fermentation, where the dominant alcohols in Cava, being in accordance with other studies [15,18,22]. Although 2-phenylethanol was found in all lees samples, isoamyl alcohol was only identified in L-CGR1 (Cava Gran Reserva) and L-CV1 (cellar waste), with a low

relative abundance (1.11 ± 0.12 and 2.03 ± 0.62, respectively). 2-butanol, 1-hexanol, (Z)-3-hexen-1-ol and 2-methylpropanol were only found in Cava. Moreover, 2-butanol and 2-methylpropanol were exclusive of rosé Cava, while 1-octanol and (Z)-3-hexen-1-ol were only identified in white Cava. On the other hand, 1,3-butanediol, 1-nonanol, 2-hexanol and 2-ethilhexanol were only obtained in lees. In fact, Gallardo et al. (2009) [3] studied the volatile profile of lees and concluded that lees have a scarce capacity of retaining higher alcohols on their surface.

As previously stated, esters were the major volatile compounds of both Cava and lees, contributing to aroma with fruity notes. Regarding Cava, they represented between 65% (CGR1) and 75% (CR1); while in lees there was a greater variability, ranging between 64% (L-CGR1) and 82% (L-CRR2). Because of their great hydrophobic capacity, esters can easily be retained on lees surface [3]. Among the detected esters, most of them were ethyl esters. Those are produced by yeasts during alcoholic fermentation, contributing with floral and fruity aromas [22]. In accordance with other studies, ethyl hexanoate, octanoate and decanoate and diethyl succinate, were the major esters found in Cava [18,22]. Similarly, they were also the most outstanding esters, in agreement with the results reported by Gallardo-Cachón et al. (2009) [3].

Finally, vitispirane A and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) were the norisoprenoids identified in Cava and lees. They are both considered ageing markers due to their concentration increase with time [3,17]. In fact, the obtained results showed a greater area percentage in lees and Cava Gran Reserva when compared to younger Cavas, being in agreement with other studies [3,15,17,19]. Similarly to esters, those compounds are hydrophobic, therefore, they have a great capacity of being retained in lees surface [3].

In general, volatile compounds of Cava and lees differ from each other (Figure 4). Regarding white Cavas, CGR1 (Gran Reserva) there is a coincidence of 53% of the compounds with respect to their lees (L-CGR1); while CR1 (Reserva) there is a 43% similarity between Cava and lees. As for rosé Cavas, between CRR1 and L-CRR1 there was a 31% coincidence, and a 35% similarity between CRR2 and L-CRR2. Thus, longer times of ageing in contact with lees may result in greater adsorption or release of compounds and, consequently, more similarity between the wine and the lees.

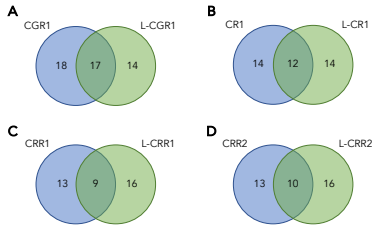

Figure 4. Venn diagram of the volatile compounds Shared by each Cava and their lees: A) Gran Reserva White Cava (CGR1); B) Reserva White Cava (CR1); C) Reserva Rosé Cava coupage Garnatxa-Trepat (CRR1); D) Reserva Rosé Cava Trepat (CRR2).

4. Conclusions

During biological ageing of Cava (sparkling wine) compounds are both released and retained by lees, therefore modifying the volatile and phenolic profiles of such wine product.

Different physicochemical parameters of both Cava and lees were studied. Generally, it was observed that lees presented higher values of TPI and CI, pointing towards the adsorption of phenolic compounds in the lees surface. It was found that pH values were lower for Cava and lees with a longer ageing period. Regarding the number of plasmolyzed cells in Cava lees, values were around 10^8 cells/g of lees.

On the other hand, a total of 68 volatile compounds were identified in Cava and lees, of which 19 were only found in lees samples. Most of these differences were found in L-CV1 (cellar lees waste) in which lees from different origins are mixed.

In conclusion, lees could be a potential source of flavor as a new ingredient for the food industry. That might be a new strategy for the valorization of such by-product. Therefore, future research should focus on the use of different lees in the formulation of foodstuff.

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CAPÍTOL 6. LES LIES DEL CAVA COM A INGREDIENT

6.1. Avaluació de l'efecte de les lies del Cava sobre la fermentació i el perfil volàtil de la massa mare i el pa

La massa mare és una barreja de farina i aigua que, tradicionalment, es deixa fermentar de manera espontània (sense l'ús de cultius iniciadors) per la microbiota pròpia dels ingredients utilitzats, on les farines més utilitzades són les de blat i sègol. D'altra banda, les lies del Cava són un subproducte de la indústria vitivinícola, riques en fibra i compostos antioxidants (**Secció 2.1.2.3**). Per tant, la hipòtesi de la que deriva el segon objectiu de la tesi se centra en la possible capacitat de les lies del Cava per promoure el creixement i supervivència de microorganismes amb interès tecnològic, com ho són els responsables de la fermentació de la massa mare i el pa.

De fet, en els aliments fermentats, aquests microorganismes tenen un efecte sobre la qualitat nutricional i organolèptica d'aquests productes. És més, en funció de les poblacions microbianes l'aroma d'aliments com el pa es pot veure modificat. A més a més, com s'ha comprovat les lies tenen capacitat de retenció de compostos volàtils a la seva superfície (**Secció 5.1 –** Publicació 3). Per tant, com a conseqüència de la incorporació de lies a la formulació de la massa mare i el pa es podria modificar la microbiota fermentativa i aportar aromes nous al producte, pel que podrien presentar canvis en la percepció i acceptació d'aquest.

Per tant, el segon àmbit estudiat en aquesta tesi es va dividir en diferents objectius. Per una banda, avaluar l'addició de diferents concentracions de lies del Cava (0 – 2%) sobre el creixement i supervivència de la microbiota pròpia de la massa mare (Publicació 4). A continuació, es va estudiar l'efecte d'aquest nou ingredient sobre el perfil volàtil de la massa mare utilitzant les mateixes concertacions (Publicació 5). Finalment, es va fixar una concentració del 5%, basat en estudis previs *in vitro* [29], i se'n va estudiar l'efecte sobre la microbiota i el perfil volàtil tant de la massa mare com del pa final (Publicació 6).

6.2. Publicacions científiques

PUBLICACIÓ 4

Revalorization of Cava (Spanish Sparkling wine) lees on sourdough fermentation

Alba Martín-Garcia, Montserrat Riu-Aumatell, Elvira López-Tamames.
Fermentation, **2022**, *8(3)*, 133.
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LES IDEES CLAU

L'addició de lies del Cava a la formulació de massa mare promou el creixement i supervivència dels microorganismes fermentadors i, conseqüentment, promou una disminució del pH i un increment en la producció d'àcids orgànics. Tot això pot suposar una major estabilitat microbiològica així com canvis en el sabor i aroma del pa resultant.

fermentation

MDPI

Article

Revalorization of Cava (Spanish Sparkling Wine) Lees on Sourdough Fermentation

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Abstract: Cava lees are a sparkling wine by-product formed of dead microorganisms, tartaric acid and other inorganic compounds, with a potential for enhancing microbial growth. Lees are rich in antioxidant compounds as well as β-glucans and mannoproteins. The aim of this study was to evaluate the effect of different concentrations of cava lees (0–2% *w*/*w*) on the microbiota (LAB and yeasts) responsible for sourdough fermentation (8 days) to revalorize this by-product of the wine industry. The results showed that 2% cava lees promoted microbial growth and survival in both wheat and rye sourdoughs, except for yeast growth in rye, which stopped at day 3 of fermentation. Moreover, sourdough with lees achieved lower pH values as well as higher concentrations of organic acids, especially lactic and acetic acids ($p < 0.05$). To sum up, the use of cava lees in sourdough formulation promotes the growth and survival of microorganisms, which, in consequence, promotes a lower pH and greater amounts of organic acids. This could lead to microbial stability as well as changes in bread flavor.

Keywords: cava lees; wine by-product; revalorization; sourdough; lactic acid bacteria; yeasts; prebiotic

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1. Introduction

Cava is a Spanish sparkling wine with a Certified Brand of Origin (CBO) that is produced using a traditional method, refermenting a base wine in a sealed bottle. In order to be considered Cava, wines must undergo an ageing process for a minimum of 9 months (EC Regulation 2019/934). Yeast autolysis takes place during the ageing process, releasing cell components and breakdown products into the wine [1,2].

Lees are defined as the residue formed at the bottom of receptacles containing wine, after fermentation and during storage (e.g., during the ageing process of Cava). Lees mostly consist of dead microorganisms (generally *Saccharomyces cerevisiae*), tartaric acid and other adsorbed compounds [3]. The cell wall of *S. cerevisiae* remains intact and is mainly composed of mannoproteins and branched β-glucans, as well as soluble polysaccharides [1]. Cava lees are rich in antioxidant compounds [3,4] along with soluble and insoluble dietary fiber from the yeast cell wall [5].

Each bottle of Cava contains about 1g of lees, which contains approximately 10^8 yeast cells that contribute to organoleptic properties during ageing [6]. However, yeast lees of Cava are considered a by-product of approximately 300 tons per year, representing approximately 25% of the waste by-products from the wine industry [4]. Despite being the second largest by-product in wineries, wine lees are mainly destined for distillation. However, considering their composition, those lees could also potentially acquire an added

value [5,7–9]. In fact, there is an increasing trend in the food and drinks industry to reduce food waste by revalorizing by-products and co-products, therefore contributing to a circular economy and more sustainable food production [4,8,10–16].

On the other hand, consumers are more conscious of their food consumption and their health. Moreover, artisan food products' popularity is increasing due to a clean-label trend, including the use of sourdough in bread-making [17]. In addition, Spain has recently developed new bread legislation, including the definition of sourdough and establishing some rules regarding its production and labelling (RD 308/2019) [18].

Sourdough is a mixture of flour and water, fermented by homo- and heterofermentative lactic acid bacteria (LAB) and yeasts. The traditional method consists of the spontaneous fermentation of sourdough by the microorganisms present in the flour, which are responsible for acidification, leavening and flavor formation [17,19,20]. Different flours (e.g., wheat, rye, teff, barley, etc.) may be used to produce sourdough, presenting different characteristics in the final bread, such as flavor or nutritional value. In fact, the flour composition and its quality can affect the microorganisms' dynamics and, therefore, the sensory properties of the sourdough bread [17,21–23].

Therefore, the aim of this study was to evaluate the prebiotic effect of yeast lees on the microbiota (LAB and yeasts) responsible for sourdough fermentation to revalorize this by-product of the wine industry.

2. Materials and Methods

2.1. Preparation and Propagation of Sourdoughs

A commercial wheat flour was used for sourdough preparation (Ref.: 7230 Buonpane, Molino Quaglia SpA, Padua, Italy), with the following composition (% *w*/*w*): carbohydrates 72.0, fat 1.5, fiber 2.0, protein 11.5 and moisture 15.0. A second type of sourdough was prepared using a commercial rye flour (Ref.: 50782, Molino Quaglia SpA, Padua, Italy), with the following composition (% *w*/*w*): carbohydrates 76.4, fat 0.8, fiber 4.6, protein 4.6 and moisture 15.0.

Both types of sourdough were prepared by mixing 100 g of flour and 100 mL of sterile distilled water, without the inoculation of starter culture bacteria or yeasts, and incubated at room temperature. Cava lees were provided by the winery Freixenet S.A. (Sant Sadurní d'Anoia, Spain) and lyophilized following the method described by Hernández-Macias et al., (2021) [7]. Lyophilized lees were added at different concentrations (0%, 0.5%, 1% and 2% (*w*/*w*)) to assess their effect on sourdough fermentation (Table 1). The sourdoughs were propagated daily by backslopping for 8 days, inoculating an aliquot of the previous dough into a new mixture of flour and water. All fermentations were carried out in triplicate.

Table 1. Ingredients of sourdough (flour weight basis, g).

	Flour [1]	Water	Dough [2]	Cava Lees [3]
Control	100	100	100	-
0.5% Lees	100	100	100	0.5
1% Lees	100	100	100	1
2% Lees	100	100	100	2

[1] Either wheat or rye flour; [2] Aliquot of the previous dough into the new mixture; [3] Lees were added as a percentage of flour weight in sourdough formulation in each propagation step.

2.2. Viable Counts of Lactic Acid Bacteria (LAB) and Yeasts

To assess the microbial growth of LAB and yeasts, samples of 10 g of sourdough were added to 90 mL of sterile peptone water (Ref.: 1402, Condalab, Madrid, Spain) and homogenized with a laboratory blender (Stomacher 400 Seward Ltd., Worthing, UK) for 1 min. Samples were taken daily, diluted and plated in MRS (Ref.: 1043, Condalab, Madrid, Spain) to monitor LAB populations and in Saboraud-Chloramphenicol Agar (Ref.: 01-166-500; Scharlab, Barcelona, Spain) for yeasts.

2.3. Determination of pH, Fermentation Quotient (FQ) and Organic Acids

Sourdough fermentation was monitored daily by pH using the pH meter XS PH60 VioLab (XS Instruments, Carpi, Italy). The fermentation quotient (FQ) was determined as the molar ratio between lactic and acetic acids. Organic acids (acetic, citric, D-lactic, L-lactic and L-malic acids) were determined using enzymatic detection kits supplied by BioSystems (Barcelona, Spain) and a spectrophotometer Shimadzu UV-3600 (Shimadzu Corporation, Kyoto, Japan), following each kit's instructions.

2.4. Statistical Analysis

All assays were performed in triplicate. Statistical analysis was performed using the Prism 9 v.9.1.2 (225) (GraphPad Software, LLC., San Diego, CA, USA) statistical package. The results are reported as means ± standard error (SE) for parametric data. Analysis of variance (ANOVA) and comparison of the means were conducted using Tukey's test, with a confidence interval of 95% and significant results with a p-value of ≤ 0.05. Principal component analysis (PCA) was also performed to determine the differences between sourdoughs.

3. Results and Discussion

Two types of flour (wheat and rye) were used to produce sourdoughs, and different concentrations of yeast lees (0%, 0.5%, 1% and 2% (w/w)) were added to the sourdough formulation to test its prebiotic effect on the fermenting microbiota.

3.1. Propagation of Sourdoughs

3.1.1. Effect of Cava Lees on Lactic Acid Bacteria

In both wheat and rye sourdoughs, adding 2% of cava lees resulted in major viable LAB cells (8.4 ± 0.2 log CFU/mL and 9.1 ± 0.1 log CFU/mL, respectively) at the end of fermentation (Figure 1). These results are in accordance with Hernández-Macias et al. (2021) [7], who reported higher microbial counts in vitro with 2% and 5% of yeast lees after 24h and 48h of incubation, respectively.

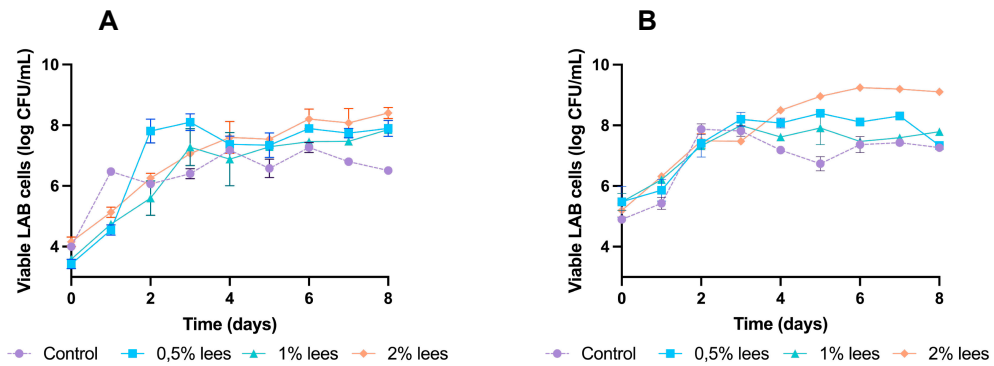

Figure 1. Growth kinetics of LAB during sourdough fermentations: (**A**) wheat sourdoughs; (**B**) rye sourdoughs.

As shown in Figure 1A, the maximum growth achieved by LAB in wheat sourdough was: 7.3 ± 0.2 log CFU/mL (day 6) in control fermentations; 8.1 ± 0.3 log CFU/mL (day 3) by adding 0.5% lees; 7.9 ± 0.1 log CFU/mL (day 8) by adding 1% lees; and 8.4 ± 0.2 log CFU/mL (day 8) by adding 2% lees.

On the other hand, Figure 1B shows the growth kinetics of rye sourdoughs, reaching their highest number of viable cells as follows: 7.9 ± 0.2 log CFU/mL (day 2) in control

fermentations; 8.4 ± 0.1 log CFU/mL (day 5) with 0.5% lees; 8.0 ± 0.1 log CFU/mL (day 3) with 1% lees; and 9.3 ± 0.1 log CFU/mL (day 6) with 2% lees.

These results suggest that the incorporation of Cava lees in the formulation of wheat and, in particular, rye sourdough improve the growth and survival of LAB. In wheat sourdough (Figure 1A), it can be observed that bacterial growth increases with lees concentration, obtaining the best results at 2% (*w*/*w*), and having statistically significant differences in all fermentations with lees regarding control.

In rye sourdough (Figure 1B), at the end of fermentation, incorporating lees in its formulation has an effect with 1% (*w*/*w*), obtaining statistically significant results with 2% (*w*/*w*) of Cava lees.

Other studies have also reported a stimulatory effect on fermenting LAB by different by-products [7,15,24–26], mainly due to oligo- and poly-saccharides. As stated by Rivas et al. (2021) [5], wine lees are the winery by-product with the highest percentage of dietary fiber (DF), over grape skins and stems. Similarly, the positive effect that Cava lees had over LAB's growth could be attributed to the use of the β-glucans and mannoproteins found in their cell wall as a carbon source. Moreover, several studies focused on the extraction and usage of β-glucans and mannoproteins from various sources (spent brewer yeasts, bacterial production or cereal origin) in order to use them as food ingredients [10,15,26–28].

3.1.2. Effect of Cava Lees on Yeasts

Figure 2 shows the growth development of yeasts in both wheat (Figure 2A) and rye (Figure 2B) sourdoughs. It can be observed that, in wheat control sourdoughs, the plate counts showed no yeast growth.

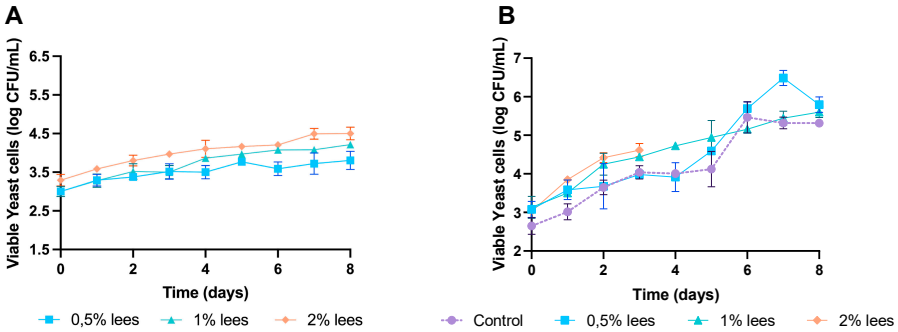

Figure 2. Growth kinetics of yeasts during sourdough fermentations: (**A**) wheat sourdoughs; (**B**) rye sourdoughs.

Regarding yeasts in wheat sourdough (Figure 3), cell viability slightly increased with lees ($p > 0.05$), being stable during the whole fermentation process, and obtained the highest number of viable cells at the end of fermentation: 3.8 ± 0.2 log CFU/mL with 0.5% lees and 4.2 ± 0.1 log CFU/mL with 1% lees; and 4.5 ± 0.2 log CFU/mL with 2% lees.

In contrast, rye sourdough (Figure 3) control samples reached a yeast cell density of 5.3 ± 0.1 log CFU/mL at the end of fermentation, whereas the samples with 2% Cava lees stopped yeast growth at day 3. The sourdough environment is considered to be stressful; consequently, the microbiota has to adapt to the variability in nutrients and the low pH [17,29]. On that account, wheat control sourdoughs had the fastest acidification (data not shown), which may explain the growth inhibition of yeasts. Yeast higher plate counts in rye sourdoughs were as follows: 5.5 ± 0.4 log CFU/mL (day 6) in control fermentations; 6.5 ± 0.2 log CFU/mL (day 7) with 0.5% lees; and 5.6 ± 0.1 log CFU/mL

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(day 8) with 1% lees. In fact, rye sourdoughs presented higher populations of yeast from the beginning than wheat (data not shown).

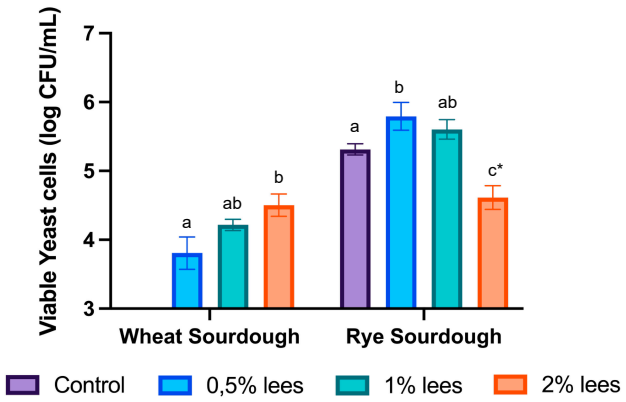

Figure 3. Yeast cell density at the end of sourdough fermentation. Different letters denote statistically significant differences ($p < 0.05$) between the sourdoughs with different amounts of lees. * Yeast plate counts in rye sourdough with 2% of cava lees ended at day 3 of fermentation, i.e., there was no further growth.

Similarly to LAB, higher yeast cell densities may be due to the fiber composition of Cava lees. Lees' main monosaccharides are glucose, mannose and rhamnose [5], whereas the carbohydrates present in the flour are sucrose, glucose, fructose and maltose [30]. Therefore, lees present an additional source of glucose that yeasts can catabolize.

In both wheat and rye sourdoughs, it took approximately 6 days to obtain a stable microbiota, which is in accordance with other studies [31,32]. The sourdough ecosystem is formed by LAB and yeasts that can interact with each other. These interactions can be synergistic (positive effects) or antagonistic (negative effects). As a result, both LAB and yeast may improve their growth kinetics or decrease them [17,30,32]. For instance, the absence of yeast growth in wheat control fermentations may be due to an antagonistic interaction with LAB, such as acidification of the medium or the production of some antifungal compounds [33].

On the other hand, in sourdough, it is very common that the association between LAB and yeast is a consequence of their metabolism preferences (e.g., a maltose-positive LAB with a maltose-negative yeast). For instance, sucrose is hydrolyzed by yeasts, releasing glucose and fructose for LAB to consume [34]. The extra nutrients from lees could enhance that type of microbial association.

Overall, the addition of Cava lees into the formulation of sourdough at 2% (w/w) improved the growth and survival of the dough microorganisms.

3.2. Physicochemical Characterization of Sourdoughs

In addition to microbial growth, pH was also monitored daily. Furthermore, at the beginning and end of fermentation, several organic acids (acetic, citric, lactic and malic acids) were analyzed, and, consequently, the fermentation quotient (FQ) was determined for both wheat and rye sourdoughs. FQ is a molar ratio between the values of lactic and acetic acids.

3.2.1. Monitoring of pH

New Spanish legislation (RD 308/2019) [18] establishes that sourdough must have a maximum pH of 4.2 before incorporation into the bread. Following those guidelines, wheat

control sourdoughs have a slightly higher final pH, with a value of 4.38 ± 0.04. On the contrary, sourdoughs including Cava lees have an acidic pH, with a difference of 0.50 relative to control. In fact, 1% (*w*/*w*) fermentations have a lower value of 3.87 ± 0.13, followed by 2% (*w*/*w*) sourdoughs (3.88 ± 0.04) and, finally, 0.5% (*w*/*w*) samples (3.98 ± 0.04). This tendency in pH reduction is in accordance with the higher LAB cell densities that increase with the addition of lees.

Conversely, all rye sourdoughs meet legislation requirements, obtaining a lower pH with 2% (*w*/*w*) Cava lees (3.66 ± 0.01), also relating to higher bacterial populations. Moreover, the addition of lees significantly decreased the initial pH with a difference of 0.66 regarding control.

Overall, both wheat and rye sourdoughs showed a reduction in pH (ranging between 1.74 and 1.95 in wheat; and 1.97 and 2.10 in rye) with the addition of Cava lees, in accordance with other studies that included Cava lees [7,8], co-products [12] or by-products [25,35] from other food industries with potential revalorization. In all cases, the ingredient added to the formulation was already acidic (e.g., citrus or orange fibers), similar to the Cava lees, which are also acidic.

3.2.2. Fermentation Quotient (FQ) and Organic Acids

Regarding organic acids (Table 2), the addition of lees to sourdough formulation resulted in significantly higher concentrations of organic acids, in both the beginning and end of fermentation in both wheat and rye formulations, following the same tendency as reported by Vriesekoop et al. (2021) [15], with the addition of brewer spent grain to sourdough bread production. In contrast, the FQ decreased with the addition of lees, with a difference in control of 6.6 in wheat (50% lower) and 3.39 in rye (52% lower), with 2% of Cava lees. In fact, the FQ was lower in all rye samples compared to wheat values. This is the result of the lower production of lactic acid and the higher concentrations of acetic acids in rye sourdoughs [31]. In fact, high values of FQ are usually found in traditional sourdoughs [30,31,36] and could be attributed to a larger presence of homofermentative and facultative heterofermentative LAB, which primarily converts glucose into lactic acid, with respect to obligate heterofermentative LAB, which also produce acetic acid [17,30].

Wheat sourdoughs (Table 2) showed an increase in initial organic acids (L-malic and citric acid) when Cava lees were added to the formulation. L-malic acid increased significantly from 0.450 ± 0.070 g/L in the control to 0.850 ± 0.021 g/L in 0.5% sourdoughs, reaching 1.000 ± 0.045 g/L in 2% sourdoughs. The concentration of citric acid ranged between 22.840 ± 2.418 mg/L (control) and 359.250 ± 11.341 mg/L (2% Cava lees). Citric acid increased significantly with the addition of at least 1% of Cava lees to the formulation (174.172 ± 12.815 mg/L), having a major impact with 2% lees.

At the end of fermentation (8 days), the use of Cava lees augmented the concentration of the quantified organic acids, having an effect at 1% and, in particular, in 2% lees sourdoughs. The production of acetic acid was five times greater in 2% cava lees sourdoughs (0.660 ± 0.023 g/L) than in the control (0.135 ± 0.025 g/L). Lactic acid concentration increased 2.5 times in 2% fermentations in comparison to the control. The higher production of organic acids is in accordance with a higher cell density and a lower pH with the addition of Cava lees.

As for FQ, there was a significant decrease in control fermentations when a minimum of 0.5% (*w*/*w*) Cava lees were added to the sourdough, although there were no differences between 1% and 2% sourdoughs.

Organic acids in rye sourdoughs (Table 2) also increased by adding Cava lees to their formulations. In comparison to wheat, L-malic and citric acid concentrations were higher in the control already and were critically augmented with the addition of lees.

Table 2. Physicochemical characterization of sourdoughs (both wheat and rye) and fermentation quotient (FQ).

Wheat Sourdoughs	**Control**	**0.5% lees**	**1% lees**	**2% lees**
	Beginning of fermentation (t = 0 days)			
pH	5.78 ± 0.04	5.72 ± 0.07	5.48 ± 0.03	5.83 ± 0.04
Acetic acid (g/L)	<0.03	<0.03	<0.03	<0.03
Lactic acid (g/L)	<0.02	<0.02	<0.02	<0.02
Malic acid (g/L)	0.45 ± 0.07 ^{a}	0.85 ± 0.02 ^{b}	0.95 ± 0.04 ^{b}	1.00 ± 0.05 ^{b}
Citric acid (mg/L)	22.84 ± 2.42 ^{a}	40.74 ± 8.70 ^{a}	174.17 ± 12.82 ^{b}	359.25 ± 11.34 ^{c}
	End of fermentation (t = 8 days)			
pH	4.38 ± 0.05 ^{a}	3.98 ± 0.04 ^{b}	3.87 ± 0.13 ^{b}	3.88 ± 0.04 ^{b}
Acetic acid (g/L)	0.14 ± 0.03 ^{a}	0.29 ± 0.08 ^{a}	0.51 ± 0.02 ^{b}	0.66 ± 0.023 ^{b}
Lactic acid (g/L)	2.58 ± 0.14 ^{a}	3.14 ± 0.34 ^{a}	4.37 ± 0.13 ^{b}	6.35 ± 0.25 ^{c}
Malic acid (g/L)	<0.03	<0.03	<0.03	<0.03
Citric acid (mg/L)	<11.00	<11.00	<11.00	<11.00
Fermentation Quotient (FQ)	13.00 ± 0.96 ^{a}	7.40 ± 0.89 ^{b}	6.26 ± 0.44 ^{b}	6.40 ± 0.02 ^{b}
Rye Sourdoughs	**Control**	**0.5% lees**	**1% lees**	**2% lees**
	Beginning of fermentation (t = 0 days)			
pH	6.29 ± 0.07 ^{a}	6.01 ± 0.03 ^{b}	5.84 ± 0.06 ^{b}	5.63 ± 0.11 ^{b}
Acetic acid (g/L)	<0.03	<0.03	<0.03	<0.03
Lactic acid (g/L)	<0.02	<0.02	<0.02	<0.02
Malic acid (g/L)	0.95 ± 0.03 ^{a}	1.20 ± 0.04 ^{b}	1.550 ± 0.05 ^{b}	2.10 ± 0.02 ^{c}
Citric acid (mg/L)	116.53 ± 8.73 ^{a}	182.41 ± 12.53 ^{a}	250.32 ± 29.51 ^{a}	638.15 ± 72.51 ^{b}
	End of fermentation (t = 8 days)			
pH	4.06 ± 0.02 ^{a}	3.92 ± 0.05 ^{b}	3.93 ± 0.01 ^{b}	3.66 ± 0.01 ^{c}
Acetic acid (g/L)	0.18 ± 0.03 ^{a}	0.49 ± 0.07 ^{b}	0.62 ± 0.04 ^{bc}	0.80 ± 0.08 ^{c}
Lactic acid (g/L)	1.70 ± 0.20 ^{a}	2.55 ± 0.191 ^{b}	3.22 ± 0.18 ^{bc}	3.67 ± 0.18 ^{c}
Malic acid (g/L)	<0.03	<0.03	<0.03	<0.03
Citric acid (mg/L)	<11.00	<11.00	<11.00	<11.00
Fermentation Quotient (FQ)	6.48 ± 0.79 ^{a}	3.53 ± 0.20 ^{b}	3.47 ± 0.23 ^{b}	3.06 ± 0.17 ^{b}

Values are mean ± standard deviation of triplicates. Significant differences between samples are indicated by different superscript letters ($p < 0.05$) for each compound.

With reference to acids at the end of fermentation (8 days), the addition of 0.5% Cava lees had a significant effect on acetic acid and L-lactic acid, with a major change occurring with the addition of 2%, compared to the control. D-lactic acid presented significance when lees were added at a minimum of 1% (w/w), compared to the control. Following the same tendency as wheat sourdoughs, rye fermentations also raised their organic acid production accordingly, with greater cell density and a lower pH, with the addition of Cava lees to their formulation.

Concerning FQ, by adding 0.5% (w/w) Cava lees, it was reduced to half, but there were no significant differences with the addition of higher concentrations of lees.

As previously stated, the microbial association due to their metabolism preferences is very common. As a consequence, the nutrient consumption affects the production of organic acids such as acetic and lactic acids, since yeasts may consume the soluble carbohydrates faster, decreasing LAB acidification because of the microbial competition [37].

Additionally, LAB metabolize malic acid and convert it to lactic acid [38]. In both wheat and rye sourdoughs, L-malic acid increases its concentration when Cava lees are added (Table 2); therefore, Cava lees may be a source of malic acid that might have been adsorbed during Cava ageing. Furthermore, some LAB strains are able to degrade tartrate, a major compound found in wine lees, into lactate and acetate [38]. Therefore, this could explain the increment in lactic acid concentration in sourdoughs with lees.

Citric acid metabolism can also produce acetate (considered an antimicrobial compound), as well as acetoin, diacetyl and butanediol. These compounds are flavor com-

pounds of the bread crumb [39,40]. Hence, a greater concentration of citric acid at the beginning of fermentation may result in an increased amount of these compounds, probably having a positive impact on flavor.

Considering the variables studied, the results obtained were subjected to a PCA (Figure 4) that confirmed the differences between the sourdoughs with and without Cava lees. The PCA shows similar behavior in sourdoughs with the same Cava lees concentration in both wheat and rye, although it can differ across samples according to the flour used to produce them. It can be observed that Component 1 separates the samples according to the percentage of lees added, whereas Component 2, which is equivalent to 22% of the variance, separates the sourdoughs according to the type of flour, wheat or rye. In fact, it shows that sourdoughs with Cava lees have higher LAB cell densities, acetic, citric and lactic acids as well as a lower pH, whereas control sourdoughs are defined with higher pH values, less microbial cell density and lower organic acids concentrations, especially wheat control sourdoughs. Therefore, according to the PCA, the addition of 2% (*w*/*w*) Cava lees to sourdough formulation has the greatest impact in its microbial populations and, consequently, to its physicochemical characteristics.

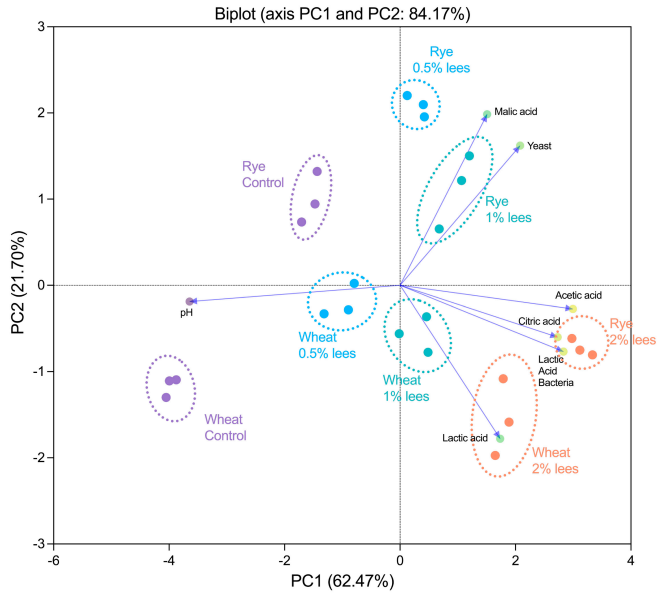

Figure 4. Principal component analysis (PCA) biplot of sourdoughs obtained at the end of fermentation.

4. Conclusions

Cava lees are a wine industry by-product containing several highly valuable compounds such as β-glucans and mannoproteins [1,4], with the potential to modify food-fermenting microbial populations, such as the ones in sourdough.

In this study, with the aim of revalorizing such by-products, it was found that the addition of 2% (*w*/*w*) Cava lees to sourdough formulation improved the growth and survival of LAB and yeasts that carry fermentation, especially in rye sourdough.

In addition, with increased microorganism cell density, there can be a greater production of organic acids and a lower pH, as shown in the PCA. Therefore, it may change sourdough bread flavor as well as other parameters such as texture and shelf life.

Since consumer acceptance is of great value, studies on sourdough bread volatiles and more complete sensory analysis should be conducted. Further studies with higher concentrations of Cava lees should also be considered, as well as the use of lees obtained from different Cava productions (e.g., different ageing times or initial coupages), which could also affect their composition and, consequently, bread flavor.

Author Contributions: Conceptualization, E.L.-T.; investigation, A.M.-G.; writing—original draft preparation, A.M.-G.; writing—review and editing, M.R.-A. and E.L.-T.; supervision, M.R.-A.; project administration, E.L.-T. All authors have read and agreed to the published version of the manuscript.

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PUBLICACIÓ 5

Changes in the Volatile Profile of Wheat Sourdough Produced with the Addition of Cava Lees

Alba Martín-Garcia, Oriol Comas-Basté, Montserrat Riu-Aumatell, Mariluz Latorre-Moratalla, Elvira López-Tamames.

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LES IDEES CLAU

L'addició de lies del Cava va resultar en un increment en la concentració de compostos volàtils (alcohols, àcids, aldehids, cetones i esters), sobretot a una concentració del 2% de lies. Addicionalment, les lies van contribuir a l'aroma de la massa mare amb compostos típics del Cava.

molecules

MDPI

Article

Changes in the Volatile Profile of Wheat Sourdough Produced with the Addition of Cava Lees

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Abstract: The volatile fraction is of great importance for the organoleptic quality and consumer acceptance of bread. The use of sourdough improves the sensory profile of bread, as well as the addition of new ingredients to the fermentation. Cava lees are a sparkling wine by-product formed of dead microorganisms, tartaric acid, and other inorganic compounds, rich in antioxidant compounds as well as β-glucans and mannoproteins. The aim of this study was to evaluate the effect of different concentrations of Cava lees (0–2% *w*/*w*) on sourdough volatile compounds to re-valorize this by-product of the wine industry. Headspace solid-phase microextraction (HS-SPME) was optimized to study the volatile fractions of sourdoughs. The parameters selected were 60 °C, 15 min of equilibrium, and 30 min of extraction. It was found that the addition of Cava lees resulted in higher concentrations of volatile compounds (alcohols, acids, aldehydes, ketones and esters), with the highest values being reached with the 2% Cava lees. Moreover, Cava lees contributed to aroma due to the compounds usually found in sparkling wine, such as 1-butanol, octanoic acid, benzaldehyde and ethyl hexanoate.

Keywords: sourdough; HS-SPME-GC-MS; volatile compounds; Cava lees; wine by-product

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1. Introduction

Sourdough is the result of fermenting a mixture of flour and water, and it is traditionally used during bread making as a leavening agent, influencing bread quality [1,2]. This process takes place by the action of the lactic acid bacteria (LAB) and yeasts present in flour, and can occur either by the addition of a starter culture or by spontaneous fermentation. The metabolic activity of the bacteria leads to acidification and flavor formation, improving nutritional and sensory characteristics in addition to increasing microbiologic stability and shelf life [3].

The volatile profile is very significant for the organoleptic quality and consumer acceptance of bread. More than 500 volatile compounds have been reported in bread [4], while sourdough (and sourdough bread) volatiles have been less studied, with less than 200 compounds having been identified [2]. Several research articles have been published in which headspace solid-phase microextraction (HS-SPME) has been used to study the volatile fraction of sourdough [5–8]. Nonetheless, there is no common base methodology.

Moreover, sourdough bread flavor strongly depends on the fermenting microbiota that produces a range of secondary metabolites, as well as on the enzymatic and autoxidation of flour lipids, and the Maillard reaction [1–3]. In addition, several bacteria and yeast strains not only produce desirable volatile compounds, but also release aromatic precursors, and

some are able to degrade undesirable compounds [7,9]. In fact, researchers are studying the use of different flours (i.e., chickpea, lentil, bean and hemp) and the addition of new ingredients (i.e., broccoli by-products and brewers' spent grain) in sourdough formulation that can improve its fermentation and the effect on sourdough and bread characteristics [7,10–13]. On that account, our research group has been focused on the valorization of wine by-products as new ingredients in bakery products. We found that the use of Cava lees in wheat and rye sourdough promoted the growth and survival of LAB and yeast in spontaneous fermentation [14].

Lees are a residue formed during the ageing process of Cava (Spanish sparkling wine) and consist, mostly, of dead microorganisms (generally *Saccharomyces cerevisiae*), tartaric acid, and other adsorbed compounds [15,16]. They are rich in antioxidant compounds [16,17] as well as dietary fiber from the yeast cell wall that is composed of mannoproteins and branched β-glucans [18,19]. Nowadays, Cava lees are produced at an amount of 300 tons per year, representing 25% of the waste generated by the wine industry [17]. Although some studies have reported that wine lees could acquire an added value due to their composition [18,20–23], they are actually destined for distillation. Moreover, there is an increasing tendency in the food industry towards reducing food waste and re-valorizing by- and co-products to contribute to a circular economy and sustainable food production [10,13,21,24].

The addition of Cava lees in the formulation of sourdough could have an important effect on the fermenting microbiota and, hence, in the volatile profiles of these products. Therefore, the aim of this study was to evaluate the impact of Cava lees on sourdough volatile compounds by an optimized method of HS-SPME-GC-MS.

2. Results

The addition of Cava lees to sourdough formulation may change the volatile profiles of such products. Hence, this study focused on the impact of different concentrations of Cava lees on the volatile fraction of wheat sourdough. Since there is no common base methodology for the extraction of volatile compounds in sourdough [5–8], a previous optimization of the HS-SPME parameters was performed.

2.1. Optimization of Headspace Solid-Phase Microextraction (HS-SPME) Parameters

Figure 1 shows the total number (TN) of volatile compounds and total area (TA) of volatile compounds identified by GC-MS analysis as a result of the modification of the extraction parameters. Figure 2 shows the TN of the different chemical families (acids, alcohols, aldehydes and ketones, and esters) identified.

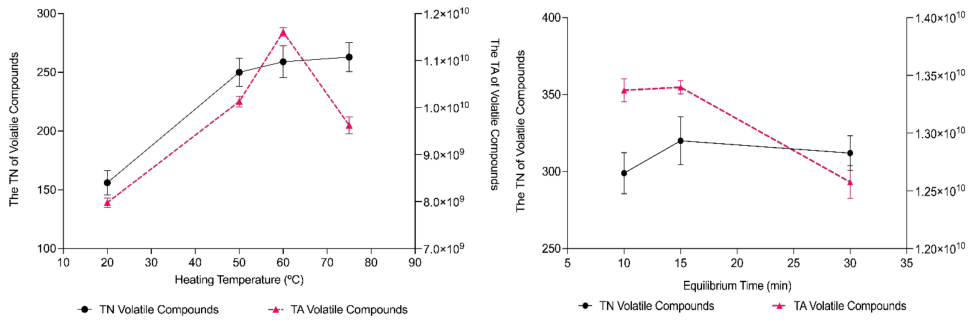
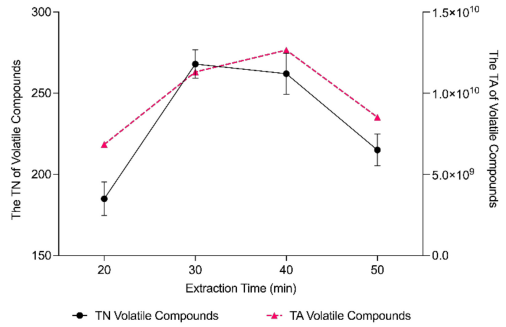
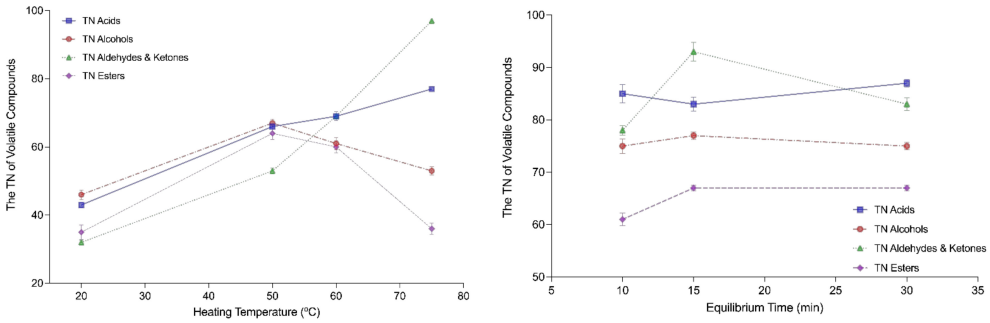


Figure 1. *Cont.*

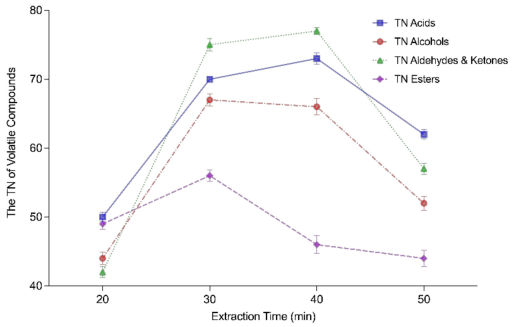


(**c**)

Figure 1. The effect of different extraction parameters of HS-SPME on the total number (TN) and total area (TA) of volatile compounds in sourdough: heating temperature (**a**); equilibrium time (**b**); extraction time (**c**).



(**a**) (**b**)



(**c**)

Figure 2. Effect of different extraction parameters of HS-SPME on the total number (TN) of compounds of the different chemical families in sourdough: heating temperature (**a**); equilibrium time (**b**); extraction time (**c**).

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2.1.1. Effect of Heating Temperature

To evaluate the effect of the heating temperature, four temperatures were selected: 20 °C, 50 °C, 60 °C, and 75 °C. The impact of heating temperature on the extraction of the volatile compounds from sourdough is shown in Figures 1a and 2a. The TN and TA of the compounds increased with temperature (Figure 1a), although there were no significant differences between the TN of compounds extracted between 50 °C (250 ± 12 identified compounds), 60 °C (259 ± 13 identified compounds), and 75 °C (263 ± 12 identified compounds). However, Figure 2a shows that when compounds were separated by chemical families, the TN did not increase with temperature for all of them. Acids, aldehydes and ketones increased with temperature. Alcohols and esters decreased, reaching the maximum performance in the TN of volatiles extracted at 50 °C (67 ± 1.2 and 64 ± 1.0 compounds, respectively), although there were no significant differences ($p > 0.05$) between 50 °C and 60 °C (63 ± 1.2 (alcohols) and 60 ± 1.0 (esters)). Since 60 °C was the temperature at which the number of compounds extracted was higher, it was the selected temperature for the HS-SPME in sourdough.

2.1.2. Effect of Equilibrium Time

For the optimization of the HS-SPME method, three periods of time were assessed for the equilibration of the samples: 10 min, 15 min and 30 min (Figures 1b and 2b). It can be observed that the equilibrium times before extraction did not lead to any significant differences in the TN identified, in general or when separating between chemical families. In view of the cost of time, an equilibrium time of 15 min was chosen as the optimal amount of time sufficient to extract the volatile compounds of the sourdough.

2.1.3. Effect of Extraction Time

Four periods of time were tested for extraction: 20 min, 30 min, 40 min and 50 min. The TN and TA of the volatile compounds extracted depending on extraction time are shown in Figure 1c. Figure 2c shows the TN of each chemical family according to different times of extraction. There was an increase in the TN of volatiles extracted when increasing the extraction time, although there was a decrease in those numbers with 50 min of extraction (Figures 1c and 2c). Therefore, the optimal extraction time was considered to be between 30 min and 40 min, which were the periods of time that showed the maximum number of volatiles identified (268 ± 8.7 and 262 ± 12.7 identified compounds, respectively). When observing the impact of time on each chemical family, the effect was similar on all of them, except for esters, which peaked at 30 min (56 ± 0.7 compounds) and began decreasing at 40 min (46 ± 1.2 compounds). Regarding the other compounds, there were no significant differences between the TN of compounds extracted at 30 min and 40 min.

As shown in Figure 3, at a lower temperature (20 °C) and shorter extraction time (20 min), the TN of components extracted was significantly reduced (33 ± 1.4 compounds) compared to the same temperature with a longer extraction time of 40 min (48 ± 1.8 compounds). Nonetheless, when the extraction time was too long (50 min), the TN of components was also lower (29 ± 1.2 compounds). The same trend was observed for all the other studied temperatures (50 °C, 60 °C and 75 °C). For the selected temperature of 60 °C, the TN increased from 36 ± 0.7 (20 min extraction) to 79 ± 0.9 (30 and 40 min) compounds. However, when the time of extraction was 50 min, the TN compounds identified decreased (65 ± 0.5). Therefore, the extraction time selected was 30 min in order to obtain the highest number of volatiles extracted from each chemical family with the shortest amount of time possible.

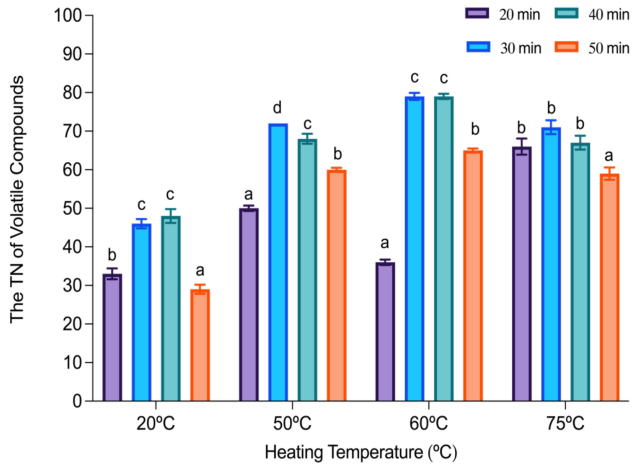

Figure 3. The total number (TN) of volatile compounds regarding heating temperature (°C) and extraction time (min). Different letters denote statistically significant differences ($p < 0.05$) between different times of extraction for each temperature.

2.2. Analysis of Volatile Compounds in Different Sourdough Samples

The effect of different percentages of Cava lees on the volatile profile were assessed following the optimized HS-SPME method (60 °C, 15 min, 30 min). The volatile compounds of Cava lees were also analyzed by HS-SPME. During the sourdough fermentation, volatile compounds such as alcohols, acids, aldehydes, ketones, and esters were formed (Table 1).

Table 1. Concentration (mg/kg) of total volatile compounds classified by the chemical family obtained with the optimized HS-SPME method in Cava lees and sourdough.

Family Compound	Cava Lees	Sourdough			
		Control	0.5% Cava Lees	1% Cava Lees	2% Cava Lees
Alcohols	95.14 ± 8.92	158.66 ± 28.81 ^{a}	283.87 ± 39.69 ^{ab}	505.29 ± 80.88 ^{b}	923.39 ± 150.02 ^{c}
Acids	143.33 ± 14.45	612.72 ± 39.37 ^{a}	386.83 ± 99.92 ^{a}	568.27 ± 132.07 ^{a}	1008.03 ± 69.33 ^{b}
Aldehydes	5.19 ± 1.77	160.44 ± 13.34 ^{a}	189.96 ± 12.18 ^{a}	165.14 ± 8.61 ^{a}	234.31 ± 19.95 ^{b}
Ketones	2.01 ± 0.58	11.74 ± 3.95 ^{a}	11.59 ± 5.41 ^{a}	32.96 ± 7.27 ^{b}	44.51 ± 4.19 ^{b}
Esters	489.38 ± 60.34	373.08 ± 44.02 ^{a}	1136.39 ± 268.77 ^{b}	3593.89 ± 737.88 ^{c}	7514.18 ± 764.37 ^{d}

Values are mean ± standard deviation of triplicates. Significant differences between sourdoughs are indicated by different superscript letters ($p < 0.05$) for each family compound.

Overall, the control and 0.5% Cava lees samples showed no significant differences in the concentration of volatile compounds reported, except for esters ($p < 0.05$). As a general rule, with higher amounts of Cava lees added to the sourdough formulation, there was a greater production of volatile compounds, especially alcohols and esters ($p < 0.05$). In fact, Cava lees were characterized by esters (489.38 ± 60.34 mg/kg).

2.2.1. Alcohols

The concentration of alcohols (Table 1) increased with the addition of lees, with values ranging between 158.66 ± 28.81 mg/kg (control) and 923.39 ± 150.02 mg/kg (2% Cava lees). The main alcohols identified in sourdough were 1-butanol, 1-pentanol, 1-hexanol, 1-octen-3-ol, 1-heptanol, 1-octanol, 1-nonanol, and 2-ethylhexanol (Table 2).

Table 2. Concentration (mg/kg) of the main alcohols obtained with the optimized extraction parameters in Cava lees and sourdough.

	Compound	CAS Num.	Odor [1]	ODT [2]	Cava Lees	Sourdough			
						Control	0.5% Cava Lees	1% Cava Lees	2% Cava Lees
1	1-Butanol	71-36-3	medicinal, fruit, wine	500	nd	27.53 ± 9.86 ^{a}	58.52 ± 9.06 ^{ab}	148.09 ± 36.70 ^{b}	390.76 ± 66.00 ^{c}
2	1-Pentanol	71-41-0	green, fruit, balsamic	4000	29.74 ± 4.62	6.62 ± 1.73 ^{a}	16.04 ± 6.87 ^{ab}	41.53 ± 6.72 ^{c}	82.42 ± 6.64 ^{d}
3	1-Hexanol	111-27-3	sweet, resin, flower	2500	52.76 ± 3.22	76.17 ± 7.93 ^{a}	126.41 ± 11.94 ^{a}	165.10 ± 21.78 ^{ab}	249.65 ± 29.86 ^{c}
4	1-Octen-3-ol	2291-86-4	mushroom, earthy	1	nd	20.49 ± 0.96 ^{a}	23.28 ± 1.70 ^{a}	25.63 ± 2.21 ^{a}	24.54 ± 4.30 ^{a}
5	1-Heptanol	111-70-6	herb, mushroom, chemical, green	3	nd	17.3 ± 2.88 ^{a}	21.91 ± 2.39 ^{ab}	28.18 ± 1.56 ^{bc}	29.47 ± 2.19 ^{cd}
6	1-Octanol	111-87-5	moss, nut, mushroom, chemical	110–130	12.32 ± 1.03	5.12 ± 2.73 ^{a}	25.30 ± 2.37 ^{ab}	62.66 ± 6.47 ^{bc}	100.57 ± 30.93 ^{cd}
7	1-Nonanol	143-08-8	fat, green, oily, floral	50	nd	2.60 ± 2.59 ^{a}	9.64 ± 3.49 ^{ab}	24.63 ± 4.32 ^{bc}	34.69 ± 8.16 ^{cd}
8	2-Ethylhexanol	104-76-7	citrus, fatty	na	0.32 ± 0.05	2.83 ± 0.13 ^{a}	2.77 ± 1.87 ^{ab}	9.47 ± 1.12 ^{c}	11.29 ± 1.94 ^{cd}

[1] From [25]. [2] ODT: Odor Detection Threshold (in water) from [26]. Expressed as mg/kg. Values are mean ± standard deviation of triplicates. Significant differences between sourdoughs are indicated by different superscript letters ($p < 0.05$) for each compound. nd: not detected; na: not available.

Most of the alcohols identified in the sourdough samples increased their concentration with the addition of Cava lees, reaching the highest values at 2% Cava lees ($p < 0.05$). The dominant alcohol quantified was 1-hexanol in sourdoughs formulated with and without Cava lees. Additionally, 1-butanol was the dominant alcohol in sourdoughs with 2% Cava lees (249.65 ± 29.86 mg/kg).

2.2.2. Acids

The total concentration of acids ranged between 386.83 ± 99.92 mg/kg and 1008.03 ± 69.33 mg/kg, depending on the Cava lees percentage in the sourdough. A total of 12 different acids were found in the sourdough samples (Table 3): acetic, butanoic, pentanoic, hexanoic, heptanoic, octanoic, nonanoic, decanoic, benzoic, tetradecanoic, hexadecenoic, and octadecanoic acid.

Although butanoic acid (269.41 ± 9.36 mg/kg) was the most prevalent acid in the control sourdough, its concentration decreased with the addition of Cava lees, and was not detected in the 2% Cava lees sourdough. Pentanoic and heptanoic acid followed the same trend, being identified in the control sourdough but not in the sourdoughs with Cava lees. The opposite occurred to acetic acid, with a lower concentration in the control sourdough (66.08 ± 9.99 mg/kg) that increased with lees, reaching values of 246.10 ± 14.64 mg/kg with the 2% Cava lees.

2.2.3. Aldehydes and Ketones

The aldehydes identified in sourdough (Table 4) were hexanal, benzaldehyde, nonanal, (E)-2-heptenal, (E)-2-octenal, and decanal. In the control and 0.5% lees sourdoughs, the most prevalent aldehyde was (E)-2-octenal (72.65 ± 3.32 mg/kg and 75.18 ± 5.44 mg/kg, respectively), while in 1% and 2% lees sourdoughs this aldehyde was not detected. Generally, hexanal, nonanal and decanal increased their concentration with the addition of Cava lees, adding 1% to 2% (*w*/*w*). The ketones quantified in this study were acetoin and 2-undecanone, representing 1% of the total volatile fractions in all samples (Table 1). Acetoin was the main ketone identified in all samples.

2.2.4. Esters

Esters were the most prevalent compounds in all types of sourdough (Table 1), especially when Cava lees were added, with values ranging between 373.40 mg/kg in the control sourdough (33% of the total volatile compounds) and 7514.18 mg/kg in the 2% Cava lees sourdough (77% of the total volatile compounds). The esters present in the sourdough (Table 5) were butyl acetate, butyl butyrate, butyl hexanoate, butyl benzoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl laurate, ethyl palmitate, hexyl acetate, hexyl butyrate, octyl acetate, and decyl acetate. The most prevalent esters found in the sourdough samples were butyl butyrate (96.76 ± 3.36 mg/kg–689.04 ± 12.35 mg/kg) and ethyl decanoate (80.07 ± 15.41 mg/kg–3,330.26 ± 314.82 mg/kg) in all sourdoughs. Moreover, the sourdoughs with Cava lees presented high concentrations of ethyl octanoate (170.91 ± 94.63 mg/kg–2,629.71 ± 316.18 mg/kg).

Table 3. Concentration (mg/kg) of main acids obtained with the optimized extraction parameters in Cava lees and sourdough.

	Compound	CAS Num.	Odor [1]	ODT [2]	Cava Lees	Sourdough			
						Control	0.5% Cava Lees	1% Cava Lees	2% Cava Lees
9	Acetic acid	64-19-7	pungent, sour	na	32.58 ± 1.24	66.08 ± 9.99 ^{a}	132.11 ± 46.11 ^{a}	168.78 ± 78.48 ^{a}	246.10 ± 14.64 ^{ab}
10	Butanoic acid	107-92-6	sweaty, rancid	240	nd	269.41 ± 9.36 ^{a}	80.43 ± 14.13 ^{b}	29.55 ± 4.26 ^{c}	nd
11	Pentanoic acid	109-52-4	-	3000	nd	6.18 ± 2.04	nd	nd	nd
12	Hexanoic acid	142-62-1	-	3000	nd	55.56 ± 2.09 ^{ab}	31.60 ± 9.58 ^{a}	32.39 ± 3.08 ^{a}	41.73 ± 5.97 ^{ab}
13	Heptanoic acid	111-14-8	-	3000	nd	8.28 ± 1.23	nd	nd	nd
14	Octanoic acid	124-07-2	oily, rancid	3000	49.45 ± 4.98	8.72 ± 3.18 ^{a}	48.40 ± 22.33 ^{ab}	135.98 ± 13.91 ^{c}	253.58 ± 22.36 ^{d}
15	Nonanoic acid	112-05-0	fatty, mild, nutlike	3000	nd	3.03 ± 0.80 ^{a}	17.78 ± 3.17 ^{b}	28.21 ± 7.51 ^{b}	40.99 ± 1.93 ^{c}
16	Decanoic acid	334-48-5	sour, fatty	10,000	31.68 ± 5.84	nd	23.27 ± 1.14 ^{a}	85.70 ± 11.43 ^{b}	209.27 ± 4.98 ^{c}
17	Benzoic acid	1863-63-4	-	na	nd	1.33 ± 0.05 ^{a}	0.61 ± 0.33 ^{b}	1.50 ± 0.02 ^{a}	1.56 ± 0.04 ^{a}
18	Tetradecanoic acid	544-63-8	waxy, oily, faint	10,000	nd	5.55 ± 0.17 ^{a}	nd	11.37 ± 1.36 ^{b}	29.36 ± 4.98 ^{c}
19	Hexadecanoic acid	57-10-3	-	10,000	29.62 ± 2.39	8.57 ± 5.82 ^{a}	52.63 ± 3.13 ^{bc}	74.79 ± 12.02 ^{bc}	164.35 ± 10.79 ^{d}
20	Octadecanoic acid	57-11-4	-	20,000	nd	nd	nd	nd	21.09 ± 3.64

[1] From [25]. [2] ODT: Odor Detection Threshold (in water) from [26]. Expressed as mg/kg. Values are mean ± standard deviation of triplicates. Significant differences between sourdoughs are indicated by different superscript letters ($p < 0.05$) for each compound. nd: not detected; na: not available.

Table 4. Concentration (mg/kg) of main aldehydes and ketones obtained with the optimized extraction parameters in Cava lees and sourdough.

	Compound	CAS Num.	Odor [1]	ODT [2]	Cava Lees	Sourdough			
						Control	0.5% Cava Lees	1% Cava Lees	2% Cava Lees
21	Hexanal	66-25-1	fatty, green, grassy	4.5–5	nd	45.32 ± 3.11 ^{a}	50.85 ± 1.73 ^{ab}	60.55 ± 2.55 ^{bc}	69.19 ± 5.00 ^{bc}
22	Acetoin	513-86-0	butter, cream	800	nd	11.74 ± 3.95 ^{a}	8.28 ± 4.44 ^{a}	24.09 ± 3.99 ^{b}	29.29 ± 1.59 ^{b}
23	Benzaldehyde	100-52-7	cherry, candy	350–3500	nd	nd	nd	0.55 ± 0.08	2.59 ± 0.34
24	2-Undecanone	112-12-9	citrus, rose, iris	7	2.01 ± 0.58	nd	3.31 ± 0.98 ^{a}	8.86 ± 3.28 ^{a}	14.86 ± 2.60 ^{b}
25	Nonanal	124-19-6	piney, floral, citrusy, fat	1	3.21 ± 1.10	9.94 ± 2.65 ^{a}	20.08 ± 1.85 ^{ab}	46.65 ± 3.08 ^{c}	88.28 ± 9.06 ^{d}
26	(E)-2-Heptenal	18829-55-5	green, sweet, fresh, fruity, apple	13	nd	nd	5.13 ± 0.72 ^{a}	6.71 ± 0.55 ^{a}	7.41 ± 0.92 ^{ab}
27	(E)-2-Octenal	2548-87-0	green, nut, fat, leaf, walnut	3	nd	72.62 ± 3.32 ^{a}	75.18 ± 5.44 ^{a}	nd	nd
28	Decanal	112-31-2	beefy, musty, marine, cucumber	0.1–2	1.98 ± 0.67	32.56 ± 4.27 ^{a}	38.72 ± 2.43 ^{ab}	50.68 ± 2.35 ^{c}	66.84 ± 4.63 ^{d}

[1] From [25]. [2] ODT: Odor Detection Threshold (in water) from [26]. Expressed as mg/kg. Values are mean ± standard deviation of triplicates. Significant differences between sourdoughs are indicated by different superscript letters ($p < 0.05$) for each compound. nd: not detected.

Table 5. Concentration (mg/kg) of major esters obtained with the optimized extraction parameters in Cava lees and sourdough.

	Compound	CAS Num.	Odor [1]	ODT [2]	Cava Lees	Sourdough			
						Control	0.5% Cava Lees	1% Cava Lees	2% Cava Lees
29	Butyl acetate	123-86-4	sweet, ripe, fruity, green	66	nd	31.01 ± 5.70 ^{a}	43.39 ± 4.03 ^{b}	55.62 ± 3.84 ^{b}	73.55 ± 4.90 ^{c}
30	Butyl butyrate	109-21-7	fruity, sweet	100	nd	96.76 ± 3.36 ^{a}	222.68 ± 41.42 ^{b}	597.88 ± 36.26 ^{c}	689.04 ± 12.35 ^{d}
31	Butyl hexanoate	626-82-4	fruity, winey, berry, green	700	nd	67.34 ± 10.18 ^{a}	63.24 ± 3.82 ^{a}	76.95 ± 3.75 ^{a}	79.26 ± 2.87 ^{a}
32	Butyl benzoate	136-60-7	amber, balsamic, fruity	na	nd	56.68 ± 2.10 ^{a}	69.93 ± 6.29 ^{b}	82.88 ± 3.76 ^{c}	91.73 ± 2.23 ^{c}
33	Ethyl hexanoate	123-66-0	fruity	70–84	nd	nd	31.66 ± 5.89 ^{a}	49.81 ± 9.52 ^{a}	100.75 ± 14.96 ^{b}
34	Ethyl octanoate	106-32-1	fruity, floral	na	139.63 ± 12.67	6.98 ± 1.65 ^{a}	170.91 ± 94.63 ^{a}	1503.39 ± 275.45 ^{b}	2,629.71 ± 316.18 ^{c}
35	Ethyl decanoate	110-38-3	sweet, oily, nutlike	na	250.07 ± 17.95	80.07 ± 15.41 ^{a}	404.93 ± 79.33 ^{ab}	930.25 ± 351.91 ^{b}	3,330.26 ± 314.82 ^{c}
36	Ethyl laurate	106-33-2	sweet, waxy, creamy, floral	na	41.33 ± 13.46	nd	32.54 ± 15.66 ^{a}	70.19 ± 16.67 ^{a}	189.99 ± 48.46 ^{b}
37	Ethyl palmitate	628-97-7	waxy, fruity, creamy, vanilla, balsamic	>2000	13.59 ± 1.58	4.75 ± 1.58 ^{a}	5.14 ± 2.49 ^{a}	21.33 ± 13.77 ^{a}	60.40 ± 6.59 ^{b}
38	Hexyl acetate	142-92-7	sweet, fruity, herb	2	24.79 ± 8.45	3.99 ± 1.24 ^{a}	42.61 ± 2.97 ^{b}	88.99 ± 8.01 ^{c}	115.10 ± 7.22 ^{d}
39	Hexyl butyrate	2639-63-6	green, fruity, vegetable, waxy	250	nd	25.50 ± 2.80 ^{a}	41.99 ± 8.76 ^{a}	79.49 ± 8.90 ^{b}	104.75 ± 19.55 ^{bc}
40	Octyl acetate	112-14-1	fruity, fatty	12	nd	nd	1.75 ± 0.80 ^{a}	19.87 ± 4.41 ^{b}	49.70 ± 6.24 ^{c}
41	Decyl acetate	112-17-4	sweet, fatty, fruity	na	19.97 ± 6.23	nd	5.62 ± 2.67 ^{a}	17.25 ± 1.63 ^{b}	29.94 ± 8.00 ^{c}

[1] From [25]. [2] ODT: Odor Detection Threshold (in water) from [26]. Expressed as mg/kg. Values are mean ± standard deviation of triplicates. Significant differences between sourdoughs are indicated by different superscript letters ($p < 0.05$) for each compound. nd: not detected; na: not available.

The results obtained were subjected to a PCA to group the different sourdoughs produced based on their similarities or differences in the volatile fraction. Figure 4 shows the result of a previous correlation analysis and Figure 5 presents the PCA biplot obtained. It can be observed that, in general, most compounds had a positive correlation between them, except for the SCFAs (butanoic, pentanoic and hexanoic acids) and heptanoic acid (Figure 4). These compounds were also the ones that characterized the control sourdoughs (Figure 5). Indeed, samples were grouped according to the concentration of Cava lees added to the formulation. The PC1 and PC2 explained 84.40% of the total variability. The first principal component (PC1) explained 75.26% of the samples variances while the second one (PC2) explained 9.15%. All volatile compounds were found on the positive side of PC1, except for butanoic, pentanoic, hexanoic, and heptanoic acids (short- and medium-chain fatty acids), and 2-undecanone (ketone) and (E)-2-Octenal (aldehyde). These compounds were considered to characterize the control and 0.5% Cava lees sourdoughs. On the other hand, it can be observed that the sourdoughs with 1% and 2% Cava lees had the highest concentrations of all volatile compounds, especially esters. PC2 showed a positive correlation with alcohols, aldehydes and ketones, whereas the negative axis contained esters mainly characterizing sourdoughs formulated with 2% lees and acids.

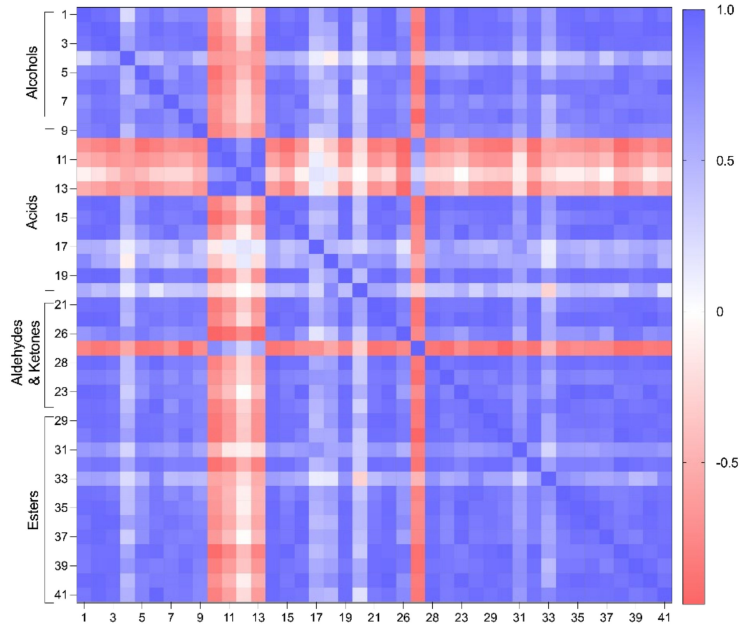

Figure 4. Heatmap of the correlation matrix of the volatile compounds ($p < 0.05$). Numbers in loadings correspond to the volatile compounds identified in sourdoughs: 1–8 alcohols (Table 2); 9–20 acids (Table 3); 21–28 aldehydes and ketones (Table 4); and 29–41 esters (Table 5). Positive correlations are shown in blue; negative correlations in red; absence of correlation in white.

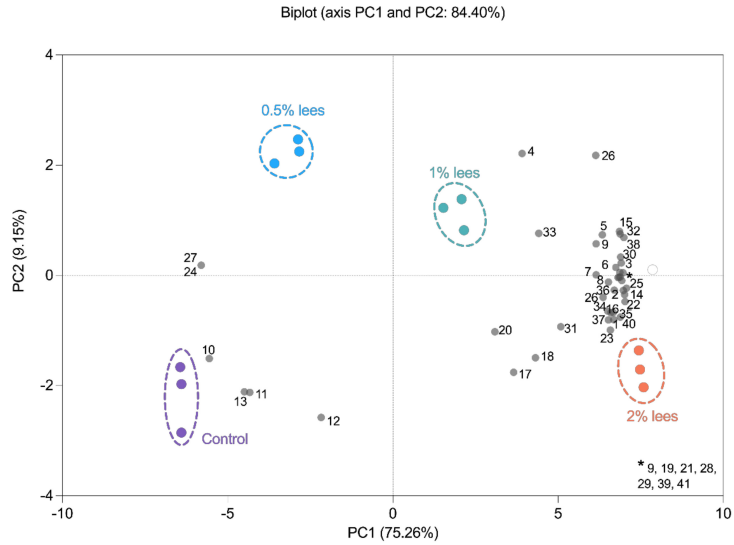

Figure 5. Principal component analysis (PCA) loadings for 41 volatile compounds (grey) and scores for the different sourdoughs at the end of the fermentation period (purple—control; blue—0.5% Cava lees; green—1% Cava lees; and orange—2% Cava lees). Numbers in loadings correspond to the volatile compounds identified in sourdoughs: 1–8 alcohols (Table 2); 9–20 acids (Table 3); 21–28 aldehydes and ketones (Table 4); and 29–41 esters (Table 5).

3. Discussion

3.1. Optimization of Headspace Solid-Phase Microextraction (HS-SPME) Parameters

As previously stated, although multiple studies have used HS-SPME to extract and analyze the volatile fraction of sourdough [5–8], there is no common base methodology. Consequently, we conducted an optimization process for the HS-SPME parameters. This included the selection and evaluation of three parameters that influence extraction: heating temperature, equilibrium time, and extraction time.

Overall, the higher the TN of compounds identified, the greater the TA and concentration of the compounds will likely be [27], as can be observed in Figure 1. Moreover, the increase in heating temperature resulted in a greater composition (TN) and content (TA) of volatilized compounds. This temperature rise could have the ability to facilitate the volatilization of the molecules from the sourdough matrix, improving the vapor pressure and diffusion coefficients of the analytes being absorbed by the fiber coating [27,28].

Before extraction, samples are usually equilibrated for a period of time to enable molecules into the headspace, which leads to a potentially greater recovery of the compounds [27,29]. Three sets of time were tested (10 min, 15 min, and 30 min), although there were no statistically significant differences between them.

Lastly, extraction time is of importance since it is the time that compounds need to reach equilibrium between the headspace and the fiber [29]. In fact, longer extraction times can be beneficial, with more analytes occupying more sites on the fiber, but exceeding these times may trigger a desorption of the analytes [27,28]. Additionally, extraction temperature and time are closely related [29], since increasing extraction temperature can accelerate the volatilization of compounds; consequently, extraction time can be reduced. In fact, a three-factor analysis was performed on the results obtained from the optimization to observe any possible interactions between the variables (temperature, equilibrium, and extraction time).

It was found that temperature and extraction time presented an interaction, being related to one another in accordance with Garvey et al. (2020) [28]. On that account, the selected HS-SPME parameters for the extraction of volatile compounds in wheat sourdough were 60 °C, 15 min of equilibrium, and 30 min of extraction.

3.2. Analysis of Volatile Compounds in Different Sourdough Samples

Volatile compounds in sourdough are developed during the fermentation process, and many come from precursors such as carbohydrates and amino acids. Lipid oxidation also produces aldehydes and ketones from the decomposition of triglycerides and fatty acids. Additionally, LAB also release aroma precursors, such as amino acids that can be transformed into aldehydes or the corresponding alcohols [30].

It was observed that, with the addition of Cava lees, there was a greater production of volatile compounds (Table 1), including the products of microbial metabolism. In fact, previous studies have focused on the effect of Cava lees on the growth and survival of LAB, concluding that they have a positive effect on the fermenting microbiota [14,20,21]. Therefore, higher microbial populations in formulations with Cava lees might induce greater concentrations of volatile compounds as a consequence of LAB and yeast fermentation in sourdough.

3.2.1. Alcohols

Alcohols can be produced by both sugar fermentation (short-chain alcohols) and amino acid metabolism (long-chain alcohols) [30,31], and are usually characterized by green and herbaceous odor notes [32]. In fact, microbial amino acid metabolism may be increased during the back-slopping steps of fermentation as a protection against acidic stress and to maintain the redox balance, transforming peptides and amino acids into higher alcohols [33].

The dominant alcohol quantified was 1-hexanol in the control sourdoughs and in the sourdoughs formulated with Cava lees (Table 2). 1-Hexanol is usually one of the dominant alcohols produced in sourdough [5,7,8], as well as in sparkling wines [31,34–36], along with the other alcohols reported in this study, such as 1-pentanol, 1-octanol and 2-ethylhexanol [32,35]. It contributes odors of cut grass, sweetness, resin, flowers and green, and it originates from fermentation and lipid oxidation (linoleic and linolenic acids) [2,5]. Actually, 1-hexanol was also identified in the lees samples (Table 2), which can support the fact that Cava lees seem to retain volatile compounds on their surface during the biological ageing process [15,36]. In addition, it has been reported that heterofermentative bacteria produce a greater quantity of hexanol than homofermentative LAB [5,31,37,38]. In fact, Liu et al. (2020) [38] proposed that facultatively heterofermentative LAB, such as *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*), can produce 1-hexanol via pathways other than the reduction of hexanal and that it can facilitate the production of hexanal, resulting in more substrate to transform into the corresponding alcohol.

1-Butanol was the dominant alcohol in sourdoughs with 2% Cava lees (249.65 ± 29.86 mg/kg). It can be observed that the mentioned compound increases its concentration by the addition of Cava lees. This higher alcohol has been reported in wine fermentation [39,40] and is also commonly found in sparkling wines [32,35,36,41].

In summary, most of the alcohols identified in the sourdough samples increased their concentration with the addition of Cava lees (Table 1). In addition, the alcohols found in Cava lees increased their concentration in sourdoughs formulated with lees. The highest values were reached at 2% Cava lees ($p < 0.05$), which may be due to higher survival rates among the microorganisms fermenting the sourdough. In fact, it has recently been reported that Cava lees have a growth-promoting effect on different species of LAB in vitro and in sourdough [14,20].

3.2.2. Acids

Acids are produced during fermentation throughout the catabolism of long-chain fatty acids [31]. The total concentration of acids was higher with the addition of 2% Cava lees ($p < 0.05$) (Table 1). In general, high concentrations of organic acids exhibit antimicrobial activity, contributing to the extended shelf-life of bread made with sourdough [3]. In this sense, acetic acid in sourdough has a positive effect because, besides improving its sensory properties, it also possesses anti-ripeness and anti-mold activity [5]. In the same manner, it has also been observed in sparkling wine that acids tend to increase in concentration during biological ageing in contact with lees [36].

Moreover, there are acids that were not detected in the control sourdoughs that increased in concentration with the addition of lees, as was the case of decanoic acid (Table 3). Decanoic acid has been reported as a major volatile compound found in wine lees surfaces [42]. We identified this compound in Cava lees along with other organic acids, such as acetic, octanoic, dodecanoic, and hexadecanoic acid (Table 3). It can be observed in Table 3 that all of these compounds increased in concentration in the sourdoughs formulated with lees. Since Cava lees can promote the growth and survival of sourdough microbiota [30], this increase may be a consequence of a higher production of microbial metabolites coming from the lees surface, since they are able to retain certain volatile compounds [15,42].

Nevertheless, short-chain fatty acids (SCFAs) (butyric and pentanoic acids) decreased in concentration with the addition of lees. In fact, pentanoic acid was not detected in the sourdoughs with Cava lees, even though both SCFAs are volatiles of fermentation origin that have been reported in wheat sourdough [2,7]. Indeed, butyric acid has been associated with the metabolism of acetic bacteria (such as *Acetobacter cerevisiae*) [7]. Since the sourdoughs produced were fermented spontaneously and analyzed shortly after microbial stabilization, it may be assumed that a greater presence of wild bacterial strains may be conditioned by the addition of lees.

3.2.3. Aldehydes and Ketones

Aldehydes are formed by unsaturated fatty acid decarboxylation as well as lipid oxidation [2,5,7,31]. Hexanal was one of the dominant aldehydes in this study (Table 4). It produces fatty, green, grassy, powerful, and tallow odors and has an odor threshold in water of 4.5–5 ppb [2]. Although lipid oxidation products such as hexanal have been reported several times and in high concentrations in bread crumbs, they generally produce off-flavors [43].

Nonanal, an aldehyde that has also been reported in sparkling wine [32], showed the greatest increment in samples with lees compared to the control, increasing eight times its value when 2% Cava lees (w/w) were added to the sourdough formulation. Moreover, benzaldehyde was only identified in sourdoughs with 1% and 2% Cava lees. It is an aldehyde commonly found in sparkling wine [31,32] and it has been reported in other foodstuff formulated with wine lees [23]. Nevertheless, the absence of certain aldehydes or their low production may be explained by the ability of heterofermentative LAB to reduce aldehydes to other compounds [37,38].

Regarding ketones, acetoin was the main one in all samples (Table 4). Acetoin is a key aroma in bread formed during fermentation, with a positive correlation with wheat bread; therefore, the higher the concentration, the better the acceptance by consumers [43]. It is characterized by a buttery and creamy odor and comes from the bacterial conversion of citrate into pyruvate, which then results in acetoin in order to equilibrate the redox balance of the cell metabolism [44].

As for 2-undecanone, this ketone has only been reported once in gluten-free hemp-enriched sourdough bread [11], but it has been identified in wine as well [45–47]. In this study, 2-undecanone was only found in samples with Cava lees, and it was the only ketone identified in lees (Table 4). This could indicate that it comes from lees, perhaps being attached to their surface during the ageing of the sparkling wine.

3.2.4. Esters

Esters were the most prevalent volatiles in all types of sourdough, especially in samples with Cava lees (Table 1). As a general rule, esters are characterized by a fruity odor, and are a result of the reaction of alcohols (mainly ethanol) and acetyl co-A derivatives of fatty acids [8]. Additionally, ester production is predominantly due to heterofermentative LAB [29,37,38]. In addition, esters are released by the degradation of yeast cells in sparkling wine, which could explain the concentration increase in samples with Cava lees [32,48,49]. Along with other substances, esters improve the flavor characteristics of sourdough bread [3]. Adding Cava lees to sourdough fermentation presents an increment in ester concentration. Therefore, the flavor of the breads produced with these sourdoughs could be more complex.

Some of the esters reported in sourdough with Cava lees were not found in the control (ethyl laurate and decyl acetate). These compounds have previously been reported in wine and sparkling wine, being dependent of the yeast strain as well as the grapes used [32,50–52]. So, it can be assumed that these esters originate the Cava lees added to sourdough fermentation.

Overall, all sourdoughs shared 14 volatile compounds (Figure 6). Moreover, 13 volatiles were identified in both sourdoughs and Cava lees including 2-ethylhexanol, 1-hexanol, 1-pentanol, and 1-octanol (alcohols); hexadecenoic, octanoic, and acetic acid (acids); decanal and nonanal (aldehydes); and ethyl octanoate, hexyl acetate, ethyl decanoate, and ethyl palmitate (esters). Furthermore, ethyl laurate, decyl acetate, 2-undecanone, and decanoic acid were compounds found in the sourdoughs with lees that were also identified in Cava lees. Nevertheless, heptanoic and pentanoic acids were only detected in the control sourdoughs. To summarize, the addition of Cava lees resulted in sourdoughs with a greater diversity of aldehydes and esters, as well as higher concentrations of all chemical families (Table 1). For instance, benzaldehyde, (E)-2-heptanal, (E)-2-octenal (aldehydes), ethyl hexanoate, ethyl laurate, octyl acetate, and decyl acetate (esters) were only produced in sourdoughs with Cava lees.

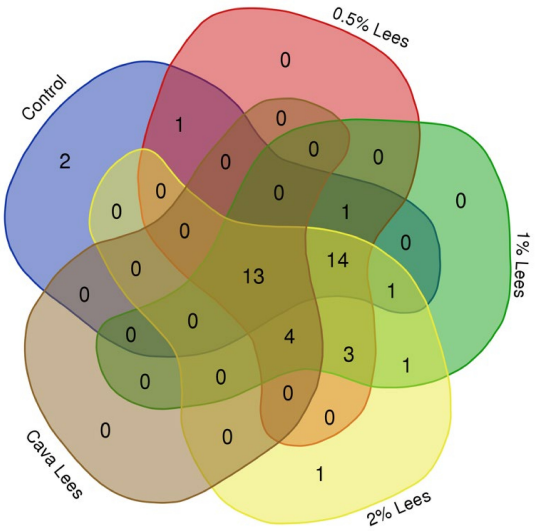

Figure 6. Venn diagram of the volatile compounds shared between the different sourdoughs produced and Cava lees.

Lastly, a PCA was performed with the aim to observe the differentiation between the produced sourdoughs (Figure 5). After the analysis, the PCA showed that there were differences between the sourdoughs according to the percentage of Cava lees added to the formulations. It showed that sourdoughs with 1% and 2% Cava lees were described by esters. Oppositely, the control and 0.5% lees sourdoughs were only characterized by short- and medium-chain carboxylic acids. Finally, it is known that there are several factors influencing the volatile characteristics of sparkling wine, such as the grape used, the fermenting yeast, and the terroir [31]. This may also modify the characteristics of the corresponding lees; therefore, further studies should focus on how different lees may impact sourdough and sourdough bread flavor as well as its microbial population and physicochemical characteristics.

4. Materials and Methods

4.1. Preparation and Propagation of Sourdoughs

For the sourdough formulation, a commercial wheat flour was used (7230 Buonpane, Molino Quaglia SpA, Padua, Italy) with the following composition (g/100 g): carbohydrates 72.0, fat 1.5, fibre 2.0, protein 11.5, and moisture 15.0.

Sourdoughs were prepared by mixing 100 g of flour and 100 mL of sterile distilled water, without the inoculation of starter culture bacteria or yeasts, and incubated at room temperature for 24 h, following the method described by Martín-Garcia et al. (2022) [29]. Briefly, Cava lees were provided by the winery Freixenet S.A. (Sant Sadurní d'Anoia, Spain) and lyophilized following the method described by Hernández-Macias et al. (2021) [20]. They were added as a percentage of flour weight at different concentrations (0%, 0.5%, 1%, and 2%) to assess their effect on the volatile compounds. Sourdoughs were propagated by backslopping for 8 days and inoculating an aliquot of the previous dough into a new mixture of flour and water. Three different sourdoughs were prepared and analyzed in triplicate.

4.2. Optimization of Headspace Solid-Phase Microextraction (HS-SPME) Parameters

The optimization of extraction of volatile compounds was performed using headspace solid-phase microextraction (HS-SPME) and it was carried out using a 2 cm long divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber supplied by Supelco (Bellefonte, PA, USA). To that end, a control sourdough was produced, and samples of 5 g were prepared. Before extraction, the fiber was conditioned according to the manufacturer's recommendations. After equilibration at a specified temperature (20, 50, 60 and 75 °C) for a specified time (10, 15 and 30 min), the fiber was exposed to the sample headspace for a specified time (20, 30, 40 and 50 min). A total of 48 runs were analyzed in triplicate for the optimization procedure based on a multilevel factorial design. Once the HS-SPME method was optimized, it was applied to the assessment of the different sourdoughs produced with and without lees. An internal standard (4-methyl-2-pentanol (CAS: 108-11-2, TCI Ltd., Eschborn, Germany), 100 μg/mL) was added (100 μL) for semi-quantification.

4.3. Analysis of Volatile Compounds by Gas Chromatography–Mass Spectrometry (GC-MS)

Chromatographic analysis was carried out in a 6890N Network GC system (Agilent, Palo Alto, CA, USA) coupled to an MS Agilent technologies 5973 Network selective detector (Thermo Fischer Scientific, Waltham, MA, USA). Helium was used as a carrier gas. Separations were accomplished in a DB Wax USN 125-7031 column (30 m × 0.25 mm × 0.25 μm) (Agilent, Palo Alto, CA, USA). A splitless injector suitable for SPME was used. After extraction, the fiber was removed from the headspace vial and inserted directly into the injection port of the GC. The SPME fiber was thermally desorbed for 2.5 min at 260 °C.

The initial temperature was 40 °C for 5 min, and this was subsequently increased at 4 °C/min using the splitless injection mode for 5 min up to 250 °C. GC-MS detection was performed in complete scanning mode (SCAN) in the 40–350 amu mass range with two scans per second. Electron impact mass spectra were recorded at an ionization voltage of

70 eV and an ion source of 280 °C. The volatile concentrations reported were calculated by dividing the peak area of the compounds of interest by the peak area of the internal standard (normalized area). The relative response factor was considered to be 1. Identification was performed by comparison of the mass spectra with the mass spectra library database Wiley 6.0., and retention times with those of pure standards when they were available.

4.4. Statistical Analysis

All assays were performed in triplicate and in a randomized run order. The statistical analysis was performed using the Prism 9 version 9.1.2 (225) (GraphPad Software, LLC., San Diego, CA, USA) statistical package. The results are reported as the means ± standard error (SE) for parametric data. A three-factor analysis was conducted on the optimization results. A one-way ANOVA and comparison of the means were conducted using Tukey's test with a confidence interval of 95%. Significant results were identified with a p-value of ≤ 0.05. Principal component analysis (PCA) was also performed to determine the differences between the sourdoughs.

5. Conclusions

After the optimization of the HS-SPME parameters, it was found that the best temperature of extraction was 60 °C, with 15 min of equilibrium and 30 min of extraction for wheat sourdough. Then, when applied to the studied sourdoughs, it was found that acids and esters were the most prevalent compounds quantified, followed by alcohols, aldehydes, and finally ketones. Regarding particular compounds, butyl butyrate, ethyl octanoate, ethyl decanoate, octanoic acid, and 1-hexanol were the most prevalent volatiles quantified.

In general, the addition of Cava lees caused an increase in the concentration of the volatile compounds typically found in sourdough, such as 1-hexanol, acetic acid, hexanal, and ethyl decanoate. Additionally, compounds usually reported in sparkling wines were also identified in sourdough samples formulated with Cava lees, such as 1-butanol, octanoic acid, benzaldehyde, and ethyl hexanoate. Therefore, it can be concluded that Cava lees not only promote the production of sourdough volatile compounds, but they also provide volatiles frequently found in sparkling wines, which supports the fact that lees can retain volatile compounds on their surface. Moreover, the ability of Cava lees to retain odorous volatile compounds could be of great interest for the food and aroma industries that could revalorize and use such by-products, contributing to a circular economy.

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By-Product Revalorization: Cava Lees Can Improve the Fermentation Process and Change the Volatile Profile of Bread

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LES IDEES CLAU

L'addició de lies del Cava al 5% va tenir un impacte positiu sobre el creixement i supervivència dels microorganismes fermentadors en la formulació del pa. A més a més, afegint un 5% de lies es va augmentar la concentració dels compostos volàtils i aquest ingredient va aportar nous compostos propis del Cava al pa.

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By-Product Revalorization: Cava Lees Can Improve the Fermentation Process and Change the Volatile Profile of Bread

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Abstract: Wine lees are a by-product that represents a 25% of the total winery waste. Although lees are rich in antioxidant compounds and dietary fiber, they have no added value and are considered a residue. The aim of this study was to evaluate the effect of Cava lees (0 and 5% *w*/*w*) on microbial populations during sourdough and bread fermentation and the volatile fraction of the final bread. The results showed that 5% Cava lees promoted the growth of both lactic acid bacteria (LAB) and yeast in short fermentations (bread) but did not improve microbial growth in long fermentations (sourdough). Regarding volatile compounds, the addition of Cava lees increased the concentration of volatiles typically found in those products. Also, some compounds reported in sparkling wines were also identified in samples with Cava lees adsorbed on their surface. To sum up, the addition of Cava lees to sourdough and, especially, bread formulation may be a new strategy to revalorize such by-product.

Keywords: sourdough bread; volatile compounds; lactic acid bacteria; cava lees; revalorization; wine by-product

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1. Introduction

In the EU, around 129 Mt of food waste is generated annually in the food supply chain [1]. It not only has economic repercussions, but it also presents an environmental impact as a consequence of the management and disposal of the food waste [2,3]. The current situation demands for a change from a linear economy to a circular economy where by-products acquire an added value and re-enter the production cycle in order to decrease the environmental impact of industries [4].

It is particularly concerning to the winemaking industry, which includes the production of Cava. Cava is sparkling wine with Protected Denomination of Origin (PDO) that requires a second fermentation in the bottle with a biological ageing process in contact with lees for a minimum of 9 months [5]. Wine elaboration produces approximately 25 kg of waste for every 100 kg of processed grapes. Actually, the main solid residues produced by winemaking are grape pomace (60%), lees (25%) and stalks (15%) [2,3].

Lees are the residue formed during wine fermentation and consist, mainly, of naturally plasmolyzed cells of *Saccharomcyces cerevisiae*, tartaric acid and other adsorbed compounds [6,7]. It is estimated that the production of Cava lees is about 300 tones per year [8]. Those lees are rich in phenolic compounds as well as fiber and proteins from the cell wall of *S. cerevisiae* [2,5–7]. Indeed, the use of by-products with high contents of fiber and other bioactive compounds as novel ingredients is being studied to obtain foods with greater nutritional value [9–11].

Despite their composition, lees are an undervalued by-product mostly used for the recovery of tartaric acid and distillation to obtain alcohol [12]. Nevertheless, some studies have reported the possibility of revalorizing wine lees [8,13–15].

In fact, Hernández-Macias et al. (2021) [6] reported an improvement of growth and survival of lactic acid bacteria (LAB) with the addition of Cava lees in vitro. In addition, our research group recently demonstrated that the addition of Cava lees to sourdough formulation promoted the growth and survival of microorganisms (both LAB and yeast) in spontaneous fermentation [15]. In addition, it has been reported that Cava lees can inhibit the growth of pathogens (*Salmonella* spp. and *L. monocytogenes*), improving the microbiological safety of fermented sausages [6]. Hence, Cava lees might be revalorized as a food ingredient to improve fermented foods like sourdough and bread. Moreover, modifying microbial populations of food fermentation might have an impact on its flavor [16].

Indeed, flavor and especially odor are of great importance for consumer acceptance. It must be taken into account that the addition of by- and co-products to food formulation may change its sensory properties (from texture to aroma or color). For instance, Lafarga et al. (2018) [9] added broccoli co-products (stems and leaves) to wheat bread formulation to obtain functional products with enhanced concentrations of fiber and phenolic compounds. The researchers observed that breads with broccoli presented an increased green hue as well as a higher color intensity in crumb and crust. Nevertheless, when performing sensory tests, the overall acceptance of the breads was not affected by broccoli incorporation [9]. Other by-products, such as cumin and caraway seeds by-products and cocoa dietary fiber have been added to wheat bread formulation to obtain functional products [10,11]. In both studies, researchers examined the effect of those new ingredients on the sensory properties of bread. In both cases, there were no significant differences on the overall acceptance of the fortified breads and controls, even though color, texture and aroma changed.

In that regard, lees are able to adsorb volatile compounds during the biological ageing of sparkling wine [17]. Consequently, incorporating Cava lees to sourdough and bread formulations may add new odors and other compounds to such bakery products. Therefore, the aim of this study was to evaluate the effect of Cava lees on microbial populations during sourdough and bread fermentation as well as the volatile fraction of those breads to revalorize this winery by-product.

2. Materials and Methods

2.1. Sourdough Formulation and Bread-Making

A commercial wheat flour (Harina de Fuerza Gallo, Comercial Gallo S.A., Barcelona, Spain) with the following composition (% *w*/*w*): carbohydrates 69.0, fat 1.4, fiber 4.2, protein 11.7 and moisture 15.0, was used.

A parallel study was designed in order to compare breads with and without sourdough. Sourdoughs were prepared by mixing 100 g of flour and 100 mL of sterile distilled water (Table 1), without the inoculation of microorganisms and incubated at room temperature for 24 h. Cava lees were lyophilized following the method described by Hernández-Macias, Comas-Basté, et al., 2021 [6]. They were added as a percentage of flour weight at 5% (*w*/*w*) and compared to a control without lees, based on previous in vitro studies [6]. Sourdoughs were propagated by backslopping for 8 days, inoculating an aliquot of the previous dough into a new mixture of flour and water, adding the corresponding lees percentage.

Table 1. Ingredients of sourdough (wheat flour weight basis, g).

Code	Wheat Flour	Water	Dough [1]	Cava Lees
SDC	100	100	100	-
SD+L	100	100	100	5 [2]

[1] Aliquot of the previous dough into de new mixture. [2] Lees were added as a percentage of flour weigh in sourdough formulation in each propagation step.

Breads were made with the sourdoughs produced (Table 2). Breads were prepared by mixing flour (500 g), water (285 mL), sourdough (150 g), baker's yeast (4 g) and salt (10 g). Separately, breads fermented with commercial yeast (Ref.: 36835, Sosa Ingredients S.L., Barcelona, Spain) were also prepared with the following formulation: flour (500 g), water (285 mL), Cava lees (0% and 5% (*w*/*w*)), and salt (10 g). Cava lees were also added as a percentage of flour weight. The dough was manually mixed and kneaded for 10 min. The dough temperature at the end of kneading was between 22 and 24 °C. Once formed, dough was rested for 40 min, after which the dough was knocked back and rested for another 40 min. All bread was given a final proof of 20 min at 30 °C and 80% relative humidity. Following the processing of the dough, breads were baked in a convection-steam oven (Ref.: SA-SC-623, Salva S.L.U., Guipuzkoa, Spain) at 220 °C for 30 min.

Table 2. Ingredients of bread (wheat flour weight basis, g).

Code [1]	Wheat Flour	Water	Sourdough	Baker's Yeast	Salt	Cava Lees
SBC	500	285	150	4	10	-
SB+L	500	285	150	4	10	-
BC	500	285	-	4	10	-
B+L	500	285	-	4	10	25

[1] Codes of sample series of bread types: SBC: control sourdough bread; SB+L: sourdough bread with 5% Cava lees; BC: control bread; B+L: bread with 5% Cava lees.

2.2. Microbial Populations and Fermentation Monitoring

Microbial populations were monitored following the method described by Martín-Garcia et al. (2022) [15]. Briefly, samples of 10 g were added to 90 mL of sterile peptone water (Ref.: 1402, Condalab, Madrid, Spain) and homogenized with a laboratory blender (Stomacher 400 Seward Ltd., Worthing, UK) for 1 min. Sourdough samples were taken daily, while breads were monitored every 30 min. All samples were diluted and plated in MRS (Ref.: 1043, Condalab, Madrid, Spain) to monitor LAB populations and in Saboraud-Chloramphenicol Agar (Ref.: 01-166-500, Scharlab, Barcelona, Spain) for yeasts. Also, pH was monitored in all samples using a pH meter XS PH60 VioLab (XS Instruments, Carpi MO, Italy).

2.3. Headspace Solid Phase Microextraction (HS-SPME)

The extraction of volatile compounds was performed using Headspace Solid Phase Microextraction (HS-SPME) as reported by Paraskevopoulou et al. (2012) [18] using a 2 cm long Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber supplied by Supelco (Bellefonte, PA, USA). Before extraction, the fiber was conditioned according to the manufacturer's recommendations. All breads were grinded, and samples of 3 g were placed in 20 mL vials. Then, 1 mL of a 20% NaCl solution (pH 3 adjusted with 0.05 M citric acid solution) was added to the vial. After equilibration at 60 °C for 30 min, the fiber was exposed to the sample headspace for 60 min. An internal standard [4-methyl-2-pentanol (CAS: 108-11-2, TCI Ltd., Eschborn, Germany), 100 µg/L] was used (100 µL) for semi-quantification.

2.4. Analysis of Volatile Compounds by Gas Chromatography—Mass Spectrometry (GC-MS)

Chromatographic analysis was carried out in a 6890N Network GC system coupled to MS 5973 Network selective detector (Agilent, Palo Alto, CA, USA). Helium was used as a carrier gas. Separations were accomplished in a DB Wax USN 125-7031 column (30 m × 0.25 mm × 0.25 µm) (Agilent, Palo Alto, CA, USA). A splitless injector suitable for SPME was used. After extraction, the fiber was removed from the headspace vial and manually inserted directly into the injection port of the GC. The SPME fiber was thermally desorbed for 4 min at 260 °C.

The initial temperature was held at 40 °C for 5 min and increased at from 40 °C to 190 °C at 3 °C/min and from 190 °C to 220 °C at 10 °C/min which was held for 5 min using

splitless injection mode. GC-MS detection was performed in complete scanning mode (SCAN) in the 40–350 amu mass range with two scans per second. Electron impact mass spectra were recorded at an ionization voltage of 70 eV and ion source of 280 °C. Volatile concentrations reported were calculated by dividing the peak area of the compounds of interest by the peak area of the internal standard (normalized area). The relative response factor was considered to be 1. Tentative Identification was performed by comparison of their mass spectra with those of the mass spectra library database Wiley 6.0., and their retention times with those of pure standards when they were available.

2.5. Statistical Analysis

The statistical analysis was performed using Prism 9 version 9.1.2 (225) (GraphPad Software, LLC., San Diego, CA, USA) statistical package. The results are reported as the means ± standard error (SE) of triplicates for parametric data. A one-way ANOVA and comparison of the means were conducted using Tukey's test, with a confidence interval of 95% and significant results with a p-value of $\leq$0.05. Principal component analysis (PCA) was also performed to determine differences between breads.

3. Results and Discussion

3.1. Microbial Populations and Fermentation Monitoring

A control and a fortified (5% Cava lees) sourdough were prepared to assess the effect of Cava lees on the fermenting microbiota of sourdough. Figure 1 shows the growth kinetics of lactic acid bacteria (LAB) and pH during the 8 days of sourdough propagation. Both types of sourdough (control without lees—SDC; with 5% lees—SD+L) were spontaneously fermented. Although the promoting effect of 5% (w/w) Cava lees on LAB growth has been reported in vitro [6] and up to a 2% (w/w) Cava lees in wheat and rye sourdoughs [15], it can be observed that the addition of Cava lees to sourdough formulation did not stimulate LAB growth in this particular food matrix.

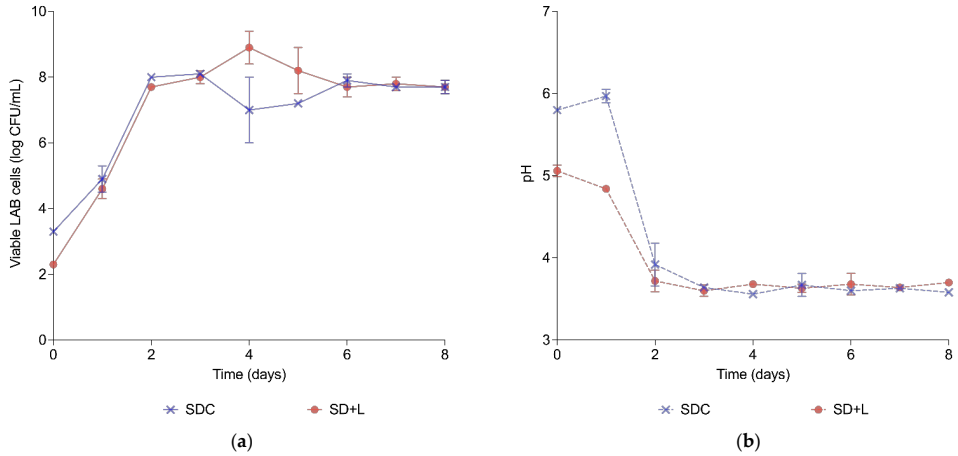

Figure 1. Growth of LAB (**a**) and pH (**b**) in sourdough without lees (SDC) and sourdough with 5% lees (SD+L).

The initial pH of sourdoughs with 5% Cava lees (w/w) was significantly lower ($p < 0.05$). In particular, SDC started the sourdough fermentation with a pH of 5.80 ± 0.01, while SD+L begun with a pH of 5.06 ± 0.07 due to the inherent acidity of Cava lees [8]. During the fermentation and propagation process of sourdough, pH decreased during the first steps of fermentation and then stabilized, obtaining values of 3.60 ± 0.04 (SDC) and

3.70 ± 0.02 (SD+L), similar to those reported in other studies [19–21] and in accordance with the pH range of traditional sourdoughs (pH 3.5–4.5) [13]. However, there were no statistically significant differences between sourdoughs. Once sourdoughs were mature (8 days), breads were prepared (Table 2) and baked.

Figure 2 presents the cell density of both LAB and yeast at the end of bread fermentation. When Cava lees were used in the formulation of bread (both fermented with and without sourdough) there was a higher cell count for both yeasts and bacteria. In fact, bread fermented without sourdough presented a difference of 0.7 log10 CFU/mL between the ones with Cava lees (B+L) and the controls (BC). Also, there was a higher cell density in SB+L (5.1 ± 0.2 log10 CFU/mL) in comparison to SBC (4.1 ± 0.1 log10 CFU/mL). Regarding yeasts, the addition of lees to formulation had the same tendency.

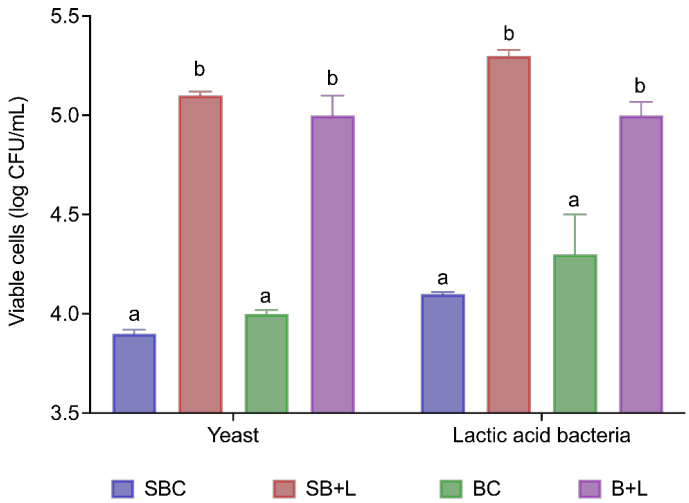

Figure 2. Microbial cell density at the end of bread fermentation. Different letters denote statistically significant differences ($p < 0.05$) between different formulations of bread. SBC: control sourdough bread; SB+L: sourdough with 5% Cava lees; BC: control bread; B+L: bread with 5% Cava lees.

Sourdough bread usually presents a pH ranging between 5.0 and 5.5 [22], which was in accordance with the results obtained in this study (Table 3). The addition of Cava lees to both sourdough bread (SB+L) and leavened bread (B+L) resulted in lower pH at the beginning of dough fermentation, and, consequently, also at the end. As previously stated, the difference in pH values between samples with and without lees was probably due to the inherent acidity of Cava lees [8]. In fact, B+L obtained the lowest pH values of all formulated doughs. Actually, the addition of Cava lees to bread formulation (B+L) resulted in the greatest drop of pH during fermentation, which could be related to the higher plate counts of both LAB and yeast (Figure 2).

Table 3. pH values at the beginning (t = 0 h) and end (t = 2 h) of bread fermentation.

pH	SBC	SB+L	BC	B+L
t = 0 h	5.48 ± 0.03 ^{a}	5.00 ± 0.01 ^{b}	5.76 ± 0.03 ^{c}	4.97 ± 0.03 ^{b}
t = 2 h	5.14 ± 0.02 ^{a}	4.75 ± 0.04 ^{b}	5.45 ± 0.02 ^{c}	4.37 ± 0.03 ^{d}

Values are mean ± standard deviation of triplicates. Significant differences between samples are indicated by different superscript letters ($p < 0.05$) for each compound. SBC: control sourdough bread; SB+L: sourdough bread with 5% Cava lees; BC: control bread; B+L: bread with 5% Cava lees.

3.2. Analysis of Volatile Compounds

In order to evaluate the effect of Cava lees on the volatile fraction of breads, HS-SPME-GC-MS was performed. A total of 74 volatile compounds were identified (Table 4), including nine acids, 16 alcohols, 11 aldehydes, five ketones, 14 esters and eight terpenes. Bread volatile compounds may result from fermentation, lipid oxidation and Maillard and caramelization reactions [20,23–25]. Alcohols, acids, esters, aldehydes and ketones were generated mainly during fermentation while some of them as alcohols, ketones and aldehydes come from lipid oxidation too.

Lastly, Maillard and caramelization reactions originate pyrazines, pyridines, pyrroles, furans, sulfur compounds, aldehydes and ketones [22]. Additionally, the volatile compounds of Cava lees were also analyzed by HS-SPME (Table S1), since wine lees are able to retain aromatic substances such as esters, aldehydes, norisoprenoids, terpenes and some phenolic compounds [17,26].

Table 4. Concentration (mg/kg) of the main volatile compounds identified in bread.

	Compound	CAS-Num.	Odor [1]	ODT [2]	SBC	SB+L	BC	B+L
	ACIDS							
1	Acetic acid	64-19-7	sharp pungent sour vinegar	-	143.83 ± 1.63 [a]	27.01 ± 8.74 [b]	132.04 ± 0.06 [a]	67.60 ± 16.22 [c]
2	Benzoic acid	65-85-0	faint balsam urine	na	nd	6.83 ± 0.98 [a]	3.89 ± 0.10 [a]	8.52 ± 2.35 [a]
3	Decanoic acid	334-48-5	sweet waxy floral soapy clean	1000	nd	5.09 ± 0.77 [a]	nd	77.19 ± 2.62 [b]
4	Dodecanoic acid	143-07-7	sweet waxy floral soapy clean	1000	nd	nd	nd	240.41 ± 83.22
5	Hexadecanoic acid	57-10-3	slightly waxy fatty	1000	7.26 ± 1.78 [a]	nd	10.20 ± 1.03 [a]	18.86 ± 2.66 [b]
6	Hexanoic acid	142-62-1	sour fatty sweat cheese	300	nd	3.76 ± 0.49 [a]	18.33 ± 0.33 [b]	46.97 ± 5.34 [c]
7	Octanoic acid	124-07-2	fatty waxy rancid oily vegetable cheesy	300	13.69 ± 2.56 [a]	nd	11.13 ± 6.27 [a]	173.53 ± 11.96 [b]
8	Isobutyric acid	79-31-2	acidic sour cheese dairy buttery rancid	810	nd	nd	18.90 ± 6.30 [a]	18.72 ± 9.86 [a]
9	Myristic acid	544-63-8	waxy fatty soapy coconut	1000	28.54 ± 3.87 [a]	12.51 ± 2.45 [b]	nd	8.66 ± 1.53 [b]
	TOTAL ACIDS				193.32 ± 9.84 [a]	55.20 ± 13.43 [a]	194.49 ± 14.09 [a]	660.46 ± 135.76 [b]
	ALCOHOLS							
10	Butyl alcohol	71-36-3	fusel oil sweet balsam whiskey	50	nd	nd	10.32 ± 1.97 [a]	11.61 ± 2.65 [a]
11	Isoamyl alcohol	123-51-3	fusel oil alcoholic whiskey fruity banana	25–30	96.16 ± 7.30 [a]	92.88 ± 6.19 [a]	82.69 ± 4.57 [a]	60.26 ± 5.17 [b]
12	1-Dodecanol	112-53-8	earthy soapy waxy fatty honey coconut	na	nd	4.76 ± 0.30	nd	nd
13	1-Hexanol	111-27-3	ethereal fusel oil fruity alcoholic sweet green	250	88.78 ± 31.46 [a]	37.95 ± 11.30 [b]	76.65 ± 11.19 [ab]	39.54 ± 7.10 [b]
14	2-Ethyl-1-hexanol	104-76-7	citrus fresh floral oily sweet	na	8.62 ± 3.20 [a]	9.81 ± 4.44 [a]	55.69 ± 9.96 [b]	64.75 ± 1.08 [b]
15	1-Octanol	111-87-5	waxy green orange aldehydic rose mushroom	11–13	18.61 ± 8.18 [a]	11.22 ± 2.88 [a]	10.23 ± 2.45 [a]	6.92 ± 2.57 [a]
16	1-Octen-3-ol	3391-86-4	mushroom earthy green oily fungal raw chicken	1	20.22 ± 2.36 [a]	8.89 ± 0.87 [b]	20.17 ± 1.25 [a]	8.06 ± 0.96 [b]
17	1-Pentanol	71-41-0	fusel oil sweet balsam	400	13.85 ± 1.48	nd	nd	nd
18	2-Methyl-1-propanol	78-83-1	ethereal winey cortex	na	8.79 ± 0.79 [a]	nd	12.27 ± 0.84 [b]	nd
19	2-Furanmethanol	98-00-0	alcoholic chemical musty sweet caramel bread coffee	na	13.79 ± 5.67 [a]	7.40 ± 2.44 [a]	24.12 ± 4.04 [b]	15.54 ± 1.78 [ab]
20	7-Octen-4-ol	53907-72-5	-	na	28.42 ± 5.87	nd	nd	nd
21	9-Decen-1-ol	13019-22-2	dewy rose waxy fresh clean aldehydic	na	27.08 ± 3.27 [a]	7.78 ± 0.86 [b]	34.13 ± 5.97 [a]	15.69 ± 3.48 [b]
22	Phenethyl alcohol	60-12-8	floral rose dried rose flower rose water	75–110	253.45 ± 3.78 [a]	100.51 ± 33.46 [b]	102.35 ± 17.03 [b]	130.47 ± 30.62 [b]
23	Benzyl alcohol	100-51-6	floral rose phenolic balsamic	1000	nd	nd	nd	11.64 ± 1.27
24	2-Phenoxy-ethanol	122-99-6	mild rose balsam cinnamyl	na	nd	nd	15.46 ± 5.11 [a]	8.76 ± 2.48 [a]
25	Heptanol	111-70-6	musty leafy violet herbal green sweet woody peony	0.3	22.69 ± 5.53 [a]	12.77 ± 1.97 [b]	24.61 ± 2.15 [a]	nd
26	Nonanol	143-08-8	fresh clean fatty floral rose orange dusty wet oily	5	10.81 ± 1.48 [a]	nd	19.48 ± 6.17 [b]	21.54 ± 1.57 [b]
	TOTAL ALCOHOLS				611.27 ± 80.37 [a]	293.97 ± 64.71 [b]	488.17 ± 72.70 [ac]	394.78 ± 60.73 [bc]

Table 4. *Cont.*

	Compound	CAS-Num.	Odor [1]	ODT [2]	SBC	SB+L	BC	B+L
	ALDEHYDES							
27	(E)-2-Heptenal	18829-55-5	pungent green vegetable fresh fatty	1.3	16.50 ± 2.36 [a]	7.58 ± 3.29 [a]	40.93 ± 20.65 [ab]	13.21 ± 3.08 [a]
28	(E)-2-Nonenal	18829-56-6	fatty green cucumber aldehydic citrus	0.08-0.1	27.29 ± 2.93 [a]	10.85 ± 0.93 [b]	nd	26.21 ± 3.28 [a]
29	(E)-2-Octenal	2548-87-0	fresh cucumber fatty green herbal banana waxy green leaf	0.3	23.02 ± 2.44 [a]	10.77 ± 3.39 [b]	17.74 ± 1.86 [ab]	12.81 ± 1.84 [b]
30	(E,E)-2,4-Decadienal	25152-84-5	oily cucumber melon citrus pumpkin nut meat	0.07	10.46 ± 2.36 [a]	nd	8.23 ± 0.94 [a]	8.92 ± 0.64 [a]
31	(E,Z)-2,4-Decadienal	25152-83-4	fried fatty geranium green waxy	na	nd	nd	24.70 ± 2.79	nd
32	Benzaldehyde	100-52-7	strong sharp sweet bitter almond cherry	35–350	41.90 ± 4.59 [a]	43.46 ± 19.19 [a]	24.36 ± 5.68 [a]	88.66 ± 11.41 [b]
33	*o*-Tolualdehyde	529-20-4	cherry	na	nd	4.57 ± 0.57	nd	nd
34	Butanal	123-72-8	pungent cocoa musty green malty bready	0.9–3.73	nd	nd	nd	2.39 ± 0.15
35	Isovaleraldehyde	590-86-3	ethereal aldehydic chocolate peach fatty	0.2–2	nd	nd	nd	2.05 ± 0.43
36	Heptanal	111-71-7	fresh aldehydic fatty green herbal wine-lee ozone	3	14.04 ± 7.68 [a]	18.19 ± 1.69 [a]	nd	nd
37	Hexanal	66-25-1	fresh green fatty aldehydic grass leafy fruity sweaty	4.5–5	56.11 ± 11.12 [a]	32.86 ± 14.76 [a]	105.01 ± 16.26 [b]	53.56 ± 11.53 [a]
38	Nonanal	124-19-6	waxy aldehydic rose fresh orris orange peel fatty peely	1	nd	8.88 ± 0.81 [a]	15.04 ± 0.74 [b]	21.80 ± 2.63 [c]
	TOTAL ALDEHYDES				189.68 ± 33.48 [a]	137.16 ± 44.63 [a]	236.01 ± 48.92 [a]	229.61 ± 34.99 [a]
	KETONES							
39	Acetoin	513-86-0	sweet buttery creamy dairy milky fatty	80	13.19 ± 6.75 [a]	14.03 ± 9.11 [a]	6.94 ± 0.97 [a]	13.38 ± 3.59 [a]
40	2-Nonanone	821-55-6	fresh sweet green weedy earthy herbal	0.5–20	nd	nd	nd	4.78 ± 1.28
41	2-Octanone	111-13-7	earthy weedy natural woody herbal	5	2.64 ± 0.45 [a]	nd	4.87 ± 1.04 [b]	nd
42	4-Methyl-2-pentanone	108-10-1	sharp solvent green herbal fruity dairy spice	na	11.24 ± 3.49 [a]	nd	14.52 ± 0.78 [a]	11.26 ± 2.37 [a]
43	2,3-Octanedione	585-25-1	dill asparagus cilantro herbal aldehydic earthy fatty cortex	na	nd	nd	6.26 ± 1.08	nd
44	Acetophenone	98-86-2	sweet pungent hawthorn mimosa almond acacia chemical	6.5	nd	nd	5.64 ± 1.11	nd
	TOTAL KETONES				27.07 ± 10.69 [ab]	14.03 ± 9.11 [b]	38.23 ± 4.98 [a]	29.42 ± 7.24 [ab]
	ESTERS							
45	Isoamyl decanoate	2306-91-4	waxy banana fruity sweet cognac green	na	nd	5.54 ± 0.83 [a]	nd	175.37 ± 20.93 [b]
46	Phenethyl acetate	103-45-7	floral rose sweet honey fruity tropical	na	6.20 ± 1.12	nd	nd	nd
47	Hexyl acetate	142-92-7	fruity green apple banana sweet	2	nd	nd	nd	12.77 ± 2.57
48	L-Bornyl acetate	5655-61-8	sweet balsamic woody fresh pine needle herbal	na	nd	6.23 ± 0.98	nd	nd
49	Diethyl succinate	123-25-1	mild fruity cooked apple ylang	na	nd	nd	nd	47.49 ± 6.39
50	Ethyl decanoate	628-97-7	mild waxy fruity creamy milky balsamic greasy oily	>200	59.56 ± 3.91 [a]	127.37 ± 13.90 [b]	nd	220.30 ± 38.41 [b]
51	Ethyl 9-hexadecenoate	54546-22-4	-	na	nd	nd	nd	23.95 ± 2.64
52	Ethyl 9-decenoate	67233-91-4	fruity fatty	na	nd	nd	nd	41.81 ± 2.93
53	Ethyl hexanoate	123-66-0	sweet fruity pineapple waxy green banana	1	9.67 ± 3.37 [a]	nd	18.64 ± 3.78 [b]	98.44 ± 3.07 [c]
54	Octyl acetate	112-14-1	green earthy mushroom herbal waxy	1.2	nd	nd	nd	8.04 ± 1.26
55	Ethyl nonadecanoate	18281-04-4	-	na	nd	nd	nd	5.43 ± 1.02
56	Isoamyl octanoate	2035-99-6	sweet oily fruity green soapy pineapple coconut	na	nd	nd	nd	181.00 ± 17.83

Table 4. *Cont.*

	Compound	CAS-Num.	Odor [1]	ODT [2]	SBC	SB+L	BC	B+L
57	Ethyl octanoate	106-32-1	fruity wine waxy sweet apricot banana brandy pear	na	nd	nd	28.72 ± 4.55 [a]	965.31 ± 167.18 [b]
58	Phenethyl isobutyrate	103-48-0	floral fruity rose tea rose peach pastry	na	nd	nd	nd	14.22 ± 2.49
59	Ethyl myristate	124-06-1	sweet waxy violet orris	na	nd	nd	nd	10.75 ± 7.36
	TOTAL ESTERS				75.43 ± 8.76 [a]	139.14 ± 15.71 [a]	47.36 ± 8.33 [a]	1804.88 ± 274.08 [b]
	TERPENES							
60	α-Terpinolene	586-62-9	fresh woody sweet pine citrus	20	nd	8.71 ± 1.25	nd	nd
61	Vitispirane	66965-94-4	floral fruity earthy woody	na	nd	7.73 ± 0.43 [a]	nd	25.03 ± 3.08 [b]
62	(E,E)-Farnesyl acetate	4128-17-0	oily waxy	na	nd	nd	nd	44.88 ± 5.60
63	dihydromyrcenol	18479-58-8	fresh citrus lime floral clean	na	2.32 ± 0.13	nd	nd	nd
64	Bornylene	464-17-5	-	na	nd	nd	37.11 ± 6.92	nd
65	d-Nerolidol	142-50-7	mild floral	na	nd	nd	nd	13.66 ± 2.01
66	DL-Limonene	138-86-3	citrus herbal terpene camphor	10	5.39 ± 1.06 [a]	9.43 ± 4.22 [a]	23.34 ± 14.40 [ab]	34.02 ± 2.30 [b]
67	Farnesol	4602-84-0	mild fresh sweet linden floral angelica	2	nd	nd	nd	24.84 ± 6.21
68	Nerolidol	7212-44-4	floral green waxy citrus woody	na	nd	nd	nd	73.31 ± 8.75
	TOTAL TERPENES				7.71 ± 1.19 [a]	25.87 ± 5.90 [ab]	60.45 ± 21.32 [b]	215.74 ± 27.95 [c]
	MISCELANEOUS							
69	2-Pentyl- furan	3777-69-3	fruity green earthy beany vegetable metallic	6	22.82 ± 1.11 [a]	59.05 ± 9.20 [b]	54.47 ± 6.62 [b]	46.31 ± 6.37 [b]
70	2-Furancarboxaldehyde	98-01-1	sweet woody almond fragrant baked bread	na	6.20 ± 3.68 [a]	6.45 ± 0.91 [a]	nd	19.55 ± 8.56 [b]
71	1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN)	30364-38-6	licorice	na	nd	43.95 ± 16.26 [a]	nd	196.29 ± 34.35 [b]
72	4-Ethylguaiacol	2785-89-9	spicy smoky bacon phenolic clove	50	nd	nd	nd	19.73 ± 6.59
73	Styrene	100-42-5	sweet balsam floral plastic	730	nd	103.69 ± 30.91 [a]	nd	12.59 ± 0.89 [b]
74	γ-Nonalactone	104-61-0	coconut creamy waxy sweet buttery oily	na	nd	nd	15.80 ± 7.79 [a]	15.10 ± 4.86 [a]
	TOTAL MISCELANEOUS				29.02 ± 4.79 [a]	213.14 ± 57.28 [b]	70.27 ± 14.41 [a]	309.56 ± 61.62 [b]

[1] From [27]. [2] ODT: Odor Detection Threshold. From [28]. Expressed as μg/mL. Values are mean ± standard deviation of triplicates. Significant differences between samples are indicated by different superscript letters ($p < 0.05$) for each compound. na: not available; nd: not detected. SBC: control sourdough bread; SB+L: sourdough bread with 5% Cava lees; BC: control bread; B+L: bread with 5% Cava lees.

In general, B+L had the highest concentration and number of different volatile compounds ($p < 0.05$), especially in acids (660.46 ± 135.75 mg/kg), esters (1804.88 ± 274.08 mg/kg) and terpenes (215.74 ± 27.95 mg/kg). Oppositely, controls (SDC and BC) were richer in alcohols (611.27 ± 80.37 and 488.17 ± 72.70 mg/kg, respectively).

Although it would be expected that sourdough bread had a richer aroma profile [29], in the present study we obtained less abundance of volatile compounds in SB+L and SBC breads. In that regard, sourdough is generally added at less than 50% of the flour content (Table 2) and afterwards there is a baking process, so volatile compounds from sourdough might be diluted in the end product [24].

Acids are a product of the fermentation process and are responsible for the acidification of the dough [24,30]. Nevertheless, organic acid production during sourdough and bread-making depends on several variables, including microbial composition as well as process parameters (dough yield, fermentation time and temperature and NaCl concentration) [16,24,30]. In B+L samples, the dominant acids were dodecanoic acid (240.41 ± 83.22 mg/kg) and octanoic acid (173.53 ± 11.96 mg/kg). In fact, octanoic acid along with decanoic acid, were the major organic acids found in Cava lees (Table S1).

Octanoic acid was also found in SBC and BC samples, and its production is related to yeast [24].

Control breads with and without sourdough (SBC and BC) presented no significant differences in organic acid concentration ($p < 0.05$). Furthermore, SBC and BC breads had the highest concentration of acetic acid (143.83 ± 1.63 and 132.04 ± 0.06 mg/kg, respectively). In fact, acetic acid is one of the main organic acids responsible form microbiological shelf-life extension since it also possesses antiripeness and antimold activity [20,30]. Moreover, acetic acid is thought to inhibit yeast growth [25], which can be related to the lower yeast cell density obtained in SBC and BC breads (Figure 2).

Alcohols are mainly produced during fermentation from flour amino acids via the Ehrlich pathway in yeast cells but may be also a product of lipid oxidation [20]. SBC showed the highest concentration of alcohols ($p < 0.05$). The dominant alcohols in all bread samples were phenethyl alcohol and isoamyl alcohol. Phenethyl alcohol is derived from the fermentation of phenylalanine by yeast, and it has been reported that prolonged fermentations increase its concentration [20,31]. SBC had the highest concentration (253.45 ± 3.78 mg/kg), which can be related to the longer fermentation of sourdough. Isoamyl alcohol is a product of the fermentation of leucine also in the yeast cell [20,24,31] and presented higher values in sourdough samples (with and without Cava lees). In addition, isoamyl alcohol can also be produced by LAB such as *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) [24,32].

Aldehydes are formed during lipid oxidation and decarboxylation of unsaturated fatty acids as well as from amino acid degradation by the Ehrlich pathway [20,33]. The most prevalent aldehydes found in the studied breads were benzaldehyde, hexanal, (E)-2-nonenal and nonanal which are commonly reported in both sourdough and bread [20,24,33].

The addition of Cava lees increased the concentration of benzaldehyde, especially in yeast leavened bread (B+L, 88.66 ± 11.41 mg/kg). This compound is the result of both fermentative reactions and lipid oxidation and has been found in bread produced with and without sourdough [20,24,34]. Benzaldehyde has been reported to be produced by yeast as well as *L. plantarum* and *L. helveticus* via amino acid (phenylalanine) conversion [24,35]. Moreover, benzaldehyde has also been found in sparkling wines [36–38], which might explain the increase in its concentration in breads with Cava lees since sparkling wine lees can retain aldehydes in their surface (Table S1) [17].

Nonanal, another compound derived from fermentation and lipid oxidation [20] has also been identified in the surface of sparkling wine lees [17]. In this study, SB+L and B+L showed significantly higher amounts of this compound, that was, indeed, also identified in Cava lees surface (Table S1). In fact, in SBC, nonanal was not detected. It must be taken into account that some heterofermentative LAB strains are able to reduce aldehydes to other compounds, which may explain the lower concentration of certain volatiles in samples with 5% Cava lees [32,35].

Regarding ketones, BC samples presented a greater variety of those compounds. Those volatile compounds are influenced by LAB in dough fermentation, and only certain homofermentative and facultatively heterofermentative bacteria are able to produce them [32]. Acetoin is a distinct aroma in bread produced during fermentation related to consumer acceptance [23]. In this study it was found that the addition of Cava lees increased its production, especially in B+L where it reached similar values to those of sourdough bread.

Esters are characterized by a fruity odor resulting from a direct esterification between ethanol and acetyl co-A derivatives of fatty acids during fermentation mainly due to heterofermentative LAB [32,35,39]. In fact, it has been observed that fermentations with LAB produce more esters than those with yeast [24]. In this study, the addition of 5% Cava lees increased the production of esters, especially in bread samples (B+L) in which 13 esters were identified, which is in accordance with higher LAB populations (Figure 2). Ethyl decanoate was the dominant ester in SB+L (127.37 ± 13.90 mg/kg), while in B+L it was ethyl octanoate (965.31 ± 167.18 mg/kg). As previously mentioned, sparkling wine lees also retain esters such as ethyl hexanoate, ethyl octanoate and isoamyl octanoate [17]. In

fact, most of the esters only identified in B+L samples have been reported in sparkling wine [36,37,40] and sparkling wine lees [17], and were also found in the Cava lees analyzed (Table S1).

Terpenes are generally characterized by a floral odor and commonly found in sparkling wine [37,38]. Furthermore, vitispirane and nerolidol, identified in this study, have also been found in sparkling wine lees [17]. Overall, it was found that the addition of Cava lees increased terpenes concentration and variability in both sourdough bread and, especially, yeast leavened bread.

TDN (1,1,6-trimethyl-1,2-dihydronaphthalene) is a C13-norisoprenoid usually found in sparkling wine [36–38]. TDN has been pointed out as an ageing marker in sparkling wine, along with diethyl succinate (ester) and vitispirane (terpene) [37]. It was identified in both SB+L and B+L but not in the respective controls. Those compounds were found by Gallardo-Chacón et al. (2009) [17] in sparkling wine lees surface, as well as in our Cava lees analysis (Table S1).

Lastly, the results obtained were subjected to a PCA to determine the differences between the breads produced with and without sourdough and Cava lees. Figure 3 shows the result of a previous correlation analysis and Figure 4 presents de PCA biplot obtained. The PC1 and PC2 explain 79.02% of the total variability. The first principal component (PC1) explains a 52.37% of the samples variances while the second one (PC2) explains a 26.64%. Most of the volatile compounds were found in the positive side of PC1, especially esters, acids, and linear aldehydes, whereas branched aldehydes, alcohols and ketones were situated on the negative axis of PC1. On the other hand, the positive axis of PC2 contained a greater number of volatiles, including alcohols, linear aldehydes, ketones, and esters; while branched aldehydes and terpenes were situated in the negative side of PC2.

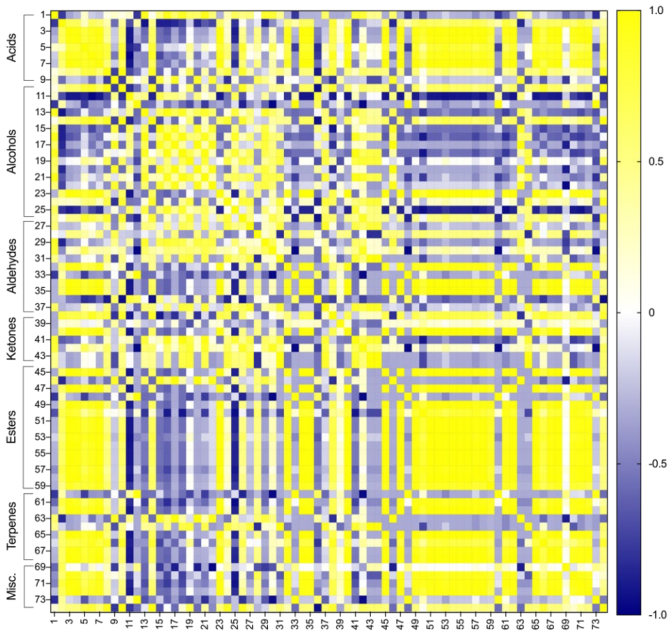

Figure 3. Heatmap of the correlation matrix of the volatile compounds ($p < 0.05$). Numbers correspond to the volatile compounds identified in Table 4. Positive correlations are shown in yellow; negative correlations in blue; absence of correlation in white.

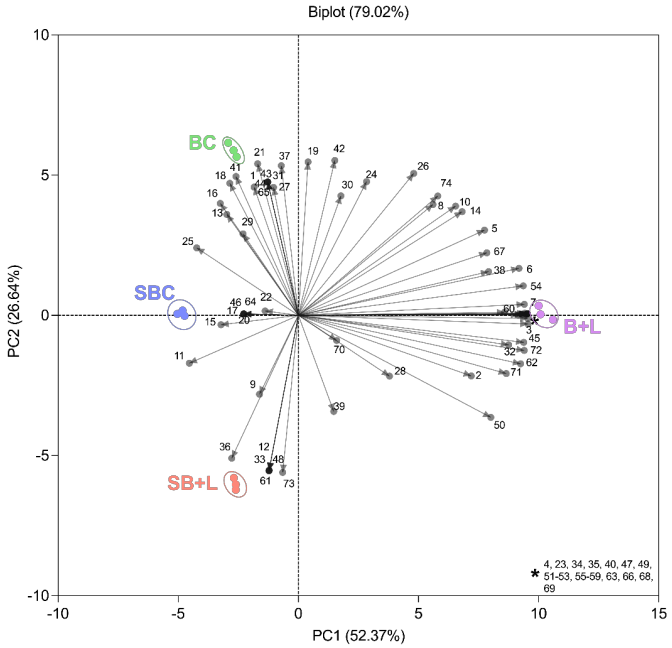

Figure 4. Principal Component Analysis (PCA) biplot of the breads obtained. SBC: control sourdough bread; SB+L: sourdough with 5% Cava lees; BC: control bread; B+L: bread with 5% Cava lees. Numbers correspond to the volatile compounds identified in Table 4.

It can be observed that both controls (SBC and BC) were placed in the same quadrant. In fact, both controls and SB+L were positioned opposite bread with Cava lees (B+L). SBC and BC were characterized by alcohols and ketones, while B+L was described by esters and terpenes. Moreover, a greater quantity of different volatiles was identified in B+L samples.

4. Conclusions

There are several factors involved in the development of bread flavor, from microbial activity to aroma precursors in the flour used. Therefore, it is important to determine the volatile fraction of the product to obtain consumers acceptance. Formulation of bread with 5% Cava lees (*w*/*w*) improved microbial growth (both LAB and yeast) in short fermentations, although there were no significant differences in prolonged fermentations (sourdough). Actually, LAB and yeast release aroma compounds as well as aroma precursors (including carbohydrates and amino acids) that can be transformed into the corresponding volatiles. Thus, higher microbial populations obtained with Cava lees might produce a greater concentration of volatile compounds due to LAB and yeast fermentation in dough. In general, the addition of Cava lees to bread increased the concentration of volatiles typically found in bread and sourdough bread. Also, some compounds usually reported in sparkling wines were also identified in samples with Cava lees, supporting the fact that yeast lees adsorb volatile compounds during wine ageing.

Therefore, it can be concluded that Cava lees promote the production of bread volatiles besides contributing with new odors from sparkling wine. Hence, the use of Cava lees as an ingredient in bread fermentation could be a new strategy to revalorize this winery by-product obtaining a new bread product. Also, further studies should focus on the effect

of Cava lees on identified microorganisms of sourdough and bread, since the fermenting microbiota can influence bread aroma and flavor.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/foods11091361/s1, Table S1: Main volatile compounds identified in Cava lees.

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CAPÍTOL 7. DISCUSSIÓ GENERAL

Segons la Organització Internacional de la Vinya i el Vi (OIV), la producció de vi a Espanya al 2019 va representar el 13% del total produït a tot el món, essent el major productor a nivell mundial. A més a més, el vi escumós, com el Cava, ha augmentat la seva producció en els últims anys[99]. Per tant, la indústria del Cava, genera grans quantitats de residus anuals. Alguns d'aquests residus, degut a la seva composició, podrien ser re-valoritzats ja que poden tenir aplicacions tecnològiques que podrien millorar altres processos dins la indústria alimentària i també efectes positius sobre la salut (Figura 10). D'aquesta manera, l'**objectiu general** d'aquesta tesi era estudiar la possible valorització de les lies del Cava com a nou ingredient en la formulació de la massa mare i el pa per tal d'afavorir-ne el procés fermentatiu, i avaluar el potencial prebiòtic de les lies sobre la microbiota intestinal.

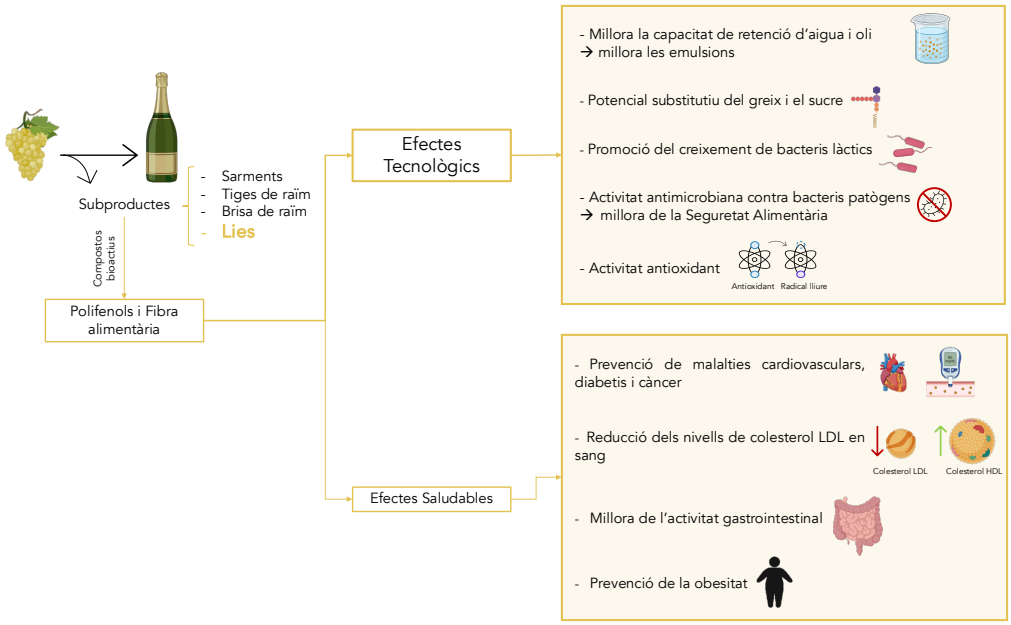


Figura 10. Efectes tecnològics i saludables dels polifenols i la fibra derivats dels diferents subproductes de l'elaboració del vi escumós. Adaptat de [100].

7.1. Potencial prebiòtic de les lies del Cava

El primer àmbit de la tesi està relacionat amb un potencial prebiòtic de les lies del Cava. Per això, es va realitzar un estudi *in vivo* en model animal (rates Wistar Han) en el que es va observar que l'administració d'una dosi de 3 x 10^6 cèl·lules de lies/Kg pes corporal diària a rates va modificar la microbiota intestinal dels animals d'estudi (Publicació 2).

Per la composició rica en fibra i altres polisacàrids, les lies no poden ser digerides per l'organisme, però si pels bacteris de la microbiota intestinal. Això els atorga un potencial prebiòtic teòric. De fet, una de les principals funcions de la microbiota intestinal és metabolitzar els compostos que arriben a l'intestí que no han pogut ser degradats pels enzims del nostre cos. D'aquesta manera, els polisacàrids més complexos i el midó poden ser digerits per aquests bacteris, promovent-ne la seva proliferació. En retorn, els microorganismes alliberen certs productes derivats del seu metabolisme (AGCC o monosacàrids) que llavors sí poden ser absorbits pel cos humà [101]. En aquest sentit, la ingesta d'aliments integrals, polifenols i fibra pot modificar positivament la microbiota intestinal, millorant la salut de l'hoste [102]. A més, un increment de la concentració d'AGCC també pot suposar una millora de la solubilitat i, per tant, l'absorció de certs minerals com per exemple el calci [96].

En general no es van trobar diferències significatives entre les rates control i les alimentades amb lies del Cava per a cap dels paràmetres generals (pes corporal, ingesta d'aliments i aigua). Així mateix, tampoc es van observar efectes adversos ni necrosi durant la continuïtat de l'estudi per a cap dels dos grups. Quan al pes dels òrgans, tampoc hi va haver diferència entre els dos grups, excepte el referent a melsa. Les rates mascle alimentades amb lies del Cava van acabar amb el pes de la melsa significativament superior al dels controls. Tot i això, els valors obtinguts per ambdós grups no van diferir dels reportats en rates sanes [103,104].

En avaluar l'efecte de les lies del Cava sobre la microbiota intestinal, es va observar que aquestes van potenciar l'abundància de bacteris pertanyents a famílies amb potencial probiòtic (p.e.: *Lactobacillaceae* o *Ruminococcaceae*)

(Figura 11). Així mateix, també es va observar una disminució en l'abundància de bacteris patògens (p.e.: *Clostridium* spp.o *Enterococcus* spp.).

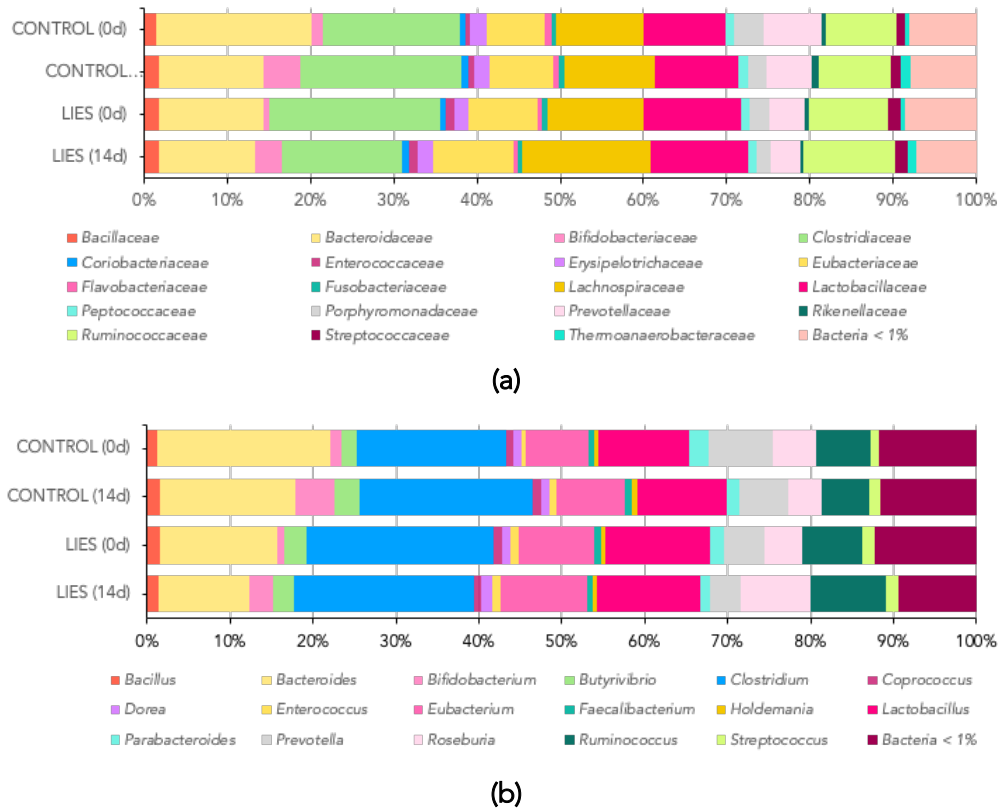


Figura 11. Abundància relativa (%) de bacteris de les diferents (**a**) famílies i (**b**) gèneres identificats a la microbiota intestinal dels animals del grup control i amb administració de lies a l'inici (0 dies) i final (14 dies) de l'estudi.

Dins els bacteris amb potencial probiòtic, *B. hansenii*, *R. intestinalis* i *R. obeum*, i diferents espècies de la família *Lactobacillaceae* van valors significativament més alts en la seva abundància respecte el control (Figura 12). Aquest tipus de bacteris, pertanyents al fílum Firmicutes, són productors d'AGCC com el butirat (majoritàriament), l'acetat o el propionat, que exerceixen diverses funcions de promoció de la salut de l'hoste [105,106].

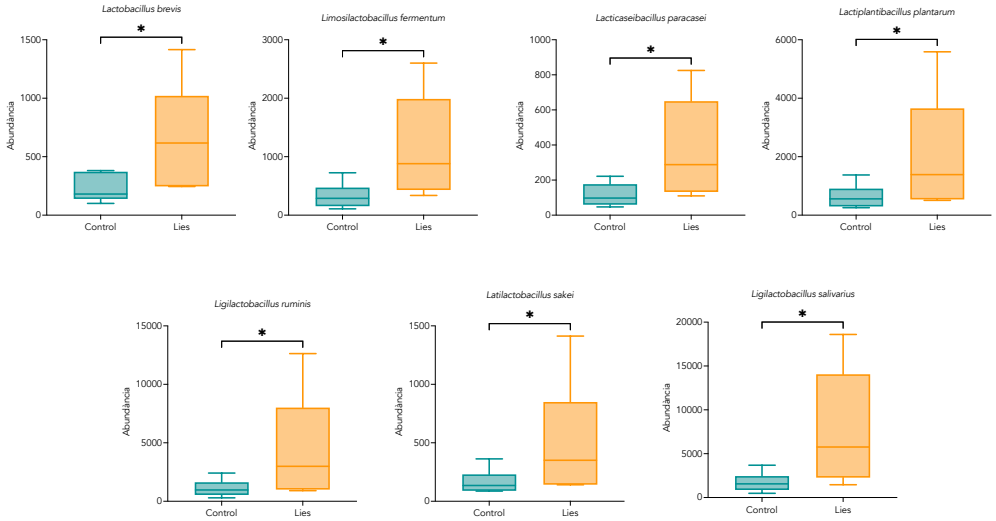


Figura 12. Diferències respecte l'abundància de bacteris de la família Lactobacillaceae amb potencial probiòtic. Les diferències significatives entre grups s'indiquen mitjançant un asteric (*).

D'aquesta manera, els resultats preliminars d'aquest estudi apunten cap a un potencial prebiòtic de les lies del Cava. A més, l'increment de l'abundància d'aquests bacteris podria resultar en un augment dels AGCC, pel que serien necessaris més estudis *in vivo* en el que també s'analitzin aquests compostos per tal de confirmar aquests resultats preliminars.

7.2. Caracterització de les lies del Cava

Com s'ha introduït anteriorment (**Secció 2.1.2.2**), durant l'envelliment del Cava es produeix l'autòlisi dels llevats. En aquest procés els llevats alliberen compostos al vi però, alhora, les lies poden adsorbir compostos a la seva superfície, com per exemple diferents compostos volàtils o fenòlics. Tot i així, hi ha pocs estudis centrats en els compostos volàtils retinguts per les lies, pel que es van haver de caracteritzar en aquest aspecte.

En general es va observar que cada Cava i les seves lies presentaven composicions molt similars entre ells (Publicació 3). La Figura 13 mostra les diferents lies obtingudes després de la liofilització. D'una banda, en mesurar l'IPT i la IC mitjançant espectroscòpia ultraviolada-visible, les lies presentaven valors

superiors respecte el Cava en ambdós paràmetres. En general, la major part de la producció de Cava són escumosos amb poc temps de criança biològica (classificats com a Cava de Guarda, aproximadament 9 mesos d'envelliment), pel que les lies residu dels cellers seran majoritàriament joves. Per tant, era d'esperar que les lies L-CV1 presentessin valors menors respecte els paràmetres estudiats.

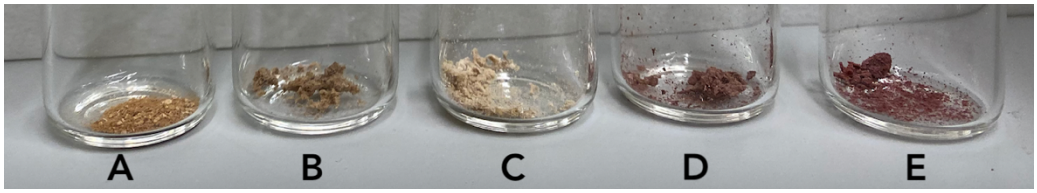
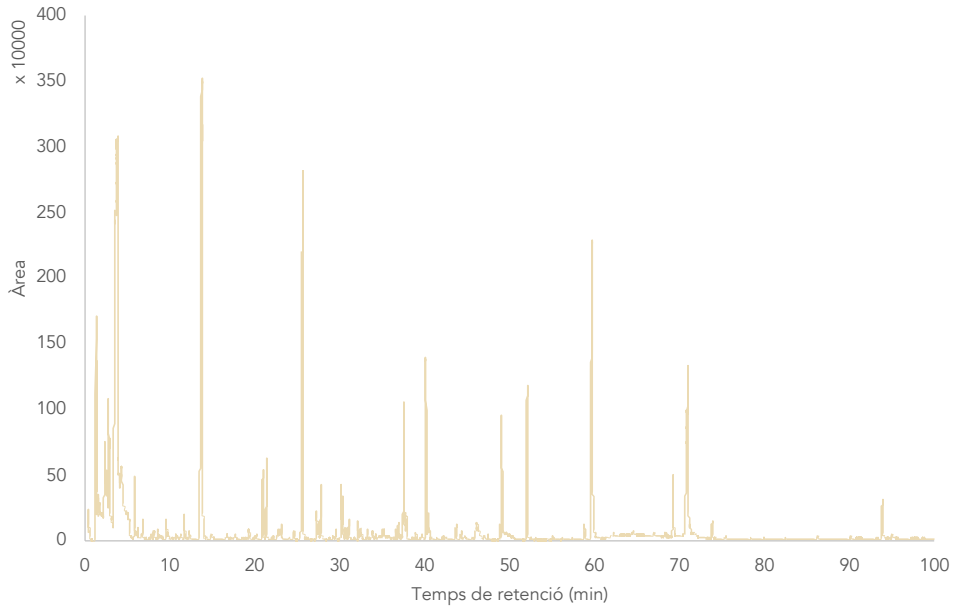


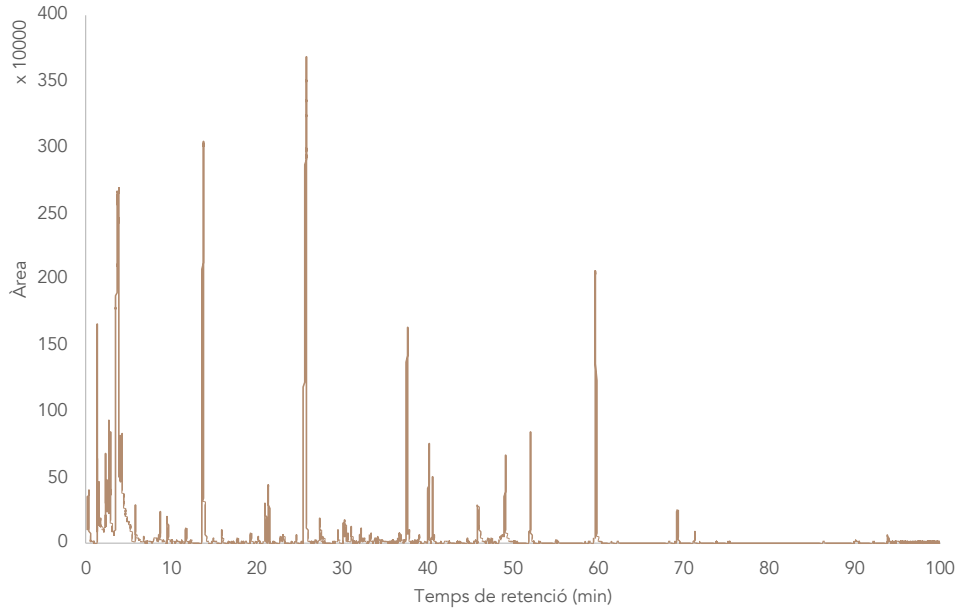
Figura 13. Lies del Cava liofilitzades: A) L-CV1 (residu celler); B) L-CGR1 (Blanc, Gran Reserva); C) L-CR1 (Blanc, Reserva); D) L-CRR1 (Rosat monovarietal, Reserva); i E) L-CRR2 (Rosat, Reserva).

Pel que fa a l'IPT, els valors obtinguts eren significativament superiors en lies blanques (9,64 ± 0,36– L-CR1 i 9,68 ± 0,48 – L-CGR1). L'IPT més baix va ser el de les lies residu (L-CV1), amb un valor de 6,36 ± 0,21. De la mateixa manera, les lies tenien una IC més alta que el Cava. Finalment, la TC presentava valors superiors en el Cava, augmentant amb el temps d'envelliment. A les mostres de lies també va augmentar en lies amb major envelliment. Tot això pot ser conseqüència de la precipitació i posterior adsorció de polifenols per part de les lies durant el temps d'envelliment del Cava [107].

D'altra banda, la fracció volàtil del vi escumós és un dels factors més rellevants en relació a la seva qualitat. De manera global, es va observar que les lies de major envelliment retenien més quantitat de compostos volàtils i presentaven una major similitud amb el seu Cava (compartint un 53% de compostos) (Figura 14). En comparar la composició de les lies del Cava separades segons el temps d'envelliment utilitzat respecte el residu del celler (L-CV1) es va observar que totes compartien un 33% del total de volàtils. Els compostos trobats únicament a les mostres L-CV1 pertanyen a la família dels terpens (**Taula 2 – Annex 1**). Els terpens, tot i ser compostos minoritaris, aporten aromes afruitats i florals al vi, a més d'olor a resina, llavors i arrels i s'han descrit com a compostos clau de l'aroma dels vins escumosos [108].



(a)



(b)

Figura 14. Cromatogrames del perfil volàtil del (**a**) Cava Gran Reserva i (**b**) les lies Gran Reserva.

A més a més, els compostos acetats (acetat d'hexil, acetat d'isoamil i acetat de 2-feniletil) i el decanoat d'etil presentaven valors més baixos d'abundància relativa en el Cava amb major envelliment. En canvi, els nivells de dietil succinat,

vitispiran i TDN van augmentar amb el temps, obtenint els valors més alts al Cava Gran Reserva (**Taula 1 – Annex 1**). De fet, Francioli et al. (2003) [109] havien proposat alguns d'aquests compostos com a marcadors d'envelliment pel Cava. En les mostres de lies es va observar una tendència similar a la del Cava, de manera que també es podrien diferenciar lies amb diferent temps d'envelliment en funció d'aquests compostos marcadors. Per tot això, les lies del Cava tenen capacitat de retenció de compostos a la seva superfície, incrementant amb el temps d'envelliment del Cava. Per tant, es considera que podrien modificar el perfil volàtil dels aliments on s'utilitzessin com a ingredient.

7.2. Les lies del Cava com a ingredient al pa

Després de caracteritzar les lies des d'un punt de vista organolèptic i determinar que aquestes podrien modificar el perfil aromàtic de l'aliment on s'utilitzin, es van aplicar a la fermentació de la massa mare i el pa. Primerament es van formular masses mare afegint lies a diferents concentracions i se'n va avaluar l'efecte sobre la fermentació (Publicació 4) i el perfil volàtil (Publicació 5). Posteriorment, fixant un percentatge del 5% de lies es va formular el pa i també es va determinar l'impacte sobre la fermentació i el perfil organolèptic (Publicació 6).

7.2.1. Promoció de la fermentació

En ambdós casos (blat i sègol), l'addició de lies del Cava entre un 0,5% i un 2% a la formulació de massa mare va augmentar els recomptes de BAL i llevats (Figura 15). Una vegada confirmada la hipòtesi de promoció de la fermentació (alcohòlica i làctica) de la massa mare formulada amb lies i basat en resultats previs *in vitro* (**Annex 2**), es va fixar la concentració de lies a la massa mare en un 5% i es va aplicar el mateix percentatge directament al pa (Publicació 6). Tal i com s'observa a la Figura 15A, en la massa mare l'augment de concentració de lies va resultar en un augment proporcional del número de cèl·lules viables de bacteris al final de la fermentació, tot i que entre el 2% i el 5% no s'hi van trobar diferències significatives. En canvi, en el cas dels llevats (Figura 15B), en afegir un 5% de lies del Cava a la formulació de la massa mare, aquests van incrementar significativament.

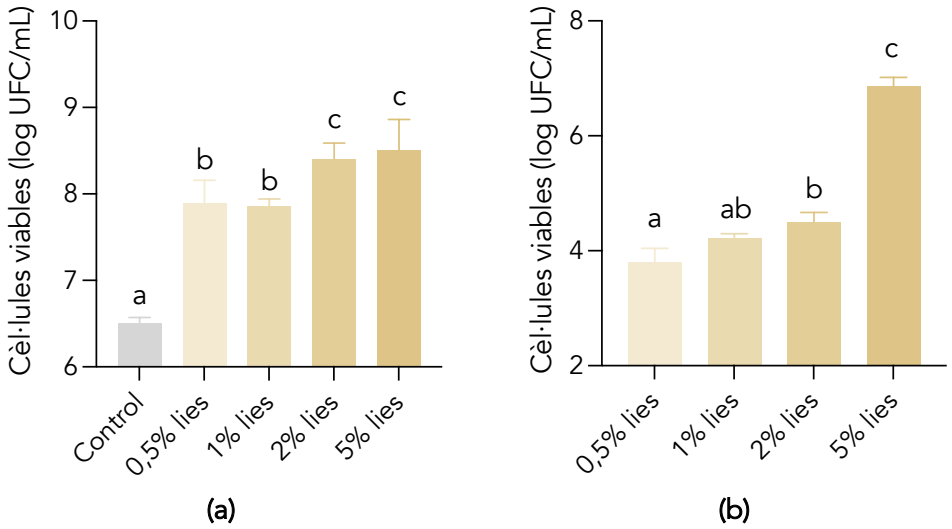


Figura 15. Cèl·lules viables de bacteris de l'àcid làctic (**a**) i llevats (**b**) al final de la fermentació de la massa mare de farina de blat formulada amb lies del Cava (0,5% - 5%). Diferents lletres indiquen diferències significatives entre les mostres.

Aquest augment del creixement de microorganismes durant la fermentació de la massa mare pot ser degut al contingut de fibra, ramnosa i manosa de les lies del Cava [2]. A més a més, alguns estudis ja havien observat *in vitro* una millora del creixement i supervivència de certes soques de bacteris entre els que s'hi troben els propis de la massa mare [2,29]. Per tant, les lies del Cava no només promocionen la fermentació làctica, sinó que també incrementen el creixement de llevats, responsables de la fermentació alcohòlica. Per això, pot ser que l'ús de les lies en altres aliments o begudes (p.e., kombutxa, iogurt o formatge) podria promocionar-hi la microbiota fermentativa.

Tot i així, en un ecosistema complex com és el de la massa mare, les poblacions de microorganismes evolucionen de manera constant. Aquests canvis respecte els microorganismes inicials de la farina i l'aigua són conseqüència de la manipulació i fermentació de la massa, els ingredients utilitzats (on es podria incloure l'addició de lies) i l'ambient (destacant la temperatura) [81]. De fet, la microbiota inicial passa per diverses fases de selecció natural on prevalen els microorganismes adaptats a l'acidesa, la temperatura i les poques fonts de nutrients pròpies de la massa [79,81]. És per això que en futures investigacions seria interesant la caracterització i tipificació mitjançant tècniques moleculars dels

diferents bacteris i llevats que es donen durant la fermentació de la massa mare amb la incorporació de lies del Cava.

Posteriorment es van aplicar les masses mare amb un 5% de lies com a cultiu iniciador de la fermentació del pa i paral·lelament es va formular pa fermentat amb un llevat comercial, amb i sense lies al 5%. En aquest cas, els recomptes de microorganismes, tant bacteris com llevats, en els pans amb lies van ser significativament superiors que en els controls, independentment de l'ús de la massa mare.

7.2.2. Millora de les característiques organolèptiques

La promoció dels microorganismes fermentadors també va derivar en canvis fisicoquímics de la massa mare (pH i àcids orgànics) i, conseqüentment, en el quocient de fermentació (QF). En referència al pH, les lies del Cava ja tenen un caràcter àcid (Publicació 3), pel que era d'esperar que acidifiquessin la massa mare.

El QF és la ràtio molar entre l'àcid làctic i l'àcid acètic i depèn directament de la composició microbiana de la massa mare i la seva activitat metabòlica [110]. Segons s'ha descrit, un QF d'entre 3 i 4 és característic de la fermentació làctica i té efectes positius sobre el perfil sensorial de la massa mare [110,111]. Per tant, en afegir lies del Cava a la formulació de la massa mare resulta en un QF menor (Figura 16), més proper als valors descrits en la literatura, tot i que no hi ha diferències significatives entre les diferents concentracions aplicades.

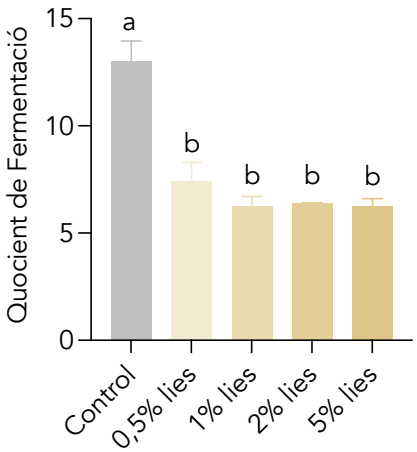


Figura 16. Quocient de fermentació (QF, ràtio molar entre l'àcid làctic i l'àcid acètic) de les masses mare formulades amb farina de blat amb diferents concentracions de lies del Cava (0,5% - 5%). Diferents lletres indiquen diferències significatives entre les mostres.

Addicionalment, la concentració d'àcid cítric i àcid màlic van augmentar proporcionalment a la quantitat de lies afegida (Publicació 4). D'una banda, els BAL poden metabolitzar l'àcid màlic en àcid làctic, pel que aquesta podria ser una de les causes de l'augment d'àcid làctic a la massa mare i, conseqüentment, la modificació del QF. A més, la presència de diferents àcids orgànics (acètic, làctic, cítric, etc.) poden allargar la vida útil del pa, ja que tenen activitat antimicrobiana [112]. D'altra banda, el metabolisme de l'àcid cítric per part dels bacteris produeix, entre d'altres, àcid acètic i acetoïna. Ambdós compostos van incrementar la seva concentració en mostres de massa mare amb lies del Cava (Publicació 4 i 5). De fet, l'acetoïna és un compost característic dels productes de forneria que proporciona aromes mantegosos.

Així mateix, l'addició de lies del Cava a la massa mare va suposar un increment per la majoria de compostos volàtils propis d'aquesta fermentació (Figura 17). Al mateix temps, es va observar que les lies del Cava i la massa mare compartien diversos compostos volàtils, com per exemple el 1-hexanol, l'hexanoat d'etil o el decanoat d'etil (Publicació 5). De fet, les masses mare amb lies del Cava van presentar perfils més complexes i amb major diversitat d'aldehids i èsters, respecte el control. Per tant, l'augment d'aquests compostos podria ser causa de l'increment de l'activitat metabòlica dels microorganismes fermentadors però també poden provenir directament les lies.

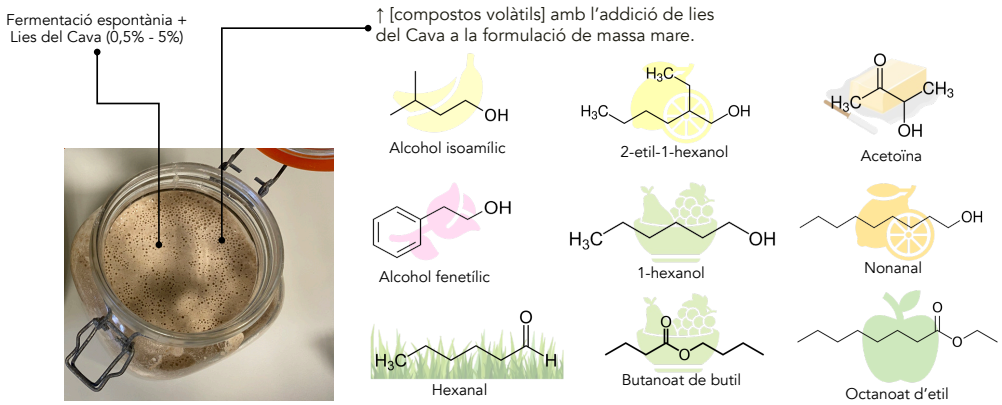


Figura 17. Compostos volàtils que van incrementar la seva concentració amb l'addició de lies del Cava.

A més a més, les lies del Cava també van aportar compostos propis dels vins escumosos, aportant un aroma diferencial a les masses mare (Figura 18). Quant als compostos volàtils del pa, l'addició de lies al 5% també va suposar un increment en la concentració dels compostos propis d'aquest aliment, així com l'aportació de volàtils típics del Cava (Figura 18). En general, per la majoria de paràmetres estudiats es va observar un major efecte de les lies sobre el pa fermentat amb llevats que sobre el formulat amb massa mare. Això és degut que tot i haver demostrat propietats de millora de la fermentació i els volàtils a la massa mare (Publicacions 4 i 5), quan s'utilitza com a cultiu iniciador aquesta s'afegeix en un percentatge menor del 30% del pes de la massa, pel que queda diluïda [113].

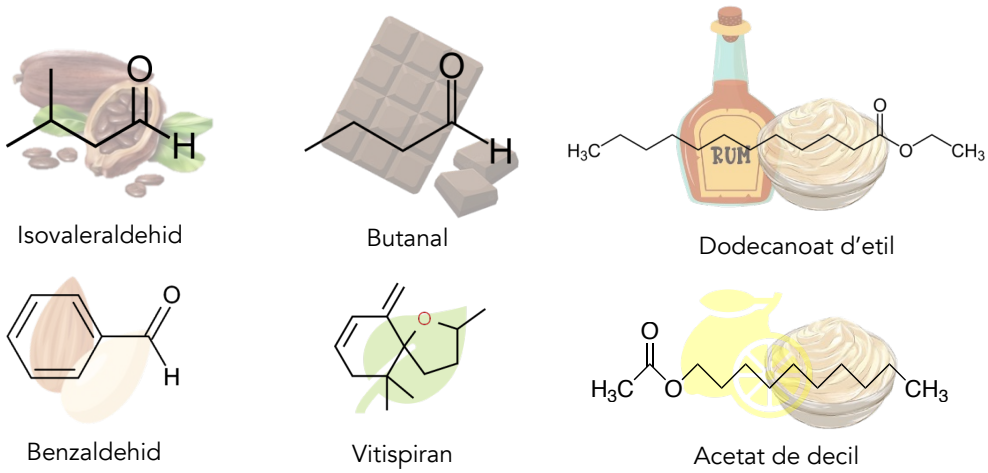


Figura 18. Compostos volàtils identificats només en masses mare i pa amb addició de lies del Cava.

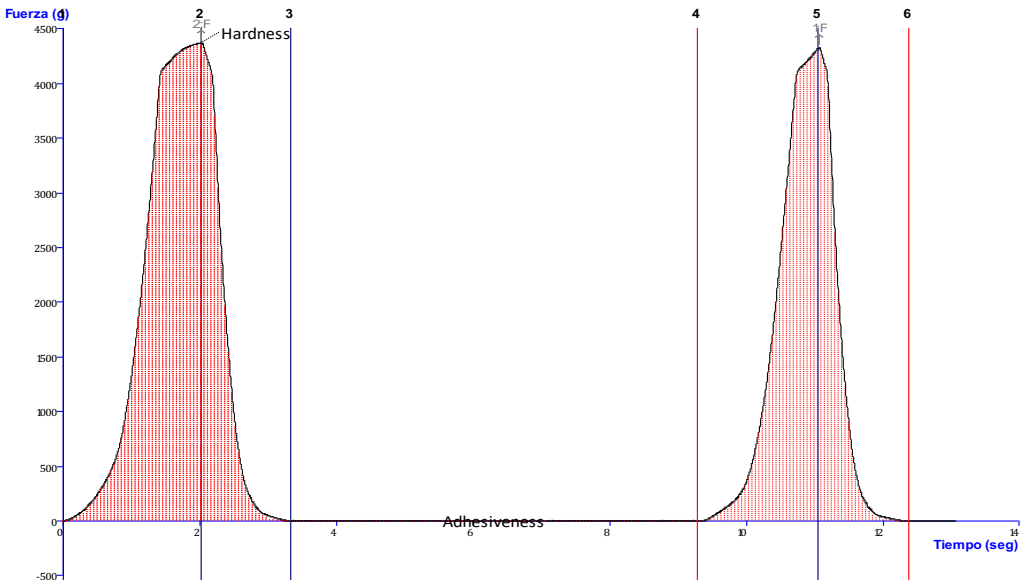
Així mateix també es analitzar el TPA dels diferents pans formulats (**¡Error! No se encuentra el origen de la referencia.**). En general, es van observar diferències significatives entre els pans amb i sense massa mare, independentment de l'addició de lies (Figura 19). Per tots els paràmetres de TPA, es van trobar diferències significatives entre els pans en funció de l'ús de massa mare, independentment de l'addició de lies del Cava. De fet, els pans sense massa mare presentaven valors superiors respecte els pans amb massa mare, especialment quant a duresa i masticació, pel que els pans elaborats amb massa mare eren pans més tous.

Taula 5. Anàlisi de textura (TPA) dels diferents pans formulats amb i sense massa mare i lies del Cava (5%).

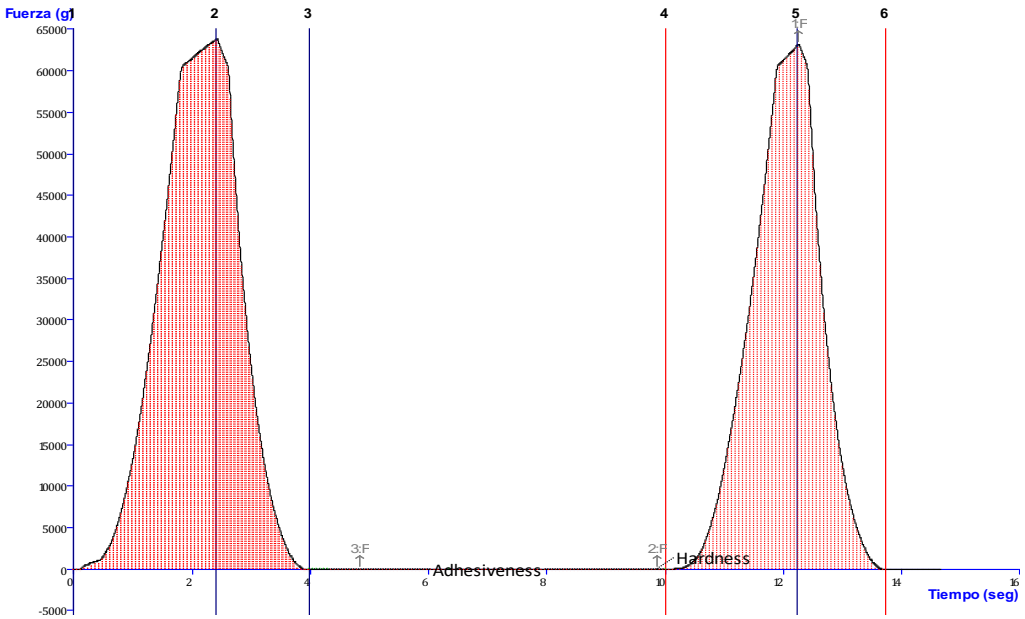
	Pa amb Massa Mare	Pa amb Massa Mare i Lies	Pa	Pa i Lies
Duresa (g)	43,7 ± 0,9 ^{a}	44,6 ± 1,3 ^{a}	53,0 ± 1,5 ^{b}	51,7 ± 1,9 ^{b}
Elasticitat	0,68 ± 0,03 ^{a}	0,65 ± 0,05 ^{a}	0,88 ± 0,04 ^{b}	0,91 ± 0,02 ^{b}
Resistència	0,44 ± 0,03 ^{ab}	0,35 ± 0,06 ^{b}	0,54 ± 0,03 ^{a}	0,50 ± 0,04 ^{a}
Cohesió	0,53 ± 0,04 ^{a}	0,61 ± 0,03 ^{a}	0,83 ± 0,01 ^{b}	0,82 ± 0,04 ^{b}
Masticació (g)	15,6 ± 0,6 ^{a}	17,5 ± 0,7 ^{a}	38,4 ± 0,9 ^{b}	37,2 ± 1,1 ^{b}

Els valors són la mitjana ± desviació estàndard de triplicats. Les diferències significatives entre mostres es troben indicades amb lletres al superíndex ($p < 0,05$) per a cada propietat.

Tant la cohesió com la masticació són paràmetres relacionats amb la consistència del pa i l'energia necessària per mastegar-lo [114,115]. Els valors obtinguts van mostrar que els pans elaborats amb massa mare tenien un grau de cohesió menor que els pans fermentats amb llevat, i requerien menor energia per mastegar-los. D'aquesta manera, els valors de cohesió i masticació indicaven que el pa amb massa mare és de més fàcil masticació.



(a)



(b)

Figura 19. Anàlisi del perfil de textura (TPA) del (**a**) pa fermentat amb massa mare i (**b**) pa fermentat amb llevat comercial.

Tot i això, degut que la formulació amb aquest tipus de subproductes podria alterar el perfil organolèptic de l'aliment [116–118], es van realitzar anàlisis sensorials dels pans elaborats amb i sense lies del Cava (Figura 20). També es va realitzar un anàlisi sensorial dels pans elaborats amb les diferents masses mare, però tal i com s'ha comentat anteriorment, la massa mare s'incorpora en menys d'un 30%, pel que l'impacte és menor i no s'hi van trobar diferències significatives respecte el control.

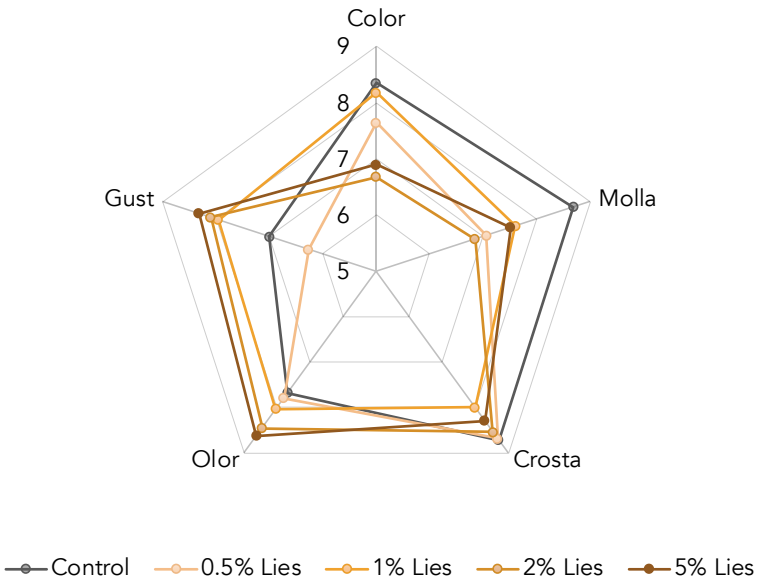


Figura 20. *Spider-web* de l'anàlisi sensorial de pa elaborat amb i sense lies del Cava.

Tot i ser una fracció important per l'acceptació per part dels consumidors, no s'han realitzat gaires anàlisis sensorials de productes elaborats amb lies. Així mateix, els pocs estudis on sí s'han realitzat, els tastadors van atorgar majors puntuacions en quant a textura i olor a les hamburgueses [116] i gelats [118] elaborats amb lies.

En el resultats obtinguts d'aquesta tesi, el panel de tastadors van puntuar positivament el gust i l'olor dels pans elaborats amb lies del Cava, especialment els del 2% i 5% de lies. Contràriament, el color i la molla del pa van rebre menor

puntuació en aquests pans. En general, els tastadors van destacar que en afegir les lies del Cava al pa de blat el color es tornava més fosc , arribant a comparar-lo amb el del pa de farina de sègol. Tot i així, es va valorar molt positivament el canvi en l'aroma, que recordava al raïm i el vi, recolzant els resultats obtinguts per HS-SPME-GC-MS (Publicacions 5 i 6), resultant en la obtenció d'un producte diferencial. Per tant, els compostos adsorbits a la superfície de les lies tenen un impacte directe sobre el perfil volàtil dels aliments on s'incorporen. Així mateix, també seria d'interès per a futures investigacions l'ús de diferents lies (en funció del tipus de raïm utilitzats) per avaluar l'efecte d'aquestes sobre el color del producte final, ja que s'ha observat diferent composició entre lies amb diferent origen (Publicació 3).

CAPÍTOL 8. CONCLUSIONS

Potencial prebiòtic de les lies del Cava

1. La ingesta de lies del Cava resulta en un augment de l'abundància relativa de bacteris probiòtics, pel que les lies poden tenir un potencial efecte prebiòtic sobre la microbiota intestinal.

Caracterització de les lies del Cava

2. Durant l'envelliment biològic del Cava les lies retenen compostos a la seva superfície (volàtils i fenòlics).

3. Les lies del Cava poden ser una font potencial d'aromes i utilitzar-se com a nou ingredient per a la formulació d'aliments.

Les lies del Cava com a ingredient

4. L'addició de lies del Cava a la formulació de la massa mare en un 2% i en el pa en un 5% promou la fermentació làctica, provocant un increment de la producció d'àcids orgànics com l'acètic o el làctic, i, per tant, a l'acidesa de la massa. Això contribueix a una major estabilitat microbiològica promovent la seguretat alimentària del producte.

5. L'addició de lies del Cava a la massa mare i el pa augmenta la concentració dels compostos volàtils del producte i aporta compostos propis del Cava i el vi. A major concentració de lies, major concentració de volàtils.

L'ús de les lies del Cava com a ingredient en aliments fermentats és una potencial estratègia per la revalorització de dit subproducte, contribuint a una economia circular i als ODS.

CAPÍTOL 9. REFERÈNCIES

Llistat de les contribucions científiques derivades de la tesi doctoral

Publicació 1: **Martín-Garcia, A.**; Riu-Aumatell, M.; López-Tamames, E. Influence of Process Parameters on Sourdough Microbiota, Physical Properties and Sensory Profile. *Food Reviews International* **2021**, 1–15, doi:10.1080/87559129.2021.1906698

Publicació 2: **Martín-Garcia, A.**; Riu-Aumatell, M.; López-Tamames, E. Potential Prebiotic Effect of Cava Lees: Changes in Gut Microbiota. *Enviada per a la seva publicació a Fermentation, **Agost 2022***.

Publicació 3: **Martín-Garcia, A.**; Riu-Aumatell, M.; López-Tamames, E. Characterization of white and rosé sparkling wine lees surface volatiles. *Enviada per a la seva publicació a BIO Web of Conferences, **Setembre 2022***.

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CAPÍTOL 10. ANNEXES

Annex 1. Material suplementari de la Publicació 2.

Taula 1. Abundància relativa (expressada com a % d'àrea) dels compostos volàtils identificats en les mostres de Cava.

Compost	Núm. CAS	Aroma [1]	CGR1	CR1	CRR1	CRR2
ÀCIDS						
Àcid Acètic	64-19-7	vinagre, agre, picant	1,01 ± 0,39	1,30 ± 0,33	1,48 ± 0,12	1,55 ± 0,29
Àcid Hexanoic	142-62-1	formatge, agre	4,20 ± 0,74 [a]	3,46 ± 0,74 [ab]	2,46 ± 0,34 [b]	2,42 ± 0,83 [b]
Àctic Octanoic	**124-07-2**	**cera, ranci, oliós, formatge, vegetal**	**12,52 ± 2,49 [a]**	**12,30 ± 1,66 [a]**	**8,86 ± 0,37 [ab]**	**7,56 ± 1,98 [b]**
Àcid Decanoic	334-48-5	cítric, ranci	1,76 ± 0,69 [a]	1,47 ± 0,47 [ab]	0,64 ± 0,11 [b]	1,27 ± 0,15 [ab]
Àcid Tetradecanoic	544-63-8	coco	0,34 ± 0,04	nd	nd	nd
Àcid Hexadecanoic	57-10-3	cera	0,93 ± 0,53	0,44 ± 0,08	nd	nd
Total Àcids			20,76 ± 4,88 [a]	18,97 ± 3,28 [ab]	13,44 ± 0,94 [b]	12,80 ± 3,25 [b]
ALDEHIDS						
Nonanal	124-19-6	rosa, iris, pell de taronja	0,46 ± 0,01	nd	nd	nd
Acetaldehyde	75-07-0	afruitat, picant, eteri	3,41 ± 0,04	nd	nd	nd
Furfural	98-01-1	pa, ametlla, llenya	1,87 ± 0,40 [a]	0,25 ± 0,04 [b]	0,55 ± 0,11 [b]	0,57 ± 0,14 [b]
Benzaldehyde	100-52-7	cirera, ametlla amarga	0,43 ± 0,12 [a]	0,38 ± 0,03 [a]	0,65 ± 0,10 [b]	nd
2-methylbenzaldehyde	529-20-4	cirera	nd	2,88 ± 0,10	nd	nd
3-methylbenzaldehyde	620-23-5	cirera, afruitat, fenòlic	1,80 ± 0,09	nd	nd	1,50 ± 0,10
4-methylbenzaldehyde	104-87-0	afruitat, cirera	2,38 ± 0,77	nd	nd	nd
Total Aldehids			10,35 ± 1,43 [a]	3,51 ± 0,17 [b]	1,20 ± 0,21 [c]	2,07 ± 0,24 [bc]
ALCOHOLS						
2-butanol	78-92-2	afruitat, albercoc	nd	nd	nd	0,47 ± 0,12
1-hexanol	111-27-3	eteri, afruitat, alcohòlic, dolç, verd	2,06 ± 1,33	2,02 ± 1,03	1,82 ± 0,26	3,12 ± 0,98
1-octanol	111-87-5	bolets, rosa, verd, taronja	0,31 ± 0,06	0,28 ± 0,05	nd	nd
(Z)-3-hexen-1-ol	928-96-1	herba tallada, oli d'herbes, vegetal	0,66 ± 0,33	0,20 ± 0,02	nd	nd

Taula 1. *Continuació.*

Compost	Núm. CAS	Aroma [1]	CGR1	CR1	CRR1	CRR2
Isoamyl alcohol	**123-51-3**	**whisky, alcohòlic, afruitat, plàtan**	**13,57 ± 2,34** [ab]	**9,74 ± 1,91** [a]	**15,40 ± 5,13** [ab]	**18,49 ± 4,31** [b]
2-methyl-1-propanol	78-83-1	vi, whiskey	nd	nd	0,39 ± 10,18	0,59 ± 0,16
2-phenylethanol	60-12-8	rosa, floral, rosa seca, aigua de roses	4,27 ± 0,93	4,02 ± 0,64	5,06 ± 0,67	6,45 ± 2,76
Total Alcohols			20,87 ± 4,99 [ab]	16,26 ± 3,65 [a]	22,67 ± 6,24 [ab]	29,12 ± 8,33 [b]
CETONES						
2-Nonanone	821-55-6	fresh sweet green weedy earthy herbal	0,31 ± 0,14	nd	0,36 ± 0,11	nd
4-methyl-2-heptanone	6137-06-0	-	0,26 ± 0,01	nd	nd	0,25 ± 0,05
Total Cetones			0,57 ± 0,15 [a]	nd	0,36 ± 0,11 [b]	0,25 ± 0,05 [b]
ÈSTERS						
Acetic acid, ethyl ester	141-78-6	afruitat, dolç, verd, herba	3,33 ± 0,74 [a]	2,81 ± 0,12 [a]	4,90 ± 1,44 [a]	9,52 ± 2,50 [b]
Acetic acid, hexyl ester	142-92-7	afruitat, poma verda, plàtan, dolç	nd	0,42 ± 0,10	0,74 ± 0,07	nd
Butanoic acid, ethyl ester	105-54-4	afruitat, conyac, pinya	1,33 ± 0,37	0,94 ± 0,13	1,00 ± 0,32	1,26 ± 0,29
Butanoic acid, 2-methyl-, ethyl ester	7452-79-1	dolç, poma verda, afruitat	0,20 ± 0,02	nd	nd	0,32 ± 0,10
Butanoic acid, 3-methyl-, ethyl ester	108-64-5	afruitat, poma dolça, pinya	0,30 ± 0,07 [a]	1,10 ± 0,04 [b]	0,20 ± 0,04 [a]	0,54 ± 0,15 [c]
1-Butanol, 3-methyl-, acetate	123-92-2	dolç, afruitat, plàtan	0,18 ± 0,02 [a]	0,66 ± 0,17 [ab]	1,27 ± 0,14 [b]	1,31 ± 0,70 [b]
Diethyl succinate	123-25-1	poma cuita	5,44 ± 0,86	3,31 ± 0,59	3,40 ± 0,36	4,69 ± 1,88
Hexanoic acid, ethyl ester	**123-66-0**	**dolç, afruitat, pinya, plàtan verd**	**20,77 ± 8,39**	**13,41 ± 3,92**	**20,04 ± 4,20**	**19,72 ± 4,56**
2-Hydroxyethyl propionate	97-64-3	mantegós, afruitat, caramel	3,15 ± 0,16 [a]	1,73 ± 0,15 [b]	1,70 ± 0,18 [b]	nd

Taula 1. *Continuació.*

Compost	Núm. CAS	Aroma [1]	CGR1	CR1	CRR1	CRR2
Formic acid, hexyl ester	629-33-4	poma, pruna verda, plàtan, dolç	2,77 ± 0,31	nd	nd	nd
Ethyl 2-hydroxy-4-methylvalerate	10348-47-7	fresc, mora	0,25 ± 0,02	nd	nd	nd
Ethyl 2-furoate	614-99-3	balsàmic, afruitat, floral, orquídea, cremat	0,40 ± 0,11	0,29 ± 0,05	nd	0,33 ± 0,11
Octanoic acid, ethyl ester	**106-32-1**	**afruitat, vi, albercoc, plàtan, brandi, pera**	**21,90 ± 4,35** [a]	**41,46 ± 7,11** [b]	**34,22 ± 3,65** [b]	**21,77 ± 5,49** [a]
Decanoic acid, ethyl ester	110-38-3	afruitat, poma, raïm, brandi	4,62 ± 1,58 [a]	8,79 ± 2,16 [b]	5,59 ± 0,68 [ab]	6,42 ± 2,12 [ab]
Dodecanoic acid, ethyl ester	106-33-2	dolç, floral	0,32 ± 0,17	nd	nd	0,35 ± 0,02
Tetradecanoic acid, ethyl ester	124-06-1	dolç, violeta	nd	0,65 ± 0,18	nd	nd
Diethyl malate	7554-12-3	brown sugar, sweet, wine, fruity, herbal	0,40 ± 0,22	nd	nd	nd
Total Èsters			65,36 ± 17,39	75,57 ± 14,72	73,06 ± 11,08	66,23 ± 17,91
TERPENS I NORISOPRENOIDS						
Vitispirane A	6965-94-4	floral, fruity, earthy, woody	3,27 ± 0,51 [a]	1,31 ± 0,29 [b]	1,49 ± 0,42 [b]	1,14 ± 0,11 [b]
Total Terpens i Norisoprenoids			3,27 ± 0,51 [a]	1,31 ± 0,29 [b]	1,49 ± 0,42 [b]	1,14 ± 0,11 [b]

[1] Obtingut de [33]. Els valors són mitjana ± desviació estàndard de triplicats. Les diferències significatives entre mostres estan indicades amb diferents lletres en superíndex ($p < 0,05$) per a cada compost. nd: no detectat.

Taula 2. Abundància relativa (expressada com a % d'àrea) dels compostos volàtils identificats en les mostres de lies del Cava.

Compost	Núm. CAS	Aroma [1]	L-CGR1	L-CR1	L-CRR1	L-CRR2	L-CV1
ÀCIDS							
Àcid Acètic	64-19-7	vinagre, agre, picant	3,30 ± 0,90 [a]	0,74 ± 0,10 [b]	0,14 ± 0,01 [b]	0,49 ± 0,18 [b]	0,46 ± 0,11 [b]
Àctic Octanoic	124-07-2	cera, ranci, oliós, formatge, vegetal	14,41 ± 4,36 [a]	6,54 ± 1,25 [b]	4,03 ± 0,04 [b]	2,43 ± 0,19 [b]	16,25 ± 4,74 [a]
Àcid Nonanoic	112-05-0	formatge, cera, làctic	0,92 ± 0,32 [a]	0,36 ± 0,12 [b]	0,24 ± 0,13 [b]	0,20 ± 0,08 [b]	nd
Àcid Decanoic	334-48-5	cítric, ranci	8,12 ± 2,15 [a]	7,68 ± 0,37 [a]	5,09 ± 0,23 [a]	3,49 ± 0,37 [a]	15,68 ± 5,84 [b]
Àcid Dodecanoic	143-07-7	oli de coco	1,06 ± 0,38 [a]	0,76 ± 0,08 [a]	0,28 ± 0,08 [b]	0,28 ± 0,11 [b]	1,63 ± 0,17 [c]
Àcid Tetradecanoic	544-63-8	coco	1,45 ± 0,32	0,33 ± 0,06	nd	nd	nd
Àcid Hexadecanoic	57-10-3	cera	2,69 ± 0,91 [a]	0,45 ± 0,11 [b]	0,49 ± 0,12 [b]	0,12 ± 0,07 [b]	0,35 ± 0,05 [b]
Total Àcids			31,95 ± 9,34 [a]	16,85 ± 2,10 [b]	10,27 ± 0,61 [b]	7,01 ± 1,00 [b]	34,37 ± 10,91 [a]
ALDEHIDS							
Octanal	124-13-0	cítric, pell taronja, herbaci	0,61 ± 0,08 [a]	0,42 ± 0,03 [b]	0,29 ± 0,02 [c]	nd	nd
Nonanal	124-19-6	rosa, iris, pell de taronja	3,94 ± 0,18 [a]	1,40 ± 0,61 [b]	1,11 ± 0,50 [b]	0,94 ± 0,48 [b]	0,53 ± 0,27 [b]
Decanal	112-31-2	dolç, pell taronja, cítric, floral	nd	1,10 ± 0,01 [a]	0,36 ± 0,05 [b]	0,17 ± 0,08 [c]	0,46 ± 0,07 [b]
Furfural	98-01-1	pa, ametlla, llenya	10,43 ± 2,09 [a]	1,34 ± 0,46 [b]	2,11 ± 1,31 [b]	1,95 ± 0,87 [b]	nd
5-methylfurfural	620-02-0	picant, caramel, xarop, cafè, pa	2,13 ± 0,91 [a]	nd	0,52 ± 0,10 [b]	0,26 ± 0,06 [b]	nd
Benzaldehyde	100-52-7	cirera, ametlla amarga	nd	nd	nd	nd	0,38 ± 0,03
Total Aldehids			17,11 ± 3,26 [a]	4,25 ± 1,11 [b]	4,39 ± 1,98 [b]	3,32 ± 1,49 [b]	1,37 ± 0,37 [b]

Taula 2. *Continuació.*

Compost	Núm. CAS	Aroma [1]	L-CGR1	L-CR1	L-CRR1	L-CRR2	L-CV1
ALCOHOLS							
1,3-butanediol	107-88-0	-	1,16 ± 0,05	nd	nd	nd	nd
2-hexanol	626-93-7	vi, afruitat, coliflor	nd	nd	11,86 ± 2,80	2,75 ± 0,19	nd
2-ethylhexanol	104-76-7	cítric, floral, dolç	3,11 ± 0,26 [a]	1,09 ± 0,66 [b]	1,43 ± 0,86 [b]	0,64 ± 0,20 [b]	nd
1-octanol	111-87-5	bolets, rosa, verd, taronja	1,33 ± 0,23	nd	nd	nd	0,18 ± 0,02
1-nonanol	143-08-8	floral, rosa, taronja, picant	9,68 ± 1,29	nd	1,06 ± 0,47	nd	nd
Isoamyl alcohol	123-51-3	whisky, alcohòlic, afruitat, plàtan	1,11 ± 0,12	nd	nd	nd	2,03 ± 0,62
2-phenylethanol	60-12-8	rosa, floral, rosa seca, aigua de roses	4,05 ± 1,11 [a]	1,29 ± 0,41 [b]	1,72 ± 0,68 [b]	1,77 ± 1,00 [b]	1,07 ± 0,34 [b]
Total Alcohols			20,44 ± 3,06 [a]	2,37 ± 1,06 [b]	16,07 ± 4,81 [a]	5,16 ± 1,39 [b]	3,28 ± 0,98 [b]
CETONES							
2-undecanone	112-12-9	afruitat, cremós, floral, lliri	nd	nd	nd	nd	0,30 ± 0,01
4-methyl-2-pentanone	108-10-1	verd, herbaci, afruitat, làctic, picant	3,38 ± 0,63	0,72 ± 0,04	nd	nd	nd
Total Cetones			3,38 ± 0,63 [a]	0,72 ± 0,04 [b]	nd	nd	0,30 ± 0,01 [b]
ÈSTERS							
Acetic acid, hexyl ester	142-92-7	afruitat, poma verda, plàtan, dolç	nd	nd	nd	nd	1,23 ± 0,39
Acetic acid, 2-ethylhexyl ester	103-09-3	terrós, herbaci	0,64 ± 0,09	0,31 ± 0,02	nd	nd	nd
Butanoic acid, butyl ester	109-21-7	afruitat, plàtan, pinya, cirera, fruita tropical	0,40 ± 0,12	nd	nd	nd	nd

Taula 2. *Continuació.*

Compost	Núm. CAS	Aroma [1]	L-CGR1	L-CR1	L-CRR1	L-CRR2	L-CV1
Diethyl succinate	123-25-1	poma cuita	3,01 ± 0,21 [a]	0,45 ± 0,01 [b]	3,24 ± 1,83 [a]	1,95 ± 0,47 [ab]	0,57 ± 0,12 [b]
Hexanoic acid, ethyl ester	123-66-0	dolç, afruitat, pinya, plàtan verd	1,04 ± 0,26 [a]	0,26 ± 0,02 [a]	nd	1,16 ± 0,31 [a]	2,34 ± 0,87 [b]
Ethyl 2-furoate	614-99-3	balsàmic, afruitat, floral, orquídea, cremat	nd	nd	nd	nd	0,25 ± 0,04
Octanoic acid, ethyl ester	**106-32-1**	**afruitat, vi, albercoc, plàtan, brandi, pera**	**6,32 ± 1,47 [a]**	**15,26 ± 6,04 [ab]**	**17,64 ± 1,23 [b]**	**20,14 ± 7,63 [b]**	**22,89 ± 0,35 [b]**
Octanoic acid, 3-methylbutyl ester	2035-99-6	afruitat, dolç, pinya, coco, cognac	nd	2,05 ± 0,06	2,41 ± 0,70	3,44 ± 1,21	2,74 ± 0,29
Nonanoic acid, ethyl ester	123-29-5	afruitat, rosa, vi, cognac	nd	nd	nd	0,10 ± 0,02	nd
Decanoic acid, ethyl ester	**110-38-3**	**afruitat, poma, raïm, brandi**	**29,15 ± 12,53**	**39,50 ± 3,18**	**41,24 ± 11,73**	**47,18 ± 8,06**	**41,00 ± 5,58**
3-methylbutyl decanoate	2306-91-4	plàtan, afruitat, cognac, dolç	1,18 ± 0,02 [ab]	1,96 ± 0,60 [b]	1,19 ± 0,03 [ab]	1,48 ± 0,63 [ab]	1,06 ± 0,05 [a]
Dodecanoic acid, ethyl ester	106-33-2	dolç, floral	9,82 ± 2,63 [a]	8,73 ± 0,72 [ab]	6,10 ± 2,38 [ab]	5,04 ± 1,91 [b]	5,98 ± 0,03 [ab]
Tetradecanoic acid, ethyl ester	124-06-1	dolç, violeta	nd	0,67 ± 0,05 [a]	0,78 ± 0,07 [a]	0,75 ± 0,14 [a]	0,29 ± 0,07 [b]
Pentadecanoic acid, ethyl ester	41114-00-5	dolç, mel	10,10 ± 0,34	nd	nd	nd	nd
Hexadecanoic acid, ethyl ester	628-97-7	afruitat, cremós, balsàmic, greixós	1,60 ± 0,49	1,21 ± 0,37	2,11 ± 1,66	1,54 ± 0,48	0,94 ± 0,03
Diethyl malate	7554-12-3	brown sugar, sweet, wine, fruity, herbal	1,04 ± 0,09	nd	nd	nd	nd
Total Èsters			64,30 ± 18,25	70,37 ± 11,07	74,71 ± 19,63	82,78 ± 20,86	79,29 ± 7,82

Taula 2. *Continuació.*

Compost	Núm. CAS	Aroma [1]	L-CGR1	L-CR1	L-CRR1	L-CRR2	L-CV1
TERPENS I NORISOPRENOIDS							
Vitispirane A	6965-94-4	floral, fruity, earthy, woody	2,30 ± 0,17 ^{abc}	1,69 ± 0,54 ^{b}	2,52 ± 0,23 ^{c}	1,94 ± 0,12 ^{b}	2,63 ± 0,20 ^{c}
Naphthalene, 1,2-dihydro-1,1,6-trimethyl- (TDN)	30364-38-6	regalèssia	4,33 ± 1,02 ^{a}	2,90 ± 0,91 ^{a}	3,75 ± 1,49 ^{a}	4,54 ± 0,53 ^{a}	8,06 ± 0,08 ^{b}
α-Farnesene	502-61-4	llenyós, cítric, herbaci, mirra, bergamot	nd	nd	nd	0,05 ± 0,01	0,26 ± 0,06
β-Caryophyllene	87-44-5	dolç, llenyós, picant, clau	nd	nd	nd	nd	0,12 ± 0,01
Farnesol	4602-84-0	fresc, dolç, floral, til·ler	nd	nd	nd	nd	0,46 ± 0,02
Isocaryophyllene	118-65-0	llenyós, picant	nd	nd	nd	nd	0,40 ± 0,02
Nerolidol	7212-44-4	floral, cítric, llenyós	nd	nd	nd	nd	0,27 ± 0,08
Total Terpens i Norisoprenoids			6,63 ± 1,19 ^{a}	4,59 ± 1,44 ^{b}	6,27 ± 1,72 ^{ab}	6,53 ± 0,75 ^{ab}	12,20 ± 0,47 ^{c}

[1] Obtingut de [33]. Els valors són mitjana ± desviació estàndard de triplicats. Les diferències significatives entre mostres estan indicades amb diferents lletres en superíndex ($p < 0,05$) per a cada compost. nd: no detectat.

Annex 2. Material suplementari de la Publicació 5.

foods

MDPI

Supplementary Material

Table S1. Main volatile compounds identified in Cava lees.

Compound	CAS-Num.	Odor [1]	Relative peak area abundance
ALCOHOLS			
3-methyl-1-butanol	123-51-3	fusel, oil, alcoholic, whiskey, fruity, banana	2.03 ± 0.62
1-Hexanol	111-27-3	sweet, resin, flower	0.40 ± 0.07
1-Octanol	111-87-5	moss, nut, mushroom, chemical	0.18 ± 0.02
2-Dodecanol	10203-28-8	-	0.16 ± 0.01
1-Decanol	112-30-1	fatty, waxy, floral, orange, sweet	0.34 ± 0.03
Phenethyl alcohol	60-12-8	floral, rose	1.07 ± 0.34
Total Alcohols			*4.18 ± 1.09*
ACIDS			
Acetic acid	64-19-7	pungent, sour	0.46 ± 0.11
Octanoic acid	124-07-2	oily, rancid	16.25 ± 4.74
Decanoic acid	334-48-5	sour, fatty	15.68 ± 5.84
Dodecanoic acid	143-07-7	mild, fatty, coconut, oily	1.63 ± 0.17
Hexadecanoic acid	57-10-3	-	0.35 ± 0.05
Total Acids			*34.37 ± 10.91*
ALDEHYDES			
Nonanal	124-19-6	piney, floral, citrusy, fat	0.53 ± 0.27
Decanal	112-31-2	beefy, musty, marine, cucumber	0.46 ± 0.07
Benzaldehyde	100-52-7	cherry, candy	0.38 ± 0.03
Total Aldehydes			*1.37 ± 0.37*
KETONES			
2-Undecanone	112-12-9	citrus, rose, iris	0.30 ± 0.01
Total Ketones			*0.30 ± 0.01*
ESTERS			
Isoamyl acetate	123-92-2	sweet, fruity, banana	1.13 ± 0.72
Hexyl acetate	142-92-7	sweet, fruity, herb	1.23 ± 0.39
Ethyl octanoate	106-32-1	fruity, floral	14.48 ± 0.35
Isoamyl octanoate	2035-99-6	sweet, oily, fruity, green, pineapple, coconut	2.74 ± 0.29
Octyl acetate	112-14-1	fruity, fatty	0.37 ± 0.16
Methyl decanoate	110-42-9	oily, wine, fruity, floral	0.16 ± 0.09
Ethyl decanoate	110-38-3	sweet, oily, nutlike	27.27 ± 5.58
Dodecyl acetate	112-66-3	sweet, fresh	0.90 ± 0.16
Decyl acetate	112-17-4	sweet, fatty, fruity	1.02 ± 0.61
Ethyl 9-decenoate	67233-91-4	fruity, fatty	0.86 ± 0.04
Phenethyl acetate	103-45-7	floral, rose, sweet, honey, fruity, tropical	1.54 ± 0.07
Ethyl laurate	106-33-2	sweet, waxy, creamy, floral	5.98 ± 0.61
Isoamyl decanoate	2306-91-4	waxy, banana, fruity, sweet, cognac, green	1.06 ± 0.05
Ethyl decanoate	628-97-7	waxy, fruity, creamy, vanilla, balsamic	0.58 ± 0.03

Ethyl 9-hexadecenoate	54546-22-4	-	0.90 ± 0.03
Total Esters			*60.22 ± 9.18*
TERPENES & NORISOPRENOIDS			
Vitispirane A	65416-59-3	floral, fruity, earthy, woody	2.63 ± 0.20
1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)	30364-38-6	licorice	8.06 ± 0.08
β-Caryophyllene	87-44-5	sweet, woody, spice, clove	0.12 ± 0.01
Farnesol	4602-84-0	fresh, sweet, linden, floral, angelica	0.46 ± 0.02
Isocaryophyllene	118-65-0	woody, spicy	0.40 ± 0.02
Nerolidol	7212-44-4	floral, green, waxy, citrus, woody	0.27 ± 0.08
Total Terpenes			*11.94 ± 0.41*

[1] From [27]. The numbers indicate relative peak area abundance calculated against the total peak area eluted on the chromatogram, results are expressed as mean ± standard deviation of triplicates from total analysed samples.

Annex 3. Comunicacions escrites derivades de la tesi.

Resultats presentats al IV Workshop Anual INSA-UB (Santa Coloma de Gramenet, 2018)

Campus de l'Alimentació Universitat de Barcelona

Bacterial growth effect of Sparkling wine lees

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UNIVERSITAT DE BARCELONA

BACKGROUND

Lees are defined as the residues formed at the bottom of receptacles containing wine, after fermentation or during storage. Lees are composed by microorganisms (mainly yeast cells), tartaric acid, inorganic and other adsorbed compounds. Yeast lees of sparkling wine (Cava) are considered a by-product (200 tons/year) mainly destined for distillation. Each bottle of cava contains approximately 1g of lees that contributes to organoleptic properties during ageing.

Figure 1. Schematic spanish sparkling wine elaboration

OBJECTIVE

The objective of this work is the valorization of the yeast lees, after their characterization from a compositional point of view and their capacity to promote the bacterial growth

METHODOLOGY

Lees were characterized by microscopy, metagenomics and chemical analysis. Confocal laser scanning microscopy was used in order to obtain images of the yeast lees. Colorimetric methods as Bradford for proteins, Phenol-sulphuric for carbohydrates, ninhydrin for aminoacidic composition and 4-DTDP (4,4-dithiodipyridine) for thiols determination were used in order to obtain the composition of yeast lees. To evaluate *in vitro* the capacity to promote the bacterial growth *Lactobacillus plantarum* ATCC 14971 and *Lactobacillus reuteri* CECT 925 strains were evaluated by comparison of kinetic growth in the presence of different concentrations of lees (0.5%, 1%, and 2% w/v) regarding control without lees. Once the concentration of use was determined, the test was carried out over time (1, 2, 7, 14 and 20 d).

RESULTS AND DISCUSSION

Yeast lees as a oenology by-product maintain the characteristic ovoid structure of the cell and the integrity of the cell wall (Figure 2). The diameter was 3-5 µm and cell counting was $1.1 \cdot 10^{10}$ –$2.7 \cdot 10^{10}$ cells/g d.w. (62% cell wall; 38% cytoplasm). Polysaccharides were the main compounds of lees (70-75%) followed by a high proteins (9-15%). (Table 1). During aging, lees were plasmolyzed because DNA of whole yeast is degraded and not determined. The subsequent degradation to nucleotides and nucleosides could be interesting in long aged sparkling wines because they can act as a flavor enhancers.

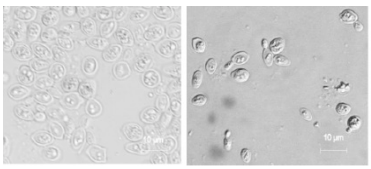

Figure 2. Images obtained by confocal laser scanning microscopy of cava lees

Table 1. Mean values for composition in dry weight for lees and dry yeast hulls (DYH). WY=Whole Yeast and YCW=yeast cell wall. (-) Value under the quantification limit.

	LEES		DYH	
FRACTION	**WY**	**YCW**	**WY**	**YCW**
Thiol groups (mg Glu-eq/g)	0.40_a	0.81_b	28.85_a	5.83_b
% Amino acids (w/w)	0.05_a	.	8.78_a	-
% Proteins (w/w)	8.5_a	9.3_a	21.2_a	28.7_b
% Sugars (w/w)	72.3_a	74.6_a	56.6_a	54.7_a

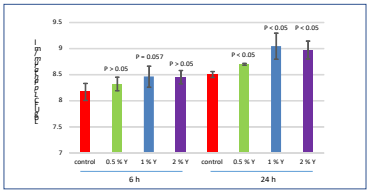

Figure 3. Promoting growth effect on *L. plantarum* at different concentration of yeast lees

Finally, in order to evaluate the capacity to promote the bacterial growth different concentration of yeast lees were assessed (0.5, 1 and 2%) in vitro culture. The promoting growth effect on *L. plantarum* was observed at 1% yeast lees concentration, showing significant differences after 24 h of incubation (Figure 3). The presence of lees, at 1% concentration, maintains the survival of *L. plantarum* culture at room temperature up to 20 days after the experiment has been performed (Figure 4). *For L. reuteri* were similar to those obtained with *L. plantarum* (data not shown).

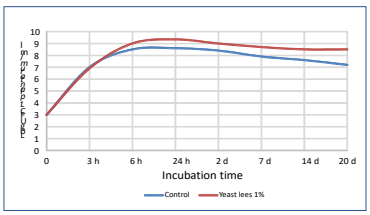

Figure 4. Survival of *L. plantarum* with 1% yeast lees carried out over time at room temperature.

CONCLUSION

It can be concluded that the sparkling wine lees improve the bacterial growth and survival on probiotic bacteria such as *L. plantarum* and *L. reuteri* and they are suitable for reused as technological adjuvant in multiple fermentation processes.

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Resultats presentats al V Workshop Anual INSA-UB (Santa Coloma de Gramenet, 2019)

EFECTO DE LAS LÍAS DEL CAVA SOBRE LA MICROBIOTA Y METABOLITOS SECUNDARIOS DE MASA MADRE

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INTRODUCCIÓN

Las lías del Cava se definen como el residuo formado después de la fermentación o durante el almacenamiento del vino (**Figura 1**) y se componen de microorganismos (principalmente células de levadura), ácido tartárico, ácidos inorgánicos y otros compuestos adsorbidos [1]. Cada botella de Cava contiene aproximadamente 1g de lías, que contribuyen a las mejoras organolépticas durante la crianza del vino. Aun así, las lías se consideran un subproducto (200 toneladas/año).

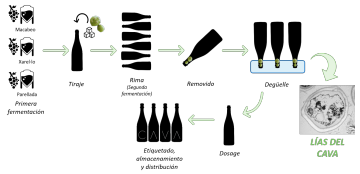

Figura 1. Esquema del proceso de elaboración del Cava.

OBJETIVO

Evaluar el efecto de las lías del Cava sobre el crecimiento, la supervivencia y la producción de metabolitos secundarios de la microbiota fermentativa de la masa madre del pan.

MATERIAL Y MÉTODOS

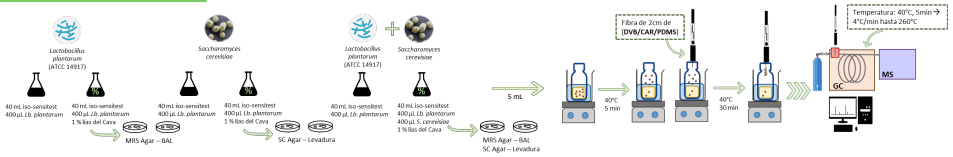

Figura 2. Esquema del diseño experimental. Se incubaron ambos microorganismos en cultivo puro y mixto a diferentes temperaturas (25, 30 y 37°C) durante 72h.

RESULTADOS

La capacidad de las lías para promover el crecimiento de los microorganismos fue evaluada a una concentración del 1% (m/v) a diferentes temperaturas. La presencia de lías aumenta el crecimiento y la supervivencia de ambos microorganismos, tanto en cultivo puro (**Figura 3**) como en cultivo mixto. *Lb. plantarum*, tuvo mayor supervivencia a temperaturas más altas (30°C y 37°C), mientras que para *S. cerevisiae* esta aumentó a temperaturas más bajas (25°C y 30°C).

Tabla 1. Ácidos grasos de cadena corta (AGCC) y media (AGCM) producidos por Lb. plantarum y S. cerevisiae: (+) presencia; (++) aumento del 10%; (+++) aumento del 20%.

		Lactobacillus plantarum						*Saccharomyces cerevisiae*					
Compuesto	**Aroma [a]**	**25°C**	**% 25°C**	**30°C**	**% 30°C**	**37°C**	**% 37°C**	**25°C**	**% 25°C**	**30°C**	**% 30°C**	**37°C**	**% 37°C**
Ácido acético	Pungente, picante	+	+++	+++	+++	+++	+++	+		+	++	+	+
Ácido propiónico	Pungente, rancio		+	+	+	+	+				+		
Ácido butírico	Sudoroso, rancio		+	+	+	+	+			+	+		
Ácido isobutírico	Mantequilla, queso		+	+	+		+						
Ácido valérico	Sudor		++	+	++	+	+			+	+		
Ácido caproico	Sudor			+	+	+	+			+	+		
Ácido caprílico	Aceitoso, rancio, sudor			+	++	+	+						
Ácido cáprico	Agrio, graso	+		+	++	+	++						

a: obtenido de Flavornet by Terry Acree & Heinrich Arn, http://www.flavornet.org, 2004 (consultado en: septiembre 2019)

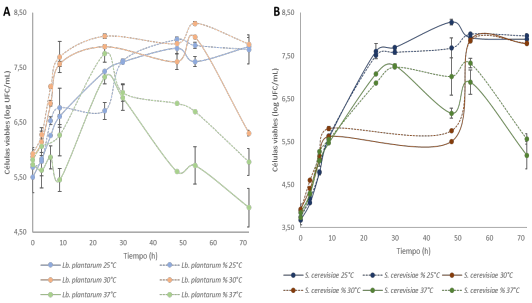

Figura 3. Crecimiento y supervivencia de Lb. plantarum y S. cerevisiae a 25°C, 30°C y 37°C.

Tabla 2. Principales metabolitos secundarios producidos por Lb. plantarum y S. cerevisiae en cultivo puro y mixto: (+) presencia; (++) aumento del 10%; (+++) aumento del 20%.

		Lactobacillus plantarum						*Saccharomyces cerevisiae*						Cultivo mixto					
Compuesto	**Aroma [a]**	**25°C**	**% 25°C**	**30°C**	**% 30°C**	**37°C**	**% 37°C**	**25°C**	**% 25°C**	**30°C**	**% 30°C**	**37°C**	**% 37°C**	**25°C**	**% 25°C**	**30°C**	**% 30°C**	**37°C**	**% 37°C**
Diacetilo	Mantequilla	+	++	+		+	+						+	+	+	+	+	+	+
2-metil-1-propanol	Regaliz							+	+	+	+	+	+	+	+	+	+	+	++
Limoneno	Cítrico, fresco				+		+		+	+				+		+	+	+	
3-metil-1-butanol	Malta	++	+	+	+	+	+	+	+	++	+++	+	+	++	++	++	++	++	++
Acetoína	Mantequilla, crema	+	+++	+	+	++	++	+		+	+			+	+	+	+	+	+

a: obtenido de Flavornet by Terry Acree & Heinrich Arn, http://www.flavornet.org, 2004 (consultado en: septiembre 2019)

CONCLUSIONES

Se puede concluir que las lías del Cava promueven el crecimiento y la supervivencia de microorganismos de interés tecnológico, como son *Lb. plantarum* y *S. cerevisiae*, siendo a 30°C la temperatura más ótpima. Esto puede deberse al contenido de β-glucanos y manoproteínas, además de fibra insoluble y otros compuestos que se encuentran en estas células plasmolizadas. Por lo tanto, se pueden revalorizar como coadyuvante tecnológico en procesos fermentativos, como, por ejemplo, la elaboración de masa madre del pan. Además, este aumento del crecimiento y supervivencia en presencia de lías también comporta una mayor producción de metabolitos secundarios por parte de dichos microorganismos.

Referencias:

[1] Gallardo-Chacón, J.J.; Vichi, S.; Urpi P.; López-Tamames, E.; Buxaderas, S., Int. J. Food Microbiol., 2010, 143, 48
[2] Shokri, H.; Asadi, F.; Khosravi, AR., Nat. Product Res., 2008, 22, 414
[3] Tudela, R.; Riu-Aumatell, M.; Castellari, M.; Buxaderas, S.; López-Tamames, E. J. Agric. Food Chem., 2013, 61, 6028
[4] Mokhtar, S; Jafari, M.; Khomeiri, M.; Maghsoudlou, Y.; Ghorbani, M. Morteza K., 2017, Food Res. Int..2017, 96, 19

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Resultats presentats al II Congreso CyTA Junior (León, 2019)

II Congreso CyTA Junior
15 de mayo 2019 - León, Facultad de Veterinaria
CIENCIA Y TECNOLOGÍA DE LOS ALIMENTOS

Effect of Sparkling wine lees on *Lactobacillus acidophilus*

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BACKGROUND

Lees are defined as the residues formed at the bottom of receptacles containing wine, after fermentation or during storage. Lees are composed by microorganisms (mainly yeast cells), tartaric acid, inorganic and other adsorbed compounds [1]. Yeast lees of sparkling wine (Cava) are considered a by-product (200 tons/year) mainly destined for distillation. Each bottle of cava contains approximately 1g of lees that contributes to organoleptic properties during ageing.

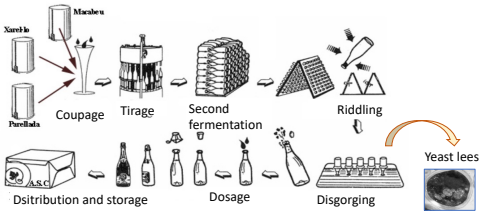

Figure 1. *Schematic spanish sparkling wine elaboration*

OBJECTIVE

The objective of this work is the revalorization of yeast lees by studying their capacity to promote *Lactobacillus acidophilus* growth at different temperatures.

METHODOLOGY

To evaluate *in vitro* the capacity to promote the bacterial growth, *Lactobacillus acidophilus* ATCC 4356 was evaluated by comparison of kinetic growth in the presence of 1% w/v regarding control without lees. The test was carried out over time (0h – 72h). To stablish the growth curves, *L. acidophilus* was incubated according to different temperatures (25°C, 30°C and 37°C) in iso-sensitest medium, and plating the appropriate dilution.

RESULTS AND DISCUSSION

The ability of yeast lees to promote bacterial growth *in vitro* culture was assessed at a 1% (w/v) concentration, at different temperatures (25°C, 30°C and 37°C). The presence of yeast lees maintains the survival of *L. acidophilus* culture at a range temperature of 25-37°C through time.

Statistically significant differences were found at a temperature of 30°C (**Figure 2**) between culture with yeast lees and control. It was observed that the presence of lees increases the growth of *L. acidophilus*, as well as bacteria survival. Similar results were obtained at 25°C and 37°C (**Table 1**).

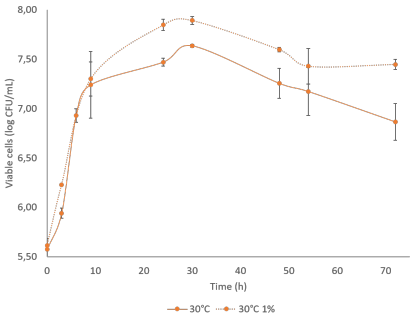

Figure 2. *Growth of Lactobacillus acidophilus in the presence of 1% (w/v) yeast lees regarding control.*

Table 1. *Growth of Lactobacillus acidophilus in the presence of 1% (w/v) yeast lees regarding control.*

TIME (h)	Viable cells L. acidophilus (log CFU/mL)			
	25°C	25°C 1%	37°C	37°C 1%
0	4,90 ± 0,04^{a}	4,93 ± 0,03^{a}	5,70 ± 0,06^{a}	5,74 ± 0,13^{a}
3	5,69 ± 0,10^{b}	5,57 ± 0,09^{b}	-	-
6	7,02 ± 0,10^{c}	6,91 ± 0,07^{c}	7,81 ± 0,04^{bc}	7,80 ± 0,02^{bc}
9	8,02 ± 0,06^{def}	7,94 ± 0,07^{def}	7,96 ± 0,07^{bc}	8,01 ± 0,05^{bc}
24	7,76 ± 0,23^{d}	7,94 ±0,02^{def}	7,93 ± 0,03^{bc}	8,03 ± 0,01^{c}
30	7,81 ± 0,01^{de}	7,92 ± 0,01^{def}	7,92 ± 0,03^{bc}	7,98 ± 0,02^{bc}
48	7,89 ± 0,05^{de}	7,99 ± 0,04^{def}	7,90 ± 0,08^{bc}	7,94 ± 0,01^{bc}
54	8,12 ± 0,03^{ef}	8,22 ± 0,11^{f}	7,75 ± 0,02^{b}	7,98 ± 0,04^{bc}
72	7,97 ± 0,08^{def}	8,10 ±0,05^{ef}	7,77 ± 0,03^{bc}	7,84 ± 0,18^{bc}

CONCLUSIONS

It can be concluded that sparkling wine lees improve the bacterial growth and survival on probiotic bacteria such as *L. acidophilus* and they are suitable for reused as technological adjuvant in multiple fermentation processes, as well as a prebiotic regarding intestinal microbiota.

References:
[1]] Gallardo-Chacón, J.J.; Vichi, S.; Urpí P.; López-Tamames, E.; Buxaderas, S., Int. J. Food Microbiol., 2010, 143, 48
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Infografia presentada al II Workshop Càtedra UB-Danone (Santa Coloma de Gramenet, 2019). **PREMI A LA MILLOR INFOGRAFIA**

¿PRE, PRO o SIM? ...Bióticos

PROBIÓTICOS

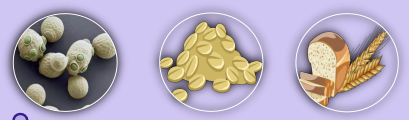
Microorganismos (MO) vivos que ofrecen beneficios para la salud del huésped

Mantienen la microbiota intestinal saludable

En alimentos fermentados, como suplemento dietético o en fármacos

Beneficios:
- Protegen vs patógenos
- Ayudan al SI
- Aportan vitaminas y enzimas
- Mejoran la digestión y la absorción de nutrientes

PREBIÓTICOS



Ingredientes alimentarios utilizados por los PROBIÓTICOS y no digeribles por los humanos

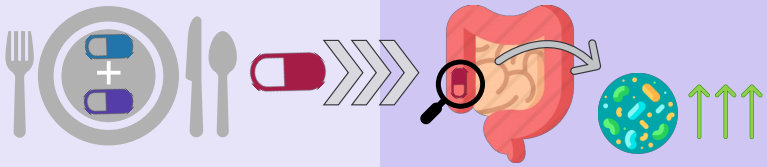
Pueden modificar la composición y/o la función de la microbiota intestinal

En alimentos de forma natural (fibra) y en suplementos alimenticios

Beneficios:
- Mejoran la función digestiva
- Ayudan a regular el balance energético y el metabolismo de la glucosa

SIMBIÓTICOS

Producto alimentario que contiene el PREBIÓTICO que favorece selectivamente al PROBIÓTICO



Infografía elaborada por **Alba Martín-Garcia** y **Salvador Hernández-Macias**, doctorandos del programa de Alimentación y Nutrición de la Universidad de Barcelona

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