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MODIFICACIÓ DEL VALOR NUTRITIU, L'ESTABILITAT OXIDATIVA I LA QUALITAT SENSORIAL DE LA CARN DE POLLASTRE MITJANÇANT LA DIETA

Memòria presentada per

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Agraïments

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Tempus fugit

ÍNDEX

I. INTRODUCCIÓ

INTRODUCCIÓ

Avui dia, tothom ha pres consciència de la importància de la dieta en la salut humana. Tot i això, els hàbits alimentaris en el món occidental moltes vegades són inapropiats. És per això que diversos organismes han posat especial èmfasi en la reducció de la quantitat i la modificació dels greixos que habitualment consumim (American Heart Association, 2001; Institute of Medicine, 2002; Department of Health, 1994). Aquestes recomanacions dietètiques van dirigides a reduir el consum de greixos amb una elevada fracció d'àcids grassos saturats i, paral·lelament augmentar el consum de les grasses insaturades, ja que els àcids grassos insaturats (AGI) que en formen part tenen un efecte positiu en la prevenció de l'aparició i desenvolupament de patologies cardiovasculars i inflamatòries (Kinsella et al., 1990; Knapp et al., 1991; Mensik et al., 1998; Ruxton et al., 2004; American Heart Association, 2002).

Per altra banda els productes carnis poden ser, a la vegada, una font molt important de vitamines i minerals (Schweigert, 1994). Entre les vitamines presents en el productes carnis es poden destacar les vitamines del grup B, especialment la B_{12} i B_6, així com la B_1, la B_2, l'àcid pantotènic i la niacina. Respecte als minerals, els productes carnis són una font excepcional de ferro, tant per la quantitat com per la biodisponibilitat d'aquest. La ingesta d'altres elements com el zinc, el fòsfor, el seleni i el coure també provenen en gran mesura del consum de productes carnis (Pennington i Young, 1991; Buss i Rose, 1992; Foster i Sumar, 1995; Subar et al., 1998). L'aport de zinc a la dieta per part dels productes carnis és semblant al de ferro ja que, apart de ser una de les fonts principals d'aquest mineral en la dieta (Subar et al., 1998; Ma i Betts, 2000; Terrés et al., 2001), aquest és altament biodisponible (Hortin et al., 1993; Lönnerdal, 2000).

Tot i que els productes carnis són consumits de manera més o menys habitual, essent per tant una font important d'aquests nutrients, cal dir que les ingestes d'algun d'aquests elements està lleugerament per sota de les recomanacions dietètiques en determinats grups de població, fins i tot en països desenvolupats (Pennington i Young, 1991). Aquest és el cas de les ingestes de zinc i seleni en la gent gran (Girodon et al., 1999; de Jong et al., 2001; Savarino et al., 2001; Ervin i Kennedy-Stephenson, 2002).

A més, les deficiències en seleni també han estat descrites en zones amb sòls deficients en seleni (British Nutrition Foundation, 2001; Venäläinen et al., 1997).

Així doncs, els productes carnis són productes d'un elevat interès nutritiu i per tant des d'aquest punt de vista es pot dir que són aliments de qualitat. Tot i això, quan es parla d'un producte carni de qualitat hi ha molts altres criteris apart dels de tipus nutritiu. El productor, l'elaborador, el distribuïdor, el venedor i finalment el consumidor avalua i cataloga la qualitat d'una carn o producte carni en funció de diferents paràmetres. Aquests paràmetres de qualitat estan relacionats amb característiques de tipus sensorial, de seguretat, tecnològiques, d'estabilitat, econòmiques i psicològiques. Aquestes característiques venen influenciades per diversos factors, com són l'espècie animal, raça, edat, sexe, dieta i manipulació *ante-* i *post-mortem* (Cross, 1994; López-Bote et al., 2001; Richardson i Mead, 1999).

Dins de les diferents característiques de tipus sensorial o organolèptiques, l'aparença, l'olor, la textura, el flavor i el color són importants en els productes carnis ja siguin crus o cuits. L'avaluació d'algunes d'aquestes característiques es pot fer mitjançant mesures de tipus físic i químic. Tot i això, aquestes mesures moltes vegades no donen una informació fidedigna del grau d'acceptació final que tindrà la carn o el producte carni per part del consumidor. De fet, l'acceptació final d'un producte carni cuit ve marcat especialment pels atributs d'aroma i flavor (Mottram, 1998).

En aquest sentit, l'oxidació lipídica és la principal causa de la deterioració dels aromes i gustos dels productes carnis, escurçant la vida útil del producte (Frankel, 1998; Mielche i Bertelsen, 1994). L'aparició d'aromes i flavors a ranci i/o a rescalfat "warmed-over" són característics d'aquests fenòmens d'oxidació (Melton, 1983; Love, 1988; Johnson i Civille, 1986; Poste et al., 1986). És en relació a la vida útil del producte on el tocoferol, un poderós antioxidant, ens pot ajudar a augmentar l'estabilitat oxidativa d'un producte carni i en la prevenció de l'aparició d'aquests aromes i flavors desagradables (de Winne i Dirinck, 1996; O'Neill et al., 1998a; Sheldon et al., 1997).

Així doncs, el desenvolupament de nous productes carnis no es pot fer solament des del punt de vista nutritiu sinó que a més ha de tenir en compte altres aspectes com són els de tipus sensorial o la seva vida útil. Per tant l'enriquiment de les carns en AGI de la sèrie n-3 pot ser una eina útil en la prevenció de diferents

patologies, així com el contingut en Zn i Se en cobrir les recomanacions dietètiques però, a més, aquest nou producte carni ha de ser acceptat pel consumidor i tenir una vida útil raonable per a la seva comercialització.

II. ANTECENDENTS BIBLIOGRÀFICS

ANTECEDENTS BIBLIOGRÀFICS

1. ALTERACIÓ DE LA CARN: OXIDACIÓ LÍPIDICA

L'alteració lipídica deguda a l'oxidació, podent-se donar per mecanismes enzimàtics i no enzimàtics, és una de les principals causes del deteriorament de la qualitat dels productes carnis emmagatzemats a temperatures de refrigeració i de congelació (Gray et al., 1996; Mielche i Bertelsen, 1994; Jensen et al., 1998a). Malgrat això, l'alteració lipídica també es pot donar per fenòmens hidrolítics sobretot provocats per contaminació microbiana.

En relació a l'oxidació lipídica, els principals components involucrats són els àcids grassos insaturats i l'oxigen. En aquest procés, l'oxigen de l'ambient reacciona amb els àcids grassos formant compostos d'oxidació primària de naturalesa inestable que, eventualment, es poden descompondre en diferents compostos anomenats d'oxidació secundària, els quals generalment són els responsables de flavors i aromes desagradables (Frankel, 1985). Molts cops tot s'inicia amb la formació de radicals lliures. Quan un radical cedeix o capta un electró d'un altra molècula, o bé, es combina amb ella, aquesta molècula esdevé un altre radical. Per tant, és característic que aquestes reaccions entre radicals i no radicals, siguin reaccions en cadena i que per tant la formació inicial d'un radical indueixi les conseqüents transformacions d'altres molècules en radicals. La complexitat de totes aquestes reaccions en matrius complexes, com poden ser les carns, ve donada no tant sols per la participació de l'oxigen i radicals lliures, sinó que a més, poden participar-hi ions metàl·lics, enzims, i determinats factors de protecció enzimàtics i no enzimàtics (Kappus, 1991).

1.1. L'Oxidació Lipídica

1.1.1. Iniciació per Mecanismes no Enzimàtics

L'oxidació primària per via no enzimàtica es du a terme bé per la formació clàssica de radicals lliures mitjançant la participació de l'oxigen triplet (anomenada autooxidació) o bé per processos mitjançant la participació de l'oxigen singulet (anomenada fotooxidació).

L'autooxidació és el procés més comú pel qual els àcids grassos insaturats i l'oxigen interactuen. És un mecanisme que necessita de la participació de l'oxigen triplet i de la formació de radicals lliures. Normalment, aquesta via d'oxidació és la que té més influència sobre les característiques nutritives i sensorials dels aliments (Frankel, 1998).

L'oxidació dels lípids pels radicals lliures es pot esquematitzar en tres etapes ben diferenciades:

Iniciació:	$RH \rightarrow R\cdot + \cdot H$	[1]	
Propagació:	$R\cdot + {}^3O_2 \rightarrow ROO\cdot$	[2]	
	$ROO\cdot + RH \rightarrow ROOH + R\cdot$	[3]	
	$RO\cdot + ROOH \rightarrow ROO\cdot + ROH$	[4]	
	$RO\cdot + RH \rightarrow ROH + R\cdot$	[5]	
	$RH + \cdot OH \rightarrow R\cdot + H_2O$	[6]	
	$ROOH \rightarrow RO\cdot + \cdot OH$	[7]	} Reaccions de multiplicació de la cadena radicalaria
	$2ROOH \rightarrow RO\cdot + ROO\cdot + H_2O$	[8]	
Acabament:	$R\cdot + R\cdot \rightarrow RR$	[9]	
	$2RO\cdot \rightarrow ROOR$	[10]	

$$ROO\cdot + R\cdot \rightarrow ROOR \qquad [11]$$

$$RO\cdot + R\cdot \rightarrow ROR \qquad [12]$$

$$2ROO\cdot \rightarrow ROOR + {}^{1}O_2 \qquad [13]$$

La reacció entre l'oxigen triplet i els AGI és termodinàmicament desfavorable (Asghar et al., 1988; Gray et al., 1996), però a partir d'aquests darrers es poden formar radicals alquil (R·) els quals poden reaccionar fàcilment amb ${}^{3}O_2$. Tot i això, la formació dels radicals alquil no és immediata i necessita d'una aportació d'energia o bé de l'acció catalítica de metalls com el ferro o el coure (Jadhav et al., 1996; Kappus, 1991) que han d'estar lliures per tal de que es produeixi la següent reacció:

$$RH + metall^{(n+1)+} \rightarrow metall^{n+} + H^{+} + R\cdot \qquad [14]$$

Un cop format el radical alquil (R·) aquest pot tenir diferents destins, el més probable en el cas d'àcids grassos poliinsaturats (AGPI) en sistemes aeròbics és patir una reorganització molecular i, després de reaccionar amb l'oxigen triplet, donar lloc a radicals peroxil (ROO·) com es veu en la reacció [2]. A continuació, els ROO· són capaços de sostreure un àtom d'hidrogen dels àcids grassos adjacents propagant d'aquesta manera la cadena d'oxidació lipídica. En aquest cas, es forma un hidroperòxid lipídic (HPL) que genèricament també s'anomena peròxid [reacció 3]. Aquests HPL, en cas de provenir d'AGPI, poden donar lloc a un hidroperòxid amb dobles enllaços conjugats i configuració *cis* o *trans* (*c* o *t*). Si aquests HPL pateixen una deshidratació es dóna lloc a la formació de diferents isòmers d'àcids grassos (Cmolík et al., 2000). Malgrat això, el més normal és que els diferents HPL, en presència de calor, radiacions i ions metàl·lics, pateixin homòlisi donant lloc a la formació de radicals peroxil (ROO·), alcoxil (RO·) o hidroxil (·OH) com es veu en les reaccions [7, 8], els quals a la vegada poden reaccionar amb altres molècules lipídiques per formar altres radicals que propaguen la cadena [reaccions 3-6]. Per això, les reaccions [7] i [8] són les anomenades reaccions de multiplicació de la cadena. La

reacció [7] és important només a altes temperatures o quan és catalitzada per molècules Fe-porfiríniques. Per contra, en la majoria d'aliments la reacció [8] no té cap paper, donat que quan s'arriba a la concentració de HPL necessària per a què es produeixi l'aliment és ja incomestible (Belitz et al., 2004).

Al final, quan s'ha reduït la quantitat d'àcids grassos i/o la presència d'oxigen disponible, predomina la unió de radicals per formar productes no radicalaris [reaccions 9-13] (Frankel, 1998; Halliwell i Chirico, 1993; Jadhav et al., 1996; Kappus, 1991; Belitz et al., 2004).

Per via no enzimàtica, hi ha un segon mecanisme d'oxidació anomenat fotooxidació. La principal diferència amb l'autooxidació es troba en com s'inicia i, també, en què generalment es dóna amb menor freqüència (Frankel, 1998). Per a què aquesta es dugui a terme es necessita de la presència de l'oxigen triplet, un sensibilitzador i llum. El sensibilitzador té capacitat d'absorbir energia de la llum (fotosensibilitzadors) i passar a un estat excitat (Min i Boff, 2002; Hamilton et al., 1997). Aquests fotosensibilitzadors són compostos amb elevat nombre de dobles enllaços (clorofil·les, porfirines, flavines) i en el seu estat excitat i en presència de l'oxigen triplet donen pas a la formació de l'oxigen singulet.

L'oxigen singulet, a diferència del triplet, reacciona fàcilment amb els AGI per formar un directament HPL [reacció 15]. Aquest HPL es pot descompondre en radicals que inicien les reaccions radicalaries.

$$RH + {}^1O_2 \rightarrow ROOH \qquad [15]$$

El sensibilitzador, un cop excitat, també pot reaccionar directament amb la molècula lipídica donant pas a la formació d'un radical (R·) el qual ja podrà reaccionar directament amb l'oxigen triplet. Aquest mecanisme d'oxidació se'n diu de tipus I mentre que el mecanisme d'oxidació per via formació de l'oxigen singulet s'anomena de tipus II (Min i Boff, 2002; Hamilton, 1997; Frankel, 1998; Belitz et al., 2004).

L'oxidació lipídica en els organismes vius també es pot iniciar per mitjà d'espècies d'oxigen reactiu (Gutteridge i Halliwell, 1990; Kanner, 1994; Deshpande et al., 1996; Benzie, 1996; Aruoma, 1998; Hamilton et al., 1997; Morrissey et al., 1998, 2003). Aquestes espècies són bàsicament radicals o molècules que contenen grups amb oxigen reactiu i es produeixen de forma normal i en petites quantitats durant el metabolisme aeròbic de les cèl·lules i també per enzims com la xantin-oxidasa. Dins d'aquestes espècies hi ha el radical anió superòxid ($O_2\cdot^-$), el peròxid d'hidrogen (H_2O_2), el radical perhidroxil ($H_2O\cdot$), el radical hidroxil ($HO\cdot$) i l'àcid hipoclorós (HClO), entre d'altres.

Gran part dels danys produïts en l'organisme per alguna d'aquestes espècies de l'oxigen reactives ($O_2\cdot^-$ i H_2O_2) es creu que són deguts a la conversió en espècies molt més reactives ($HO\cdot$) (Halliwell, 1995a). Els radicals hidroxil són extraordinàriament reactius, raó per la qual ataquen freqüentment qualsevol tipus de molècula biològica, com per exemple els lípids, abstraient un àtom d'hidrogen (Kanner, 1994; Morrissey et al., 2003; Benzie, 1996; Halliwell, 1994a, b) [reacció 6].

Aquests radicals hidroxil, a més d'originar-se *in vivo*, ja hem vist que també es poden originar durant l'oxidació lipídica [reacció 7] i també com a conseqüència de la irradiació, de la descomposició de peroxinitrit i de la reacció del peròxid d'hidrogen amb els metalls de transició (Halliwell i Chirico, 1993; Benzie, 1996; Halliwell, 1995a, b; Halliwell et al., 1995b; Deshpande et al., 1996).

Un cop l'animal ha estat sacrificat, les diferents operacions de processat i manipulació de la carn poden afavorir l'oxidació lipídica per mecanismes similars als que succeeixen *in vivo* en l'animal que ha patit estrès oxidatiu (Morrissey et al., 2003).

1.1.2. Iniciació per Mecanismes Enzimàtics

L'oxidació lipídica també es pot produir per via enzimàtica, la qual es dóna principalment per les anomenades lipooxigenases. Si bé tenen una gran importància en vegetals, aquestes també s'han identificat en carns (Erikson, 1998). De fet, es creu que la presència de 15-lipooxigenasa en el muscle de pollastre pot ser responsable del

deteriorament oxidatiu de la carn crua emmagatzemada (Frankel, 1998; Kanner, 1994; Grossman et al., 1988). Les lipooxigenases són unes metal·loproteïnes que contenen un ió ferro en el centre actiu i catalitzen l'oxidació de certs AGI a monohidroperòxids. Si bé alguns d'aquests HPL tenen la mateixa estructura que els formats per altres vies, la formació per via enzimàtica presenta totes les característiques de la catàlisi enzimàtica: especificitat de substrat, selectivitat de la peroxidació, pH i temperatura òptim d'actuació, i sensibilitat als tractaments tèrmics. Les lipooxigenases només oxiden àcids grassos que contenen un sistema 1*c*,4*c*-pentadiè (Hamilton et al., 1997; Erikson, 1998) essent l'àcid araquidònic el principal substrat en què actuen les lipooxigenases d'origen animal (Belitz et al., 2004). Malgrat que les lipooxigenases poden reaccionar amb àcids grassos esterificats la velocitat de reacció és major quan són àcids grassos lliures, que provenen principalment de l'acció de les lipases (Hamilton et al., 1997). De manera similar a la lipooxigenasa, la ciclooxigenasa forma endoperòxids a partir dels àcids grassos (Nawar, 2000; Kanner, 1994). La descomposició d'hidroperòxids i endoperòxids dóna lloc a una gran varietat de productes d'oxidació secundària amb gran importància sensorial

La mieloperoxidasa és una altre enzim present en el sistemes musculars *post-mortem* que és capaç d'iniciar la peroxidació. Normalment es troba en els neutròfils de la sang i per tant, després del sacrifici de l'animal, pot contaminar els teixits al vessar-hi la sang (Erikson, 1998).

L'oxidació lipídica en aliments carnis també es pot donar per via enzimàtica de manera indirecta (Kanner, 1994; Erikson, 1998). Aquest seria el cas d'enzims com la citocrom P450-reductasa que poden acabar generant peròxid d'hidrogen el qual activaria la metamioglobina (Rhee et al., 1984; Rhee et al., 1987; Rhee, 1988; Kanner, 1994; Erikson, 1998). L'acció d'aquests enzims generant catalitzadors en un entorn ric en lípids força insaturats és especialment important.

Sigui quina sigui la seva via de formació, els HPL, per sí mateixos, no són considerats negatius per a la qualitat sensorial de l'aliment, doncs no tenen olor ni sabor (Erikson, 1998), si bé alguns poden tenir una nota de gust amarg (Belitz et al., 2004). Un dels efectes negatius de la formació dels HPL seria la interacció dels HPL amb proteïnes i altres components dels aliments les quals representen una pèrdua del

seu valor nutritiu inicial (Belitz et al., 2004). Tanmateix, la principal raó per la qual els HPL són indesitjables és perquè quan aquests es descomposen generen radicals lliures. A la vegada, els productes de la fragmentació d'aquests HPL són els responsables principals de la generació d'olors i flavors desagradables (Frankel, 1998; Frankel, 1985; Jadhav et al., 1996). Malgrat això, es creu que diferents productes d'oxidació secundària poden estar relacionats amb la formació d'aromes característics de certes espècies i, també, contribuir a la formació de certs aromes desitjables típics de la carn cuita a través de la reacció de Maillard (Mottram, 1998; Frankel, 1998; Farmer, 1999).

1.1.3. Descomposició dels HPL

Els HPL són relativament inestables i comencen a descompondre's tant aviat es van formant. Al principi la velocitat de formació supera a la de descomposició, però a mesura que avança l'oxidació aquesta relació s'intercanvia. Les reaccions que es donen són nombroses i complexes, les quals depenen tant del substrat de descomposició com de les possibles interaccions. De fet d'aquestes reaccions de descomposició dels HPL en resulten una gran varietat de compostos de diferent pes molecular, llindars de flavor i significació biològica.

En la Figura 1 es mostra un esquema general de l'oxidació lipídica amb els diferents compostos que es poden formar.

El primer pas en la descomposició dels HPL comença amb l'escissió de l'enllaç O-O dels HPL, tal i com es mostra en la reacció [7]. El segon pas és una escissió de l'enllaç C-C del radical peroxil [reacció 16] per formar diferents aldehids i radicals alquil inestables (Frankel, 1998). La formació del radical alcoxil intermedi estaria catalitzada per metalls i compostos ferroporfirínics (Belitz et al., 2004), tal com s'observa en la Taula 1.

$$R\text{-}CHO\cdot\text{-}R' \rightarrow R\text{-}CHO + R\cdot' \quad [16]$$

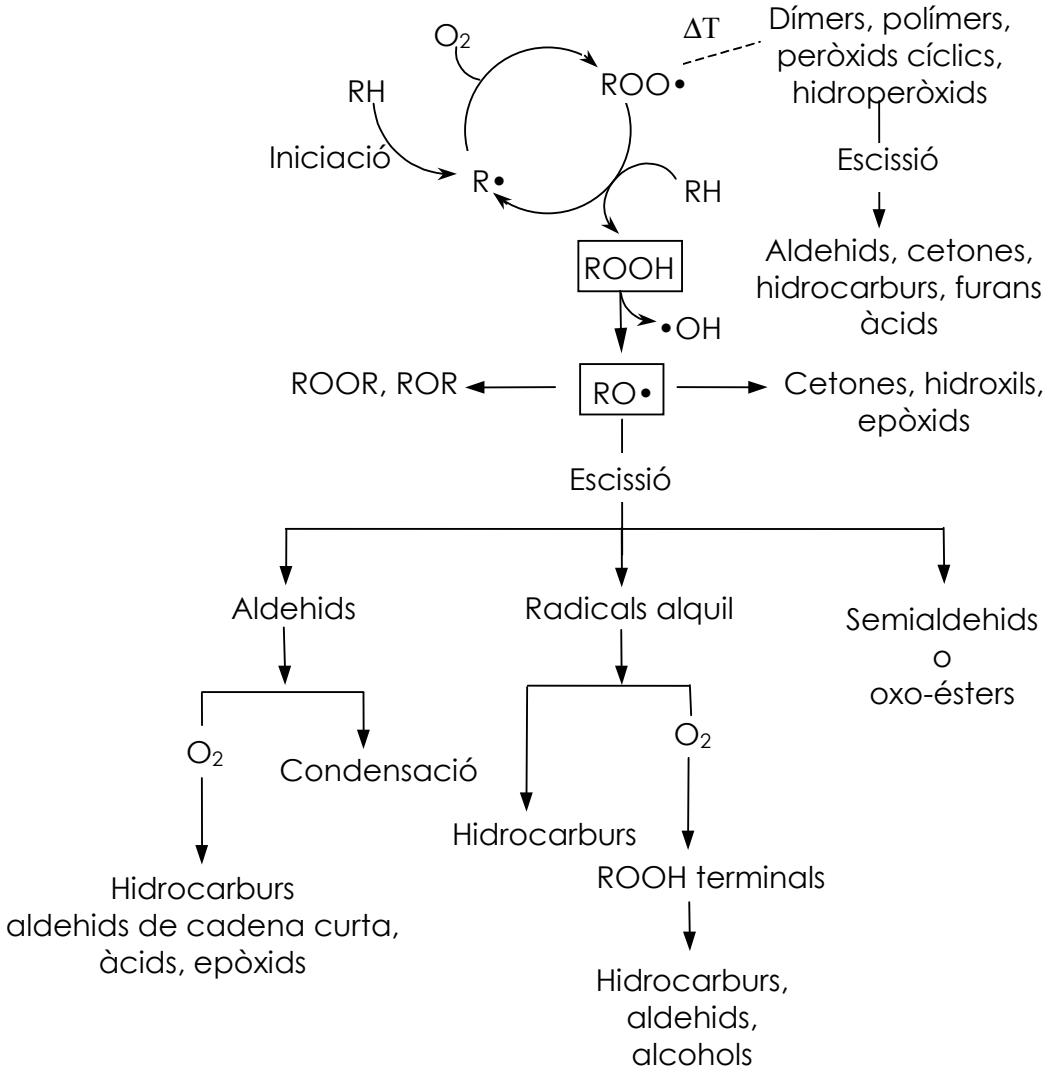

Figura 1. Esquema general de l'autooxidació i de la descomposició i polimerització a partir dels HPL (adaptada de Nawar, 2000)

Aquesta és la via de formació més important en donar lloc a la formació de productes de descomposició responsables dels flavors de deterioració dels greixos. Els radicals formats per aquesta via reaccionaran amb l'oxigen i altres radicals, per formar alcohols, aldehids, cetones, hidrocarburs i nous HPL que altre cop poden patir escissió i produir compostos de descomposició de pes molecular més baix. Apart de descompondre's donant tota una sèrie de compostos carbonílics, també poden reaccionar amb proteïnes, pigments, vitamines, colesterol, etc. (Melton, 1983; Frankel, 1998; Guardiola et al., 2002). A més, quan s'apliquen temperatures elevades poden formar dímers i polímers, monòmers d'àcids grassos cíclics i àcids grassos amb isomeria de posició i configuració (Nawar, 2000; Le Quére i Sébedio, 1996; Frankel, 1998; Sébédio et al., 1988; Sébédio et al., 1996; O'Keefe et al., 1993; Destaillats i Angers, 2002; Juanéda et al., 2003).

La formació de compostos volàtils de descomposició i les seves quantitats relatives dependran dels substrat que s'oxida, les condicions en les quals es dóna i de la via principal per la qual es du a terme l'oxidació (Frankel, 1985; Belitz et al., 2004).

Taula 1. Constant relativa de descomposició dels HPL de l'àcid linoleic per metalls o compostos amb el nucli ferroporfirínic (Belitz et al., 2004).

Metall	**Constant**		**Compost**	**Constant**	
	pH 7	**pH 5,5**	**ferroporfirínic**	**pH 7**	**pH 5,5**
Fe^{3+}	1	10^2	Hematina	$4{,}10^3$	$4{,}10^4$
Fe^{2+}	14	10^3	Metahemoglobina	$5{,}10^3$	$7{,}6.10^4$
Cu^{2+}	0,2	1,5	Citocrom C	$2{,}6.10^3$	$3{,}9.10^3$
Co^{3+}	6.10^2	1	Oxihemoglobina	$1{,}1.10^3$	
Mn^{2+}	0	0	Mioglobina	1	
			Catalasa	1	

En la Taula 2 es mostren els diferents compostos carbonílics volàtils formats per via autooxidativa a 20 ºC. Tot i la diversitat i quantitat de compostos formats, des d'un punt de vista sensorial, s'ha de tenir en compte que hi ha compostos secundaris

d'oxidació que tenen llindars de detecció molt baixos els quals repercuteixen de forma molt important en la percepció d'aromes i flavors indesitjables (Brewer i Vega, 1995). Aquest és el cas del 2*c*-nonenal, el *t*-4,5-epoxi-2*t*-decenal i la 1-octen-3-ona, productes d'oxidació de l'àcid linoleic (Belitz et al., 2004). Tot i això alguns d'aquests compostos formats poden ajudar a donar un aroma característic en certs productes cuits (Mottram, 1998; Love, 1996; Warner, 1998), com per exemple la formació del 2,4-decadienal que proporciona un aroma típic de fregit (Mottram, 1998; Warner, 1998).

Taula 2. Compostos carbonílics volàtils (µg/g) formats per autooxidació d'àcids grassos insaturats

Àcid oleic		**Àcid linoleic**		**Àcid linolènic**	
Heptanal	50	Pentà	mj	Propanal	mj
Octanal	320	Pentanal	55	1-Penten-3-ona	30
Nonanal	370	Hexanal	5.100	2*t*-Butenal	10
Decanal	80	Heptanal	50	2*t*-Pentenal	35
2*t*-Decenal	70	*2t*-Heptenal	450	2*c*-Pentenal	45
2*t*-Undecenal	85	Octanal	45	2*t*-Hexenal	10
		1-Octen-3-ona	2	3*t*-Hexenal	15
		1-Octen-3-HP	tr	3*c*-Hexenal	90
		2*c*-Octenal	990	2*t*-Heptenal	5
		2*t*-Octenal	420	2*t*,4*c*-Heptadienal	320
		3*c*-Nonenal	30	2*t*,4*t*-Heptadienal	70
		3*t*-Nonenal	30	2,5*c*-Octadienal	20
		2*c*-Nonenal	tr	3,5*c*-Octadien-2-ona	30
		2t-Nonenal	30	1,5*c*-Octadien-3-ona	tr
		2*c*-Decenal	20	1,5*c*-Octadien-3-HP	tr
		2*t*,4*t*-Nonadienal	30	2*t*,6*c*-Nonadienal	10
		2*t*,4*c*-Decadienal	250	2,4,7,*c*-Decatrienal	85
		2*t*,4*t*-Decadienal	150		
		t-4,5-Epoxi-2*t*-decenal	tr		

Mj = compost majoritari; tr = traces; *c* = *cis*; *t* = *trans*

1.2. Oxidació Lipídica a la Carn

S'ha de destacar que en els aliments, i en concret en les carns, hi ha diferents factors que poden influir en la propagació de les reaccions en cadena per radicals, la formació de HPL i la descomposició dels HPL en compostos de baix pes molecular. Dins d'aquests factors tenim la composició en AG, l'oxigen disponible, la presència de metalls i d'espècies reactives derivades de l'oxigen, la d'enzims pro i antioxidants, la de sensibilitzadors i també d'antioxidants (Decker, 1998; Ternay i Sorokin, 1997; Erikson, 1998; Morrissey et al., 1998; Kanner et al., 1988a; Kanner, 1994; Decker i Xu, 1998).

Tot s'inicia en el muscle *post-mortem* on es produeixen una sèrie de canvis bioquímics deguts a què la circulació sanguínia s'atura (Erikson, 1998; Morrissey et al., 1994; Eskin, 1990). Aquests canvis són atribuïbles a la falta d'oxigen i a l'acumulació de subproductes de reacció com són l'àcid làctic i els protons, els quals comporten una disminució del pH de la carn.

El pH final de les carns és un factor a tenir en compte doncs si aquest és elevat no s'afavoreix tant l'oxidació. Una de les raons seria la major solubilitat dels metalls de transició a pH baix. El pH es veu determinat pel sacrifici. Si l'animal abans de morir ha patit estrès, la quantitat de reserves de glucosa es veuran reduïdes i en conseqüència la formació d'àcid làctic serà menor i el pH resultant serà més elevat. El pH final, però, té un marcat efecte sobre les propietats físiques i organolèptiques. Valors de pH relativament alts produeixen un increment de la capacitat de retenir aigua i provoquen que les carns tinguin un color més fosc i una textura més grollera, per contra si el pH és baix té els efectes contraris (Pearson, 1994).

En aquesta conversió del muscle en carn, a part del canvi de pH, es produeixen altres canvis químics i físics entre els quals, relacionats amb l'oxidació, es poden destacar:

1.- La pèrdua de la capacitat de les membranes cel·lulars per mantenir la seva integritat, el que comporta una alliberament d'enzims del lisosoma, com per exemple

fosfolipases i lipases, i d'ions calci que a la vegada poden activar proteases (Erikson, 1998; Morrissey et al., 1998) .

2.- La progressiva disminució amb el temps de la concentració d'agents antioxidants com els tocoferols, l'àcid ascòrbic i el glutatió. A més, es genera peròxid d'hidrogen el qual pot alliberar el ferro de les hemoproteïnes (Erikson, 1998; Rhee et al., 1987).

3.- Si la carn emmagatzemada ha estat tallada, l'oximioglobina s'oxida ràpidament a metamioglobina. Aquesta pot reaccionar amb el peròxid d'hidrogen i donar lloc a radicals (Rhee, 1988). A més, el pas del color vermell de la mioglobina i oximioglobina cap al color marró de la metamioglobina afecta negativament a la qualitat sensorial de la carn (Frankel, 1998).

4.- Oxidació de la metamioglobina cap a un radical porfirínic, que pot reaccionar amb el peròxid d'hidrogen i convertir-se en l'espècie oxidant protoporfírina-Fe=O també anomenada oxen (Erikson, 1998; Asghar et al., 1988; Gutteridge i Halliwell, 1990; Belitz et al., 2004).

5.- Conversió de l'hipoxantina-deshidrogenasa en xantin-oxidasa, que pot iniciar l'oxidació (Erikson, 1998).

Com a resultat d'aquests canvis, just després del sacrifici de l'animal, s'inicia l'oxidació incrementant-se progressivament fins que el producte deixa de ser acceptat pel consumidor (Gray et al, 1996). Tot i que en un principi no es coneixia bé a on s'iniciava l'oxidació lipídica de les carns, avui es coneix que aquesta principalment es dóna en els fosfolípids de les membranes cel·lulars i subcel·lulars, les quals estan situades prop dels catalitzadors hemo (Fe^{2+}) de les mitocondries i microsomes (Frankel, 1998; Gray i Pearson, 1987). Els radicals hidroperoxil formats reaccionen amb grups sulfur, amines, aminoàcids, etc. provocant una pèrdua nutritiva i un posterior deteriorament del flavor (Frankel, 1998). Malgrat tots aquests factors

desencadenants de l'oxidació, la carn crua i sencera emmagatzemada a temperatures de refrigeració és relativament resistent a l'oxidació (Frankel, 1998; Pearson et al., 1977).

Quan es trenca l'estructura del muscle original l'oxidació s'accentua, doncs es trenca la integritat de les cèl·lules i s'afavoreix que els lípids quedin exposats a l'oxigen, enzims, compostos hemo i ions metàl·lics (Frankel, 1998; Erikson et al., 1998, Jadhav et al., 1996). A més, determinades tècniques de desossat mecànic i de sacrifici també poden afectar de forma diferent a l'oxidació (Buckley et al., 1995). En el cas del desossat, l'oxidació es pot veure afavorida per l'alliberament d'hemoglobina i dels lípids de la medul·la òssia, que són molt insaturats (McNeill et al., 1988), i també pot haver-hi alliberament de metalls com el ferro, que afavoriran l'oxidació.

En el cas que la carn es cogui, donat que les reaccions químiques augmenten amb la temperatura, fa que s'augmentin el processos oxidatius (Jadhav et al., 1996). Però a més, hi ha tota una altra sèrie de mecanismes pels quals la cocció afavoreix l'oxidació lipídica els quals han estat descrits per diversos autors (Decker i Xu, 1998, Erikson, 1998; Frankel, 1998; Jadhav et al., 1996). Entre ells hi ha:

1.- La desnaturalització de les metal·loproteïnes que contenen ferro, que comporta un alliberament d'aquest. La mioglobina és probablement la font més important de ferro alliberat ja que la ferritina no allibera tant fàcilment el ferro en ser desnaturalitzada (Decker i Welch, 1990).

2.- La desnaturalització d'enzims antioxidants. La catalasa queda desactivada quan la carn de vedella o de porc arriba a uns 70 ºC i la glutatió-peroxidasa (GPx) queda parcialment inactivada a aquesta temperatura (Decker i Xu, 1998). Al contrari la superòxid-dismutasa (SOD) és força termostable (Deshpande et al., 1996). Harel i Kanner (1985) atribueixen una major estabilitat de la carn crua respecte la cuita a l'acció de la catalasa, que evitaria l'activació de la metamioglobina per part del peròxid d'hidrogen.

3.- La mioglobina, pel tractament tèrmic, passa a l'espècie prooxidant oxen. A més, la desestructuració de les membranes cel·lulars fa que la separació entre els AGI i els catalitzadors metàl·lics es perdi i s'afavoreixi l'oxidació.

4.- Per altra banda, els HPL es descomponen per efecte de la calor i augmenten les reaccions d'isomerització i escissió donant finalment productes d'oxidació secundària que afavoriran la producció d'aromes i flavors moltes vegades no desitjats.

Tots aquests efectes depenen directament del temps i de la temperatura d'escalfament (Erikson, 1998; Frankel, 1998).

Amb tot, com a resultat de la cocció, tenim que les carns desenvolupen un aroma i flavor característics però, a conseqüència de l'esmentat anteriorment, també són més susceptibles en vers l'oxidació. Aquest increment de la susceptibilitat en vers l'oxidació dóna lloc a la formació de compostos volàtils, que provenen dels HPL, com per exemple alcanals, alquenals, alcadienals i cetones. A la vegada, amb l'emmagatzematge, es produeix un descens dels nivells de compostos volàtils associats amb olors carnis desitjables (furans amb substituents sulfur). La formació d'aquests compostos volàtils d'oxidació secundària és de gran importància, doncs són responsables directes dels olors desagradables i de l'enranciament de les carns (Frankel, 1998; Kerler i Grosch, 1996; Lamikanra i Dupuy, 1990; St Angelo et al., 1987; De Winne i Dirinck, 1996; Ang i Lyon, 1990).

Quan es parla del desenvolupament de la ranciesa en les carns normalment es diferencia entre l'oxidació "normal" deguda a l'oxidació de la fracció dels triacilglicerols, de l'oxidació ràpida que comporta el "flavor a rescalfat" o "warmed-over flavor" principalment atribuït a l'oxidació dels fosfolípids. L'oxidació "normal" apareix després d'emmagatzematges perllongats mentre que el "flavor a rescalfat" apareix després de cuinar la carn i posteriorment evoluciona cap a la ranciesa en augmentar l'oxidació lipídica. El "warmed-over flavor" apareix sobretot al rescalfar productes carnis cuits. En qualsevol cas, l'oxidació lipídica en les carns està associada a la pèrdua d'aromes desitjables i a la formació d'altres aromes, ara indesitjables, descrits generalment com a "cartró" o "pintura" (Love, 1988; Johnson i Civille, 1986; Spanier et al., 1988; Lyon et al., 1988).

Els productes de descomposició dels HPL, apart de donar aquestes característiques anòmales d'aroma i flavor, també poden interaccionar amb amines per produir bases de Schiff que posteriorment acaben donant melanoidines, les quals

confereixen un color fosc i, a la vegada, al ser inestables poden generar compostos volàtils que afecten a les característiques del flavor dels productes carnis durant el cuinat i el processat (Frankel, 1998). Si bé la majoria d'aquestes interaccions són negatives, des d'un punt de vista sensorial no totes ho són obligadament (Mottram, 1998).

En relació als diferents compostos formats, els compostos volàtils identificats en carn de pollastre cuita són en general els mateixos que en la carn de vedella, però tenen nivells més alts en compostos volàtils que provenen de lípids insaturats (2,4-decadienal, i γ-dodecalactona), conjuntament amb compostos d'aroma de vedella més baixos: bis-(2-metil-3-furil)-disulfur (Frankel, 1998; Farmer, 1999). De fet, de Winne i Dirinck (1996) indiquen que els aldehids són els principals contribuents a la ranciesa oxidativa de la carn de pollastre, sobretot l'hexanal, el nonanal i el 2,4-decadienal, degut a les quantitats en què es troben i al seu llindar inferior de percepció.

Si tenim en compte diferents carns, la carn de gall d'indi i de pollastre són més susceptibles a l'oxidació que la carn de vedella doncs la seva fracció fosfolípidica és més insaturada i tenen concentracions relativament més baixes de tocoferols (Frankel, 1998). A més, dins de la carn d'aviram, la carn vermella s'oxida més ràpidament que la blanca doncs té un contingut més elevat de fosfolípids i de ferro, tant hemo com no (Rhee, 1988).

1.3. Factors que Influeixen sobre el Balanç Oxidatiu en les Carns

Com ja s'ha vist en l'apartat anterior l'oxidació lipídica en carn i productes carnis està influenciada per varis factors, però a més n'hi ha molts d'altres entre els quals s'inclouen l'espècie animal, el tipus de muscle, la quantitat i el tipus de greix afegit a la dieta, la concentració d'antioxidants i prooxidants presents a la dieta i a la carn, l'estat sanitari i el processat que ha rebut la carn (Jensen et al., 1998a; Kanner, 1994). Dins de les condicions de processat i/o d'emmagatzematge hi ha diferents factors que tindran efectes evidents en l'oxidació de les carns com per exemple la conservació a baixes o altes temperatures, la protecció enfront la llum, l'envasat al buit i la pèrdua de la integritat original de la peça de carn.

Per incrementar l'estabilitat oxidativa del muscle esquelètic hem de conèixer els diferents factors que la poden afectar. Aquests els podem englobar en tres grans grups principals: els que afecten al substrat d'oxidació, els que afecten als catalitzadors i els que afecten als antioxidants.

1.3.1. Factors en Relació al Substrats

Com ja hem vist, els principals substrats de l'oxidació lipídica són, lògicament, l'oxigen i els lípids insaturats.

El control de la concentració d'oxigen és essencial en l'oxidació lipídica i es pot minimitzar envasant el producte carni al buit o en atmosferes modificades (Ahn et al., 1992; Bruun-Jensen et al., 1996).

Si bé la reacció directa i espontània de l'oxigen amb els AGI es troba fortament impedida (apartat 1.1), ja s'ha esmentat que aquesta es pot donar per enzims, per la presència de radicals lipídics i/o de l'oxigen o bé si l'oxigen present s'ha activat fotosensiblement. En aquest darrer cas és necessita d'un fotosensibilitzador o iniciador

com la riboflavina o les hematoporfirines. Els iniciadors absorbeixen la llum visible o ultravioleta i passen a estar excitats electrònicament. Aleshores són capaços de convertir l'oxigen triplet en singulet. En aquesta darrera forma, l'oxigen pot atacar directament l'àcid gras (veure apartat 1.1.1) i formar un HPL (Frankel, 1998; Jadhav et al., 1996, Min, 1998). A més, la radiació ultravioleta catalitza la descomposició de HPL, certs compostos cetònics i altres complexes de l'oxigen (Frankel, 1998).

En relació al substrat a oxidar, els àcids grassos, s'ha de tenir en compte el grau d'insaturació, especialment en els lípids de membrana que és on s'inicia l'oxidació lipídica en el muscle. La manipulació de la composició dels àcids grassos en animals no remugants es pot aconseguir canviant les fonts de greix incorporades a la dieta. Per contra la modificació del perfil lipídic en remugants és més difícil, degut a la biohidrogenació per part de les bactèries del rumen (Erikson, 1998; Hargis i Van Elswyck, 1993).

Per tant, si s'acaba incrementant la composició en àcids grassos saturats, provocarà unes carns més estables a l'oxidació, però disminuirà la seva qualitat des d'un punt de vista nutritiu i de la textura del producte. Si bé es prefereixen productes rics en AGPI des d'un punt de vista nutritiu, l'increment en la composició d'àcids grassos monoinsaturats representa una solució de compromís, doncs són més estables en front de l'oxidació (Nawar, 2000), els seus punts de fusió solen donar carns adequades, des del punt de vista de la textura, i presenten també uns efectes favorables per a la salut (Kritchevsky, 1998).

1.3.2. Factors Catalítics (Oxidants)

Dins d'aquest grup tenim tots aquells factors prooxidants, tal com els metalls de transició, les metal·loproteïnes i els enzims. Tots ells són constituents normals del muscle i indueixen l'oxidació lipídica de les membranes.

El ferro, el coure i altres metalls de transició són excel·lents promotors de les reaccions mitjançant radicals lliures, gràcies a la seva capacitat de transferència d'electrons en el seu canvi d'estat d'oxidació (Jadhav et al., 1996).

L'activitat catalítica dels metalls de transició en el muscle esquelètic està controlada mitjançant la formació de complexes estables amb proteïnes com la ferritina i transferrina pel cas de ferro, i l'albúmina sèrica, la ceruloplasmina, la carnosina i altres pèptids pel cas del coure (Deshpande et al., 1996; Kaur i Perkins, 1991). Normalment, aquests metalls estan lligats en la seva forma oxidada. A més, per raons estèriques, al estar lligats a les proteïnes, la capacitat d'aquests ions metàl·lics d'entrar en el cicle redox disminueix (Dunford, 1987). Si bé això és cert, el ferro lligat a les hemoproteïnes també pot participar en reaccions d'oxidació lipídica i de descomposició dels HPL (Belitz et al., 2004; Harel i Kanner, 1985; Rhee et al.,1987; Rhee, 1988). En concret, el complex protoporfirina-Fe^{2+} (P-Fe^{2+}) pot oxidar-se amb l'oxigen i formar el radical anió superòxid i el complex P-Fe^{3+} [veure reacció 17] que si després reacciona amb H_2O_2 pot formar l'espècie oxidant oxen (Belitz et al., 2004). L'espècie oxen pot generar radicals alquil i peroxil a partir dels àcids grassos i dels HPL, respectivament (Belitz et al., 2004; Asghar et al., 1988).

$$P\text{-}Fe^{2+} + O_2 \rightarrow P\text{-}Fe^{3+} + O_2\cdot^- \qquad [17]$$

La reducció de ADP-Fe^{3+} a ADP-Fe^{2+} es pot donar en la cadena de transport electrònic microsomal en presència de NADPH (Gutteridge i Halliwell, 1990; Halliwell i Chirico, 1993). A la vegada el complex ADP-Fe^{2+} pot formar radicals hidroxil a partir del peròxid d'hidrogen (Frankel, 1998; Belitz et al., 2004).

Tan sols una petita fracció dels metalls del muscle esquelètic no està associada a macromol·lècules (Decker i Hultin, 1992; Miller et al., 1990). Tot i això, són aquests metalls els que hom creu que tenen l'activitat catalítica principal. Això sí, diferents operacions de processat, per exemple el cuinat, poden incrementar aquesta fracció de metalls actius catalíticament i és la raó per la qual l'oxidació en la carn cuita és major (Erikson, 1998). El salat també altera la distribució i reactivitat del ferro (Kanner et al., 1991; Decker i Xu, 1998).

Un cop els metalls estan lliures, és difícil que oxidin directament la molècula lipídica per formar radicals lliures (Belitz et al., 2004). De totes maneres la seva

presència és decisiva en la formació d'espècies capaces de sostreure un protó als àcids grassos insaturats (Gray et al., 1996; Jadhav,1996). La seva actuació pot ser deguda a diferents mecanismes (Kanner et al., 1988b; Jadhav, 1996; Belitz et al., 2004; Morrissey et al., 1998; Miller et al., 1990; Halliwell et al., 1995b). Un primer mecanisme seria el que dóna lloc a formes actives de l'oxigen, sobretot en medi reductor, com el radical hidroxil [veure reacció 18]. Un altre, catalitzant la descomposició dels HPL donant lloc a radicals lliures [veure reaccions 19 i 20]. Les dues primeres reaccions [18 i 19] és donen a velocitats de reacció elevades mentre que la darrera [20] és una reacció lenta. Finalment, de manera indirecta, hi hauria un darrer mecanisme pel qual, interactuant amb agents reductors com la cisteïna, glutatió, l'ascorbat i l'α-tocoferol, aquests compostos són oxidats i perden la seva capacitat protectora.

$$H_2O_2 + \text{metall}^{n+} \xrightarrow{\textit{rápida}} \cdot OH + OH^- + \text{metall}^{(n+1)+} \qquad [18]$$

$$ROOH + \text{metall}^{n+} \xrightarrow{\textit{ràpida}} \rightarrow RO\cdot + OH^- + \text{metall}^{(n+1)+} \qquad [19]$$

$$ROOH + \text{metall}^{(n+1)+} \xrightarrow{\textit{lenta}} ROO\cdot + H^+ + \text{metall}^{n+} \qquad [20]$$

Per tant, els metalls poden afavorir l'aparició del radical hidroxil que és molt reactiu i pot atacar diferents molècules orgàniques presents en les cèl·lules (Halliwell, 1991; Kanner et al., 1988a, b; Kaur i Perkins, 1991; Belitz et al., 2004). A la vegada, la descomposició dels HPL mitjançant els metalls, sobretot ferro i coure, fa que siguin un factor determinant pel desenvolupament de l'oxidació degut a la formació de radicals lliures a partir dels HPL (Jadhav, 1996; Kanner et al., 1988a, b).

En relació als factors enzimàtics, ja hem vist (apartat 1.1.2) que existeixen diferents enzims que poden catalitzar l'oxidació lipídica en productes carnis, com per exemple les lipooxigenases, mieloperoxidasa i determinats enzims de membrana que redueixen el ferro (Erikson, 1998). Aquests últims són importants, ja que no tan sols generen catalitzadors si no que a més ho fan a prop de les membranes que són altament insaturades.

1.3.3. Factors Antioxidants

El teixit muscular té un sistema de defensa antioxidant per combatre els efectes negatius dels prooxidants. Aquest sistema antioxidant múltiple és pot classificar en components de naturalesa liposolube (1), de naturalesa hidrosoluble (2) i de tipus enzimàtic (3). La seva funció és controlar els prooxidants, segrestar radicals lliures i desactivar espècies reactives de l'oxigen. La capacitat antioxidant de les carns pot ser incrementada controlant les operacions de processat, amb l'ús d'additius i a través de la dieta.

(1) Liposolubles. Els principals compostos antioxidants presents en el teixit muscular, de naturalesa liposoluble, són els tocoferols. D'entre els tocoferols, l'α-tocoferol és el predominant en la carn de vedella, porc, pollastre i en els peixos (Erikson, 1998).

L'α-tocoferol i altres compostos relacionats, tant tocoferols com tocotrienols, tenen en comú un anell 6-cromanol que pot captar radicals mitjançant la formació del radical tocoferoxil. Aquest és poc reactiu i no promou més la cadena radicalaria. A més, té la particularitat que aquest radical pot ser reduït de nou, per l'acció combinada de l'àcid ascòrbic i el glutatió reduït, i per tant regenerar el tocoferol, o bé pot ser oxidat de nou (Diplock et al., 1998; Deshpande et al., 1996; Chow, 2001). Els animals no poden sintetitzar tocoferols i per tant els han d'obtenir a partir de la dieta. A més, són relativament estables a moltes operacions de processat com per exemple els tractaments tèrmics. L'α-tocoferol, a més d'ésser considerat un excel·lent captador de radicals, també pot temperar l'estat energètic excitat de l'oxigen singulet (Diplock et al., 1998; Erikson, 1998; Min i Boff, 2002).

L'altre gran grup d'antioxidants naturals són els carotens i la seva actuació contra l'oxidació es deu principalment a la seva gran capacitat per desactivar l'oxigen singulet (Min i Boff, 2002). També poden actuar captant radicals i formant un nou radical estabilitzat per ressonància, degut al gran nombre dels dobles enllaços de la seva molècula (Yanishlieva et al., 1998). Tanmateix, la capacitat per captar radicals és menor que la de l'α-tocoferol (Ternay i Sorokin, 1997). El fet que l'oxidació per part

de l'oxigen singulet sigui poc important fa que el seu paper en la protecció de productes carnis sigui més limitada que en el cas de l'α-tocoferol (Decker i Xu, 1998). A més, a elevades concentracions d'oxigen s'ha descrit que poden actuar com a prooxidants (Erikson, 1998; Madhavi et al., 1996). Els carotens, però, també són relativament estables als tractaments tèrmics.

La ubiquinona, també anomenada coenzim Q, és troba en les mitocondries. Aquesta inhibeix l'oxidació lipídica mitjançant la captació de radicals lliures (Cabrini et al., 1986; Papas, 1999) i podria estar relacionada amb la regeneració del radical tocoferoxil cap a tocoferol dins de les membranes o de les lipoproteïnes (Halliwell, 1994b; Papas, 1999; Halliwell i Gutteridge, 1999). No es coneix massa d'ella però es creu que el seu paper pot ser important en les carns vermelles (Decker i Xu, 1998).

(2) Hidrosolubles. Aquests es troben dins del citosol. L'àcid ascòrbic és el més important dins dels compostos hidrosolubles i no és un nutrient essencial per l'aviram, doncs el poden sintetitzar (Russell, 1989). La seva acció antioxidant en la carn és controvertida doncs la seva funció depèn de la concentració en què es troba. A baixes concentracions (200-300 mg/kg) s'ha descrit que té activitat prooxidant ja que redueix el Fe^{3+} a Fe^{2+}, el qual descomposa més ràpidament els peròxids presents [reaccions 19 i 20]. Per contra, a concentracions més elevades actua com a antioxidant gràcies al fet que predomina la seva capacitat de captar oxigen i/o radical lliures lipídics (Decker i Xu, 1998, Frankel, 1998; Grau et al., 2001a; Halliwell, 1994b; Halliwell i Gutteridge, 1999).

A més de l'anterior capacitat prooxidant per la seva acció sobre el ferro, l'àcid ascòrbic pot provocar l'alliberament de ferro unit a la ferritina, lípids de membrana i proteïnes insolubles (Decker i Welch, 1990; Decker et al., 1993; Frankel, 1998). També s'ha vist que l'àcid ascòrbic pot inhibir la formació i l'activitat de l'espècie prooxidant oxen, per tant, en el muscle esquelètic, l'àcid ascòrbic té un doble paper com anti i prooxidant (Decker i Xu, 1998). L'àcid ascòrbic és descompost fàcilment durant els tractaments tèrmics i l'emmagatzematge.

Potser el més important en sistemes biològics és el sinergisme descrit entre el α-tocoferol i l'àcid ascòrbic. L'α-tocoferol, lipòfil, es troba en els membranes cel·lulars i en les lipoproteïnes plasmàtiques. Això fa que reaccioni més fàcilment amb

els radicals lipídics que no pas l'àcid ascòrbic que és hidrosoluble. El tocoferol, un cop ha reaccionat amb els radicals lliures provinents de l'oxidació, es converteix en radical tocoferoxil i migra cap a la superfície de la membrana i és regenerat de nou gràcies a l'acció de l'àcid ascòrbic (Jadhav et al., 1996; Halliwell, 1996; Halliwell, 1994a). L'àcid ascòrbic oxidat per la captació de radicals passa a la forma de dehidroascòrbic i, aleshores, és regenerat per enzims a expenses del glutatió reduït (GSH) (Halliwell i Gutteridge, 1999; Decker, 1998).

El glutatió és un tripèptid que inhibeix l'oxidació tant per la captació de radicals com pel subministrament d'electrons que permet que l'enzim GPx descomposi el peròxid d'hidrogen i els HPL en compostos sense activitat prooxidant (Sciuto, 1997). En carn de gall d'indi, s'ha vist que el glutatió, si bé es veu poc afectat pels tractaments tèrmics, aquest s'esgota durant l'emmagatzematge. El mateix passa amb la carnosina.

La carnosina és un dipèptid (β-alanil-L-histidina) present de forma natural en el muscle esquelètic (Erikson, 1998). La seva funció en el muscle no es coneix exactament, però es creu que té una doble funció regulant el pH i actuant com antioxidant (Chan i Decker, 1994). La seva acció antioxidant ve donada per diversos factors, ja que pot actuar com a captador de radicals lliures en el citosol, com a donador de protons i com a quelant (Chan i Decker, 1994; Decker i Faraji, 1990). Tot i això, la capacitat quelant sembla ser específica pel coure. A més, la carnosina sembla capaç de combinar-se amb productes d'oxidació aldehídica que donen lloc a la ranciesa del producte (Zhou et al., 1998). En relació amb això, s'ha vist que l'addició de carnosina en el processat dels productes és capaç d'inhibir el desenvolupament de l'oxidació lipídica en carn de gall d'indi crua i en carn de porc cuita salada i sense salar (Morrissey et al., 1998). També s'ha vist que la seva addició, en hamburgueses de pollastre salades, inhibeix l'oxidació dels àcids grassos i del colesterol (O'Neill et al., 1998b, 1999).

A la vegada, en el muscle hi ha diverses molècules amb capacitat per quelar metalls prooxidants com per exemple l'albúmina sèrica, la histidina i altres pèptids que contenen aquest aminoàcid, que actuarien sobre el coure, mentre que la transferrina, la lactoferrina i la ferritina actuarien sobre el ferro.

També s'ha de destacar que, a més del glutatió, diferents compostos, com per exemple pèptids i proteïnes tenen capacitat antioxidant, gràcies als grups –SH. Aquest grup pot captar diferents radicals d'espècies derivades de l'oxigen (Halliwell i Gutteridge, 1999; Sen i Packer, 2000), segons la següent reacció:

$$RSH + \cdot OH \rightarrow RS\cdot + H_2O \quad [21]$$

Aquesta reacció implica la transformació d'un radical hidroxil en un radical tioil menys reactiu i podria contribuir a explicar l'activitat antioxidant descrita per la metal·lotioneina (Coyle et al., 2002; Maret, 2000, 2003; Klotz et al., 2003). D'altra banda aquesta proteïna del citosol, que també regula el metabolisme del Zn, és rica en cisteïna i actua quelant diferents cations divalents (Halliwell i Gutteridge, 1999; Nordberg, 1998; Maret et al., 1999; Maret, 2003).

Malgrat això, els grups tiol també tenen poder reductor i per tant, en presència de metalls lliures, es poden formar radicals tioil [reaccions 22 i 23] de gran reactivitat (Desphande et al., 1996; Halliwell i Gutteridge, 1999).

$$RSH + Fe^{3+} \rightarrow RS\cdot + Fe^{2+} + H^{+} \quad [22]$$
$$RSH + Cu^{2+} \rightarrow RS\cdot + Cu^{1+} + H^{+} \quad [23]$$

A més la reducció del Fe^{3+} i Cu^{2+} a Fe^{2+} i Cu^{1+} promou la formació ràpida de radicals hidroxil a partir dels HPL [reacció 19]. La protecció i la competència per aquests grups tiol enfront del coure i el ferro explicaria alguna de les propietats antioxidants descrites pel zinc (Powell, 2000; Zago i Oteiza, 2001).

Entre d'altres compostos, les poliamines i l'àcid úric sembla que també podrien tenir efectes antioxidants en les carns mitjançant diversos mecanismes (Decker i Xu, 1998; Deshpande et al., 1996).

(3) Enzims antioxidants. Dins dels factors enzimàtics tenim la superòxid-dismutasa (SOD), la catalasa, i la glutatió-peroxidasa (GPx). L'oxigen pot convertir-se en radical anió superòxid, que per l'addició d'un electró és altament reactiu. Aquest sorgeix en els sistemes de transferència d'electrons de les membranes, de l'oxidació de la oximioglobina cap a metamioglobina, de l'activació de varis leucòcits presents en el sistema vascular del teixit muscular i de l'oxidació de l'àcid ascòrbic i altres components reductors per l'acció del ferro lliure (Kanner, 1994). La SOD, que té com a cofactors el coure i el zinc es localitza en el citosol, mentre que en les mitocondries dels mamífers n'hi ha una altra que conté manganès. Aquestes catalitzen el pas de radical superòxid a peròxid d'hidrogen que és una espècie menys reactiva, segons la reacció:

$$2O_2^{\cdot -} + 2H+ \xrightarrow{SOD} H_2O_2 + O_2 \qquad [24]$$

El peròxid d'hidrogen, però, pot ser ràpidament descompost pel metalls de transició i formar el radical hidroxil (Halliwell i Gutteridge, 1990) [veure reacció 18]. Aquest radical és un radical lliure extremadament reactiu, que no pot ser eliminat enzimàticament (Deshpande et al., 1996). Abans de que això passi, la catalasa, una hemoproteïna present sobretot en els peroxisomes, segons la reacció [25], i la GPx cel·lular en el citosol, segons la reacció [26], s'encarreguen de transformar el H_2O_2 en H_2O. Així doncs, els tres enzims treballen conjuntament per mantenir en condicions fisiològiques una concentració estable i acceptable d'espècies actives de l'oxigen (Jadhav et al., 1996).

$$2H_2O_2 \xrightarrow{CATALASA} 2H_2O + O_2 \qquad [25]$$

$$2H_2O_2 + 2GSH \xrightarrow{GPx} 2H_2O + GSSG \qquad [26]$$

Per altra banda, la GPx, que conté seleni com a cofactor i que utilitza el GSH per oxidar-lo a GSSG, també pot transformar els HPL i reduir-los als corresponents alcohols [reacció 27].

$$ROOH + 2GSH \xrightarrow{GPx} ROH + GSSG + H_2O \qquad [27]$$

Posteriorment, la glutatió-transferrasa acetila els alcohols per poder-los eliminar a través de l'orina (Kaur i Perkins, 1991; Mathews i Van Holde, 1998).

En la Figura 2, s'esquematitzen els factors més importants que entren en joc en el balanç oxidatiu a les carns.

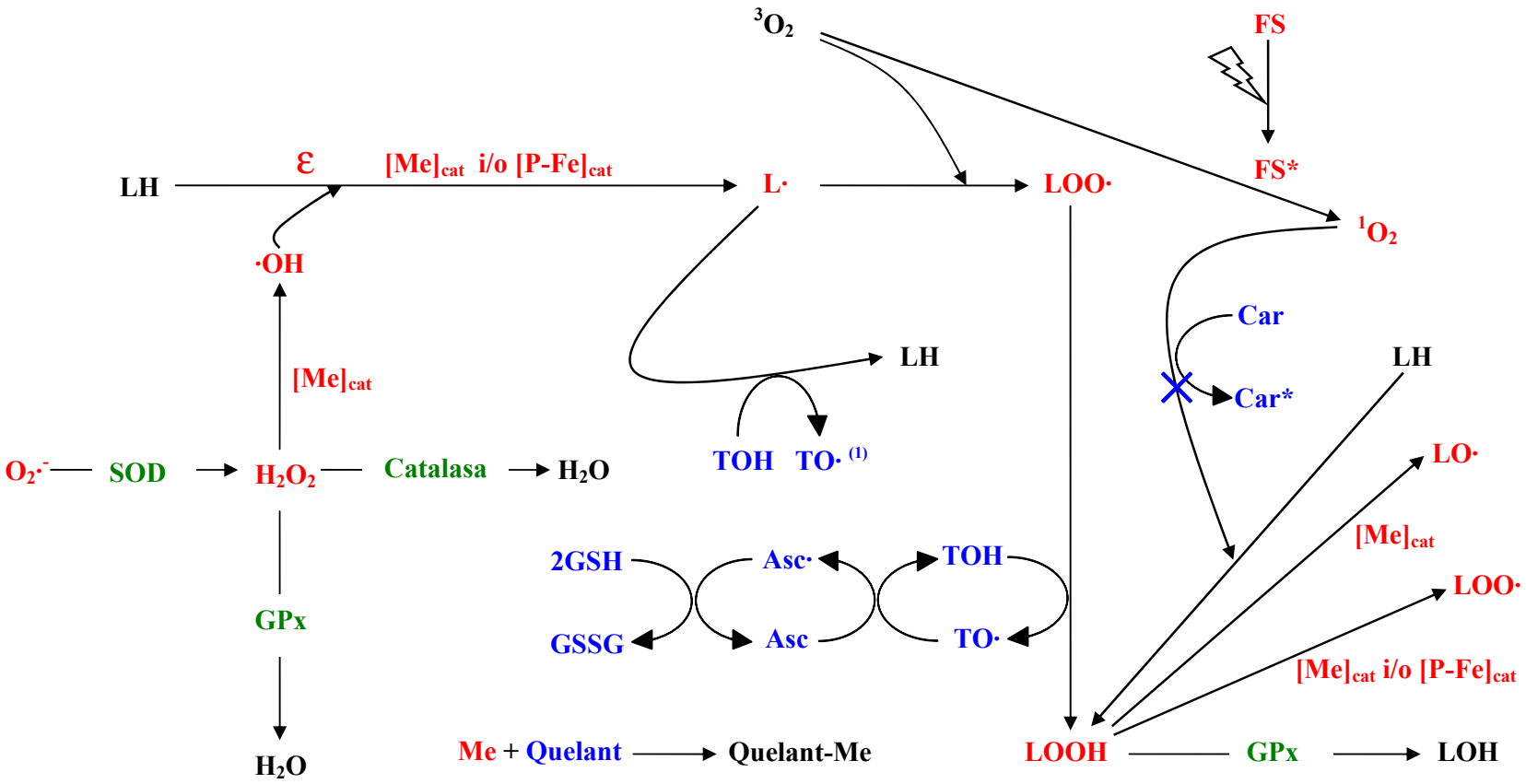

Figura 2. Esquema dels principals factors que influeixen sobre l'oxidació lipídica en carns amb les principals reaccions implicades.

Lletres en vermell indiquen factors prooxidants, en blau factors antioxidants, en verd enzims antioxidants i en negre compostos sense activitat pro o antioxidant. LH = lípid insaturat; L· = radical alquil; LO· = radical alcoxil; LOO· = radical peroxil; LOOH = hidroperòxid; FS = fotosensibilitzant; FS* = fotosensibilitzant excitat; Car = carotenoid; Car* = carotenoid excitat; LOH = hidroxiàcid; ε = energia; SOD = superòxid-dismutasa; GPx = glutatió-peroxidasa; Me = metalls lliures; $[Me]_{cat}$ = reacció catalitzada per metalls lliures; [P-Fe] molècules amb el nucli ferroporfirínic; $[P\text{-}Fe]_{cat}$ = reacció catalitzada per molècules amb el nucli ferroporfirínic; ·OH = radical hidroxil; $O_2·^-$ = radical anió superòxid; TOH = tocoferol; TO· = radical tocoferoxil; Asc = àcid ascòrbic; Asc· = radical ascorbil; GSH = glutatió reduït; GSSG = glutatió oxidat.
(1) Pot ser regenerat mitjançant ascorbic i glutatió.

Amb independència de tot el que s'ha esmentat anteriorment, s'ha de tenir en compte que les diferents operacions de processat poden modificar, de manera diferent, a tots aquests factors i per tant afectar al balanç oxidatiu. Així doncs, el trossejat de la carn afavoreix la presència d'oxigen facilitant l'oxidació. És per això que l'envasat amb atmosferes amb baix contingut d'oxigen o al buit perllonguen l'estabilitat oxidativa, encara que la formació de metamioglobina, que es pot formar a baixes pressions d'oxigen, dóna una coloració no desitjable en les carns. No obstant, el color de les carns es pot estabilitzar amb l'ús d'atmosferes modificades (Foegeding, 2000) i al mateix temps ésser eficaces enfront l'oxidació i la formació d'aromes indesitjables (Erikson, 1998; Mielche i Bertelsen, 1994).

Un altra operació de processat que pot influir en l'oxidació és la cocció. Els tractaments tèrmics són més aviat oxidants, però si s'escalfa per sobre de 100 ºC, a la superfície del producte es poden formar productes d'enfosquiment, per la reacció de Maillard. Aquests compostos inhibeixen l'oxidació lipídica i el desenvolupament del flavor a rescalfat o "warmed-over". Això és degut a que poden trencar la cadena radicalaria o bé quelar ions metàl·lics (Frankel, 1998; Jadhav et al., 1996).

Altres operacions, com per exemple el fumat, protegeixen de l'oxidació gràcies a la presència de fenols en el fum que tenen activitat antioxidant (Erikson, 1998). També es poden afegir extractes naturals o espècies que contenen antioxidants naturals com flavonoids i polifenols, com per exemple l'extracte de romaní (Frankel, 1998). Malauradament, això fa que el seu aroma i gust inicial es vegi modificat per l'addició d'aquests extractes.

A la vegada, i pensant en afavorir el balanç pro i antioxidatiu de les carns, hom pot addicionar al producte antioxidants sintètics de tipus fenòlic com el BHA, BHT, galat de propil, i el TBHQ que protegeixen de forma eficaç enfront l'oxidació segrestant radicals lliures. Tot i això la seva addició no està permesa en carns. Normalment el consumidor prefereix els additius naturals com per exemple els tocoferols, en aquest cas la seva activitat antioxidant és major quan aquest antioxidant s'afegeix a la dieta, ja que s'incorpora directament a la membrana cel·lular que és on s'inicia l'oxidació (Jensen et al., 1998a).

L'oxidació també pot ser controlada de manera efectiva controlant l'activitat dels metalls prooxidants amb l'addició de quelants. Els més comuns són els fosfats i

l'àcid cítric. L'ús de l'àcid ascòrbic és escàs en productes carnis doncs manté la forma reduïda de la mioglobina afectant a la percepció de frescor de la carn. A més, com ja ha estat comentat, pot actuar com anti i prooxidant, encara que sembla que a dosis elevades (0,5%) predominen les seves propietats antixodidants (Mielche i Bertelsen, 1994; Decker i Xu, 1998).

2. FACTORS DIETÈTICS: EFECTES EN EL VALOR NUTRITIU I LA QUALITAT SENSORIAL

La carn de pollastre és àmpliament consumida a tot el món i el consumidor la considera saludable en especial pel seu baix contingut en greix quan es menja sense pell. En la Taula 3 es mostra la composició nutritiva normal de la cuixa i del pit de pollastre.

La composició del greix, però, és força variable. De fet fa molt temps que es coneix que la composició en àcids grassos de la dieta modifica la composició dels àcids grassos en diferents teixits del pollastre (Marion i Woodroof, 1963; Miller i Robisch, 1969). Això és degut a que els àcids grassos, a l'igual que la resta d'animals monogàstrics, són absorbits en l'intestí sense patir cap modificació i incorporats en els diferents teixits (Wood i Enser, 1997). És per aquesta raó que el contingut a la canal en AGPI essencials per l'animal, com són l'àcid linoleic i el linolènic, respon ràpidament als canvis dietètics. Per contra, una dieta rica en àcids grassos saturats i monoinsaturats, els quals si que poden ser sintetitzats per l'animal, té menys repercussió en la modificació del seu perfil lipídic.

Com ja s'ha comentat en els apartats anteriors, l'increment en AGPI pot esdevenir un problema a nivell de l'estabilitat oxidativa i de les característiques sensorials del producte carni. En aquest sentit, cal esmentar que el contingut en α-tocoferol de la carn de pollastre no és massa elevat respecte al seu contingut en AGPI, particularment si es compara amb altres carns i els ous (Taula 4).

A més, el fet que diversos organismes nacionals i internacionals hagin posat un especial èmfasi en les seves recomanacions, tant en la reducció de la quantitat de greix que ingerim com en la composició d'aquest (Taula 5), ha fet créixer l'interès per part del consumidor en aliments més magres i saludables (Bruhn et al., 1992).

Aquests organismes donen diferents recomanacions en les quals es troben les quantitats en AGPI de la sèrie n-3, doncs se'ls atribueixen efectes beneficiosos en el tractament de malalties inflamatòries (Page, 1993; Barlow i Pike, 1991; Calder, 1996; Ruxton et al., 2004), en la reducció de la incidència de malalties cardiovasculars (Leaf

Taula 3.- Composició de la cuixa i del pit de pollastre crus, amb i sense pell expressada per 100 g de fracció comestible.

Nutrient	Cuixa amb pell[1]	Cuixa sense pell[2]	Pit amb pell[1]	Pit sense pell[2]
Aigua (g)	69,5	75,8	70,4	74,2
Proteïna (g)	18,2	20,9	22,2	24,0
Greix (g)	11,2	2,8	6,20	1,1
Àcids grassos saturats (g)	3,67	0,8	1,91	0,3
Àcids grassos monoinsaturats (g)	3,22	1,3	1,96	0,5
Àcids grassos poliinsaturats (g)	2,60	0,6	1,51	0,2
Hidrats de carboni (g)	0	0	0	0
Energia (Kcal)	173	109	145	106
Minerals				
Ca (mg)	15	7	14	7
Fe (mg)	1,8	0,8	1,1	0,5
Mg (mg)		24		29
P (mg)	188	110	212	220
K (mg)	250	390	264	370
Na (mg)	95	90	66	60
Zn (mg)		1,7		0,7
Cu (mg)		0,02		0,05
Mn (mg)		0,01		0,01
Se (µg)	7,3	14	6,5	12
Vitamines				
Àcid ascòrbic (mg)		0		0
Tiamina (mg)	0,10	0,14	0,07	0,14
Riboflavina (mg)	0,24	0,22	0,09	0,14
Niacina (mg)		5,6		10,7
Àcid pantotènic (mg)	0,84	1,09	0,84	1,26
Vitamina B_6 (mg)	0,25	0,28	0,53	0,51
Folats (µg)	11	9	9	14
Vitamina B_{12} (µg)		1	0,40	Tr[3]
Retinol (µg)		20		Tr
α-tocoferol (mg)		0,17	0,25	0,13

[1] Souci et al. (2000).

[2] Food Standards Agency (2002).

[3] Tr = traces.

Taula 4.- Relació entre àcids grassos poliinsaturats (AGPI) i tocoferol en diferents carns i ous (Food Standards Agency, 2002)[1].

Nutrient	Ous	Vedella	Corder	Pollastre
Proteïna (g)	12,5	22,5	20,2	22,3
Greix (g)	11,2	4,3	8,0	2,1
AGPI (g)	1,7	0,2	0,5	0,4
Tocoferol (mg)	1,11	0,13	0,10	0,15
Tocoferol/AGPI (g/g)	0,65	0,65	0,20	0,38

[1] Composició expressada per 100 g de fracció comestible.

i Weber, 1988; Page, 1993; Kinsella et al., 1990) i estan relacionats amb el desenvolupament normal del cervell i de la salut mental (Ruxton et al., 2004). Aquests efectes estan en contraposició als provocats per un consum excessiu en àcids grassos saturats, doncs aquests és relacionen amb elevades concentracions de colesterol en sang i amb un major risc de patir malalties cardiovasculars (Grundy, 1999; Hegsted, 2000; Page, 1993).

El fet que hi hagi una demanda de productes alimentaris més rics en AGPI, malauradament, fa que aquests també siguin més susceptibles cap a l'oxidació i per tant s'afavoreixi la formació de HPL i altres productes d'oxidació.

Així doncs, la formació de productes d'oxidació a partir d'àcids grassos essencials provoca una pèrdua del valor nutritiu inicial. Però a la vegada, la ingesta de productes d'oxidació dels àcids grassos així com del colesterol representen un risc potencial per la salut humana, donat els efectes biològics negatius d'aquests productes d'oxidació (Esterbauer, 1993; Cohn, 2002; Guardiola et al., 2002) i també per l'increment de l'estrès oxidatiu (Khan-Merchant et al., 2002; Penumetcha et al., 2000).

Així doncs, s'ha d'arribar a una situació de compromís entre l'increment del valor nutritiu i l'estabilitat oxidativa del producte alimentari. A més, no s'han de perdre de vista les característiques sensorials que faran que el producte acabi sent acceptat per part del consumidor. Així doncs, si volem incrementar el contingut en AGPI de la carn de pollastre per obtenir un producte alimentari amb un valor nutritiu

Taula 5.- Recomanacions de ingesta en àcids grassos poliinsaturats per adults sans.

Referència	Àmbit	Recomanació
WHO (2003)	Internacional	Expressat com a percentatge d'energia diària: 6-10% han de ser AGPI 5-8% han de ser AGPI n-6 1-2% han de ser AGPI n-3
FAO/WHO (1994)	Internacional	La relació àcid linoleic i linolènic ha de ser entre 5:1 i 10:1
Simopoulos et al. (2000)	Publicació internacional	En una dieta de 2000 Kcal: Àcid linoleic 4,44 g/dia Àcid linolènic 2,22 g/dia EPA + DHA: 0,65 g/dia EPA: mínim 0,22 g/dia DHA: mínim 0,22 g/dia
National Heart Foundation (1999, 2001)	Austràlia	Menjar peix com a mínim dos cops per setmana
Health and Welfare (1990)	Canadà	Consumir entre 1,1-1,6 g/dia en AGPI n-3
Ministry of Health, Labor and Welfare (1999)	Japó	La relació entre àcids grassos n-3 i n-6 ha de ser 4:1
Nordic Council of Ministers (1996)	Dinamarca, Finlàndia Noruega i Suècia	Els àcids grassos essencials han d'aportar el 3% de l'energia total dels quals un mímin de 0,5% han de ser n-3
British Nutrition Foundation (1999)	Regne Unit	Expressat com a percentatge d'energia diària: mínim 1% en àcid linoleic 0,2% en àcid linolenic 1,25 g/dia en EPA + DHA
Department of Health, Committee on the Medical Aspects of Food and Nutrition Policy (COMA) (1994)	Regne Unit	La ingesta en AGPI n-3 ha de ser superior 0,2 g/dia
Scientific Advisory Committee on Nutrition (2002)	Regne Unit	La ingesta en AGPI n-3 ha de ser superior 0,2 g/dia
Health Coucil of The Netherlands (2001)	Holanda	1% de l'energia total ha de ser en AGPI n-3 i la ingesta en DHA ha de ser entre 150-200 mg/dia
Institute of Medecine, Food Nutrition Board (2002)	Estats Units	La ingesta ha de ser 1,1-1,6 g/dia en àcid linolènic dels quals un 10% poden ser substituïts per EPA i DHA
American Heart Association (2002)	Estats Units	Menjar peix com a mínim dos cops per setmana

Abreviacions: AGPI = àcids grassos poliinsaturats, EPA = àcid eicosapentaenoic (20:5 n-3), DHA = àcid docosahexaenoic (22:5 n-3).

afegit, haurem de minimitzar al màxim el risc d'oxidació. L'addició d'antioxidants a la dieta, com per exemple l'α-tocoferol, permet la incorporació d'aquest en les membranes de l'animal i d'aquesta manera pot protegir-les de manera eficient contra l'oxidació (Jensen et al., 1998a).

Tot i que la manipulació del perfil lipídic en la carn de pollastre ha estat força estudiada, poques vegades s'ha fet tenint en compte les recomanacions dietètiques. A més, un cop s'ha aconseguit un producte carni que aporti una quantitat significativa de les recomanacions dietètiques cal assegurar la seva estabilitat oxidativa i la seva qualitat sensorial. En aquest sentit, l'estat oxidatiu del greix afegit al pinso també pot repercutir en l'estabilitat oxidativa del producte (Jensen et al., 1997) i per tant en les seves característiques sensorials.

Per altra banda, en relació a la composició mineral, és coneix que els productes carnis són una de les principals fonts de Fe, Zn, Se i Cu (Buss i Rose, 1992; Foster i Sumar, 1997) i, a més, aquests elements hi són altament biodisponibles (Fairweather-Tait, 1992; Lönnerdal, 2000; Hortin et al., 1993). Això fa que els productes carnis pel seu contingut elevat en aquests minerals tinguin un especial interès, i en especial per a certs grups de població (Pennington i Young, 1991; Foster i Sumar, 1997; Brown et al., 2001, 2002). Un d'aquests grups seria la gent d'edat avançada, que el té risc de baixes ingestes en vitamines i minerals que s'explica pel baix consum energètic el qual està relacionat amb la disminució del nivell d'activitat física, l'accés limitat a aliments amb un contingut nutritiu adequat, malalties cròniques, estats psicològics, medicacions o disminució de l'apetència (Drewnowski i Schultz, 2001; Russell, 1992). De fet, les ingestes de Se i sobretot de Zn per part de la gent gran tenen risc de trobar-se lleugerament per sota de les recomanacions (Pepersack et al., 2001; Girodon et al., 1999; de Jong et al., 2001; Ervin i Kennedy-Stephenson, 2002).

Si bé el Zn i el Se tenen un paper important dins del sistema enzimàtic antioxidant (apartat 1.3.3), també es creu que poden tenir un paper important en la resposta immune (High, 1999; Girodon et al., 1999; Keen i Gershwin, 1990; Bogden et al., 1987; Mitchell et al., 2003; Kiremidjian-Schumacher et al., 1994; Nair i Schwartz, 1990). A més, pel cas del Se, s'ha observat l'associació entre baixes ingestes d'aquest element amb un increment del risc en certs tipus de càncer (Clark et al., 1996; Knekt et al., 1991, Fex et al., 1987; Foster i Sumar, 1997; Bjelakovic et al., 2004) i amb risc de

patir malalties cardiovasculars (Salonen et al., 1991; Suadicani et al., 1992; Kok et al., 1989; Mihailovic et al., 2003). Tot i això, l'enriquiment en la composició mineral de la carn ha estat poc estudiada i tampoc les seves repercussions a nivell d'estabilitat oxidativa i qualitat sensorial.

2.1. Modificació de la Fracció Lipídica Mitjançant la Dieta

El contingut en lípids de la carn de pollastre varia segons el teixit (Pikul et al., 1984; Ang et al., 1988; Ratnayake et al., 1989). Per exemple, la pell té un elevat contingut de greix (aproximadament 30 g en 100 g de pell) principalment en forma de triacilglicerols. El contingut en lípids en la carn blanca (pit de pollastre) és aproximadament la meitat de la carn vermella (cuixa) (veure Taula 3). Però a més aquests lípids presents en el pit de pollastre són majoritàriament fosfolípids, mentre que en la cuixa predominen els triacilglicerols.

A la Taula 6, es mostra la composició en àcids grassos de la fracció lipídica extraïble de pit, cuixa i pell de pollastre, alimentat amb dietes estàndard (normalment poc insaturades).

A la darrera taula s'observa que l'àcid gras majoritari en els tres teixits és l'àcid oleic. Els majors percentatges en àcids grassos monoinsaturats es troben en carn vermella i pell. Contràriament la carn blanca té el major percentatge en AGPI. Això s'explica degut a la major proporció de fosfolípids presents al pit de pollastre.

Malgrat aquesta composició tipus, el contingut i la composició dels lípids de la carn de pollastre pot presentar variacions degudes a la genètica (Leclerq i Escartin, 1987; Legrand et al., 1987), l'edat (Legrand et al., 1987), el sexe (Cahaner et al., 1986), les condicions ambientals i a diversos factors nutritius (López-Bote et al., 2001). Dins del darrer hi hauria la quantitat i la composició lipídica del greix afegit a la dieta.

La composició de la dieta té una marcada influència en la composició dels àcids grassos del teixit muscular i adipós del pollastre. Així doncs, s'ha observat que el contingut en àcids grassos saturats es pot augmentar si s'afegeix a la dieta greix de coco (Lin et al., 1989a; Asghar et al., 1990) o de palma (Cherian et al., 1996). Per contra el contingut en àcid oleic es pot augmentar amb l'addició al pinso d'oli d'oliva (Lin et al., 1989a; Asghar et al., 1990; Lauridsen et al., 1997a; O'Neill et al., 1998a; Crespo i Esteve-García 2002a, b) i el contingut en àcid linoleic amb l'addició d'oli de colza (Scaife et al., 1994; López-Ferrer et al., 1999a), de gira-sol (Cherian et al., 1996;

López-Ferrer et al., 1999a; Crespo i Esteve-García, 2002a, b) o de soja (Scaife et al., 1994; López-Ferrer et al., 1999a). Igualment, el contingut en àcid linolènic es pot incrementar amb l'addició al pinso d'oli de llinosa (Lin et al., 1989a; Asghar et al., 1990; Ajuyah et al., 1993a; Ahn et al., 1995; Cherian et al., 1996; López-Ferrer et al., 1999a, b; Crespo i Esteve-García, 2002a, b) i el contingut en àcid eicosapentaenoic (EPA) i àcid docosahexaenoic (DHA) amb olis de peix (Scaife et al., 1994; Cherian et al., 1996; López-Ferrer et al., 1999b, 2001; Surai i Sparks, 2000).

Taula 6. Composició en àcids grassos de la fracció extreta amb dissolvents, a partir de la carn blanca i vermella i de la pell de pollastres alimentats amb una dieta estàndard (Ratnayake et al., 1989).

Àcid gras	**Carn**		**Pell**
	blanca (pit)	**vermella (cuixa)**	
16:0	23,8	22,6	24,0
18:0	7,5	7,6	5,1
Total saturats	33,5	32,2	30,7
16:1	4,5	6,3	7,8
18:1	29,1	32,0	39,4
20:1	0,5	0,5	0,6
22:1	0,4	0,6	0,4
Total monoinsaturats	34,5	39,4	47,8
18:2 n-6	17,8	18,3	18,2
18:3 n-3	0,5	0,7	1,0
20:4 n-6	5,0	3,7	0,6
20:5 n-3	0,7	0,6	0,4
22:5 n-3	0,9	0,5	0,1
22:6 n-3	1,8	1,0	0,1
Total poliinsaturats n-6	27,4	25,1	19,7
Total poliinsaturats n-3	4,5	3,4	1,8
Total poliinsaturats	32,0	28,5	21,4

A més, aquestes modificacions en el perfil d'àcids grassos del teixit muscular i adipós es poden aconseguir sense alterar el contingut en lípids totals ni les proporcions relatives dels principals lípids (Phetteplace i Watkins et al., 1990; Yau et al., 1991).

El perfil d'àcids grassos dels teixits de dipòsit, com per exemple la grassa abdominal i subcutània, és més fàcil de modificar a través de la dieta que no pas el del teixit muscular (Scaife et al., 1994; López-Ferrer et al., 1999a). Aquestes diferències entre el teixit muscular i els teixits de dipòsit s'han atribuït a la funció que tenen els àcids grassos en cada teixit. La funció del teixit de dipòsit és d'emmagatzematge i per això reflexa de forma fidel la composició de la dieta (Yau et al., 1991). Contràriament, les membranes cel·lulars del teixit muscular tenen una composició en àcids grassos més constant per tal de mantenir les seves característiques físiques i les seves funcions biològiques (Gurr, 1984). Així doncs, és lògic que la seva modificació mitjançant la dieta sigui més limitada (López-Bote et al., 1997) observant-se un dipòsit preferencial en determinats AGPI (Scaife et al., 1994; Crespo i Esteve-García, 2001).

En relació a la vitamina E, aquesta també es veu modificada per la seva addició en la dieta. D'entre els diferents tocoferols i tocotrienols, l'α-tocoferol és incorporat preferentment en els diferents teixits del pollastre (Kang et al., 1998; Cherian et al., 1996) i també en el rovell de l'ou (Cherian i Sim, 2003). Quan s'addicionen al pinso suplements en forma d'acetat d'α-tocoferol, els teixits de pollastre que responen més fàcilment als suplements són el fetge i el teixit adipós mentre que el cervell i la carn blanca i vermella els que menys (Morrissey et al., 1997; Cherian et al., 1996; Surai i Sparks, 2000). Tot i això, el suplement amb acetat d'α-tocoferol provoca un augment significatiu en el contingut d'α-tocoferol en les mitocondries i microsomes del pit i de la cuixa de pollastre (Lauridsen et al., 1997a) així com en les membranes de les carns d'altres animals (Jensen et al., 1998a). Si bé, el contingut total en α-tocoferol de la carn vermella de pollastre és aproximadament el doble que el de la carn blanca (Lin et al., 1989a; Ahn et al., 1995; O'Neill et al., 1998a; Cherian et al., 1996; Kang et al., 1998).

2.1.1. Addició de Fonts Riques en AGPI de la Sèrie n-3.

Hi ha diferents fonts riques en AGPI de la sèrie n-3. La llavor de llinosa i el seu oli són rics en àcid linolènic, mentre que pràcticament no presenten AGPI de la sèrie n-3 de cadena més llarga. Per tant, si volem incrementar el contingut d'àcid linolènic en la carn de pollastre ho podem fer amb l'addició al pinso d'oli de llinosa (López-Ferrer et al., 1999b; 2001).

Si bé el pollastre és capaç de sintetitzar, a partir del linolènic, àcids grassos de cadena més llarga com l'EPA i el DHA, aquesta transformació és limitada. Així doncs, en el cas que es vulgui enriquir la carn de pollastre en AGPI de la sèrie n-3 de cadena molt llarga, es prefereix incorporar-los directament en la dieta (Hargis i Van Elswyck, 1993; Ahn et al., 1995; López-Ferrer et al., 1999a, b; González-Esquerra i Leeson, 2000). La manera més senzilla de fer-ho és afegint oli de peix en el pinso (Hulan et al., 1989; Hargis i Van Elswyck, 1993; Scaife et al., 1994; López-Ferrer et al., 1999b, 2001; Gonzalez-Esquerra i Leeson, 2000). Tot i això s'ha de tenir en compte que la composició en àcids grassos pot variar en funció de l'origen de l'oli de peix. També, per tal d'enriquir la carn de pollastre en AGPI de la sèrie n-3 de cadena molt llarga, s'han provat l'ús d'altres fonts com per exemple les algues (Mooney et al., 1998; Gonzalez-Esquerra i Leeson, 2001).

Com a resultat de l'addició de fonts riques en AGPI de la sèrie n-3 a la dieta s'obté una carn de pollastre més rica en aquests àcids grassos i, per tant, interessant des d'un punt de vista nutritiu. Malauradament, aquest fet comporta que les canals siguin més olioses, més susceptibles a l'oxidació, i que fàcilment apareguin olors desagradables.

Les canals toves i/o olioses es deuen a la disminució del punt de fusió del seu greix al augmentar el grau d'insaturació dels dipòsits lipídics i són molts cops rebutjades pel consumidor (Bartov et al., 1974; Wood i Enser, 1997). Per evitar aquest problema és necessari controlar el tipus i la dosi de greix afegit al pinso, doncs afectaran a la textura del teixit adipós. És per aquesta raó que es recomana substituir els olis afegits als pinsos per greixos més saturats durant els últims dies de producció (Sanz et al., 2000) encara que la substitució de l'oli de peix per altres fonts de greix

provoquen una certa disminució en el contingut d'àcids grassos com el EPA i el DHA (Hargis i van Elswyck, 1993).

En relació a les olors desagradables, que apareixen en la carn de pollastres que han rebut pinsos enriquits amb olis de peix, aquestes poden provenir de l'olor a peix que té l'oli per ell mateix, el qual està lligat a la presència de compostos nitrogenats com la trimetilamina que es formen durant el deteriorament del peix. Per altra banda, les olors poden provenir dels productes resultants de l'oxidació dels àcids grassos (Leskanisch i Noble, 1997; Hargis i van Elswyck, 1993). L'oxidació dels AGPI, a més, representa una pèrdua evident del valor nutritiu de la carn. Respecte a l'aparició d'aquests olors i flavors desagradables, aquests s'aprecien més fàcilment en la cuixa d'animals alimentats amb olis de peix que no pas en el pit (Gonzalez-Esquerra i Leeson, 2000; López-Ferrer et al., 1999b). Aquest fet vindria explicat pel major contingut en greix i ferro que té la cuixa en comparació amb el pit el qual facilita l'oxidació dels àcids grassos.

De fet, per evitar la presència d'olors desagradables, es recomana afegir oli de peix o bé farines de peix que aportin una quantitat equivalent d'oli de peix per sota de 20g de greix /kg de pinso (Hargis van Elswyck, 1993). Així doncs, ajustar la dosi d'oli de peix que s'ha d'afegir al pinso seria un primer pas per evitar l'aparició d'olors i flavors desagradables.

Tanmateix, s'han descrit diferents estratègies a fi d'enriquir la carn de pollastre en àcids grassos de la sèrie n-3 però intentant salvaguardar la qualitat sensorial (López-Ferrer et al., 1999b, 2001; González-Esquerra i Leeson, 2000, 2001). Aquestes estratègies es basen en combinar diferents dosis d'antioxidants (per exemple el α-tocoferol) amb diferents dosis d'oli de peix, en barreges d'oli de peix amb altres olis vegetals o llavors i en la substitució de la dosi d'oli peix afegida al pinso per altres lípids uns dies abans del sacrifici (també anomenat pinso de retirada).

2.1.2 Addició d'Olis Oxidats

En el darrers anys ha anat augmentant la proporció afegida de greix a les dietes comercials dels pollastres, per tal d'incrementar la seva energia i així obtenir majors rendiments de canal. Si bé els olis vegetals rics en AGPI són altament digestibles i són fonts tradicionals en les dietes dels pollastres, l'addició d'aquests olis així com d'olis de peix, fa que també siguin més susceptibles a l'oxidació durant el emmagatzematge i per tant és d'interès la determinació de la qualitat d'aquest greix de la dieta i dels seus possibles efectes. Per altra banda, el fet que en alimentació animal, hi hagi una alta demanda en greix i juntament amb la necessitat en abaratir els costs de producció, fa que de vegades s'usin o s'hagin usat greixos de rebuig que provenen d'operacions de fregit o bé siguin subproductes del refinat d'olis comestibles (Wiseman, 1986).

L'alimentació de pollastres amb olis molt oxidats és malauradament controvertida, doncs s'ha descrit que pot reduir la velocitat de creixement dels pollastres (Engberg et al., 1996; Lin et al., 1989b; Sheehy et al., 1993, 1994), afectar al pes normal del fetge i ronyons i provocar alteracions en els sistemes enzimàtics de diferents espècies animals (Billek, 2000; Márquez-Ruiz i Dobarganes, 1996; Mahungu et al., 1999). En la composició de la carn de pollastre, l'addició d'olis oxidats en el pinso pot provocar una pèrdua del valor nutritiu per la pèrdua de tocoferol i d'àcids grassos insaturats (Jensen et al., 1997; Sheehy et al., 1993). La pèrdua en tocoferols pot ser tant important que, fins i tot, pot provocar encefalopatia nutritiva en l'animal (Budowski et al., 1979) que és un símptoma de la deficiència en tocoferols.

Com ja s'ha vist en apartats anteriors, són molts els compostos que es deriven de l'oxidació dels lípids i per tant la presència d'un o varis compostos d'oxidació i la quantitat i temps en què s'han afegit al pinso determinaran els efectes perjudicials de la seva inclusió en la dieta (Kubow, 1993; Billek, 2000).

Els HPL són els primers en formar-se i només tenen una presència destacada en els primers estadis d'oxidació, sobretot quan aquesta és dóna a temperatures relativament baixes, com per exemple en l'emmagatzematge de pinsos i olis. Si bé aquest compostos són altament tòxics (Billek, 2000) l'acció de la GPx gastrointestinal està altament especialitzada en la protecció enfront d'hidroperòxids que provenen de la

dieta, convertint-los en els seus respectius hidroxiàcids (Arthur, 2000; Esterbauer, 1993). Aquests hidroxiàcids, però, sembla que poden ser absorbits (Staprans et al., 1996; Wilson et al., 2002; Penumetcha et al., 2000) i tenir efectes biològics negatius (Khan-Merchant et al., 2002; Penumetcha et al., 2000, 2002).

En quant als compostos d'oxidació secundària de baix pes molecular, com per exemple el aldehids insaturats, aquests poden ser absorbits a l'intestí (Grootveld et al., 1998; Esterbauer, 1993) i concretament el malondialdehid i el 4-hidroxinonenal estan àmpliament estudiats pels seus efectes citotòxics i genotòxics (Esterbauer et al., 1991, 1993). Altres compostos com per exemple els polímers, tot i que no són absorbits, poden provocar diarrea (Clark i Serbia, 1991) i també afectar a la hidròlisi dels triacilglicerols intactes (Márquez-Ruiz et al., 1998).

Molts dels treballs que descriuen efectes perniciosos de la ingesta de greixos oxidats tenen com a inconvenient que els greixos emprats estan extraordinàriament degradats i, per tant, no són representatius, per exemple, dels olis de rebuig que s'obtenen sota les pràctiques habituals (Billek, 2000; Márquez-Ruiz i Dobarganes, 1996). Les disposicions legals sobre el rebuig d'un greix o oli de fregitel·la situen el límit legal en un 25-30% el contingut en compostos polars que equivaldria a un màxim d'un 10-18% de contingut en polímers (Firestone, 1996; Paul i Mittal, 1997; Boatella et al., 2000). Altres estudis, que també descriuen aquests efectes perniciosos, s'han fet afegint olis oxidats en dietes no equilibrades, com seria el cas de presentar deficiències en àcids grassos essencials o en α-tocoferol (Billek, 2000; Kubow, 1993).

Tanmateix, sembla que l'addició d'olis oxidats de rebuig en dosis semblants a les dels olis sense oxidar, no presenten efectes tòxics o perniciosos per a la salut dels animals (Billek, 2000; Clark i Serbia, 1991). Malgrat això quan s'han afegit olis oxidats a les dietes de pollastres, s'ha pogut observar una disminució en els nivells d'α-tocoferol en el muscle i altres teixits (Engber et al., 1996; Galvin et al., 1997; Lin et al., 1989b; Jensen et al., 1997). Aquestes reduccions en els nivells d'α-tocoferol han estat atribuïdes a diverses raons: la primera a la destrucció de l'α-tocoferol durant l'escalfament de l'oli o l'emmagatzematge del pinso, segon a la destrucció de l'α-tocoferol en el tracte gastrointestinal com a conseqüència dels radicals presents en l'oli oxidat, i en tercer lloc a l'absorció de compostos d'oxidació, els quals induirien un major estrès oxidatiu i per tant a un major consum de l'α-tocoferol *in vivo* (Galvin et

al., 1997; Jensen et al., 1997; Monahan et al., 1992; Sheehy et al., 1993, 1994). Les dues darreres explicarien els menors continguts d'α-tocoferol trobats en carn i plasma de pollastres alimentats amb pinsos que contenien olis oxidats en el quals s'havien rectificat les pèrdues en tocoferol produïdes per l'escalfament dels olis (Sheehy et al., 1993, 1994).

També diversos estudis en carn de pollastre revelen que després de l'addició d'olis oxidats s'observen valors d'oxidació majors, mitjançant l'índex de l'àcid tiobarbitúric (índex de l'ATB), i una menor estabilitat oxidativa, mitjançant l'índex de l'ATB induït, en comparació als valors obtinguts quan s'afegeix el mateix oli sense oxidar (Galvin et al., 1997; Lin et al., 1989b; Jensen et al., 1997; Sheehy et al., 1993, 1994). Aquest fet estaria d'acord amb la menor quantitat d'α-tocoferol present en aquests muscles. Segons aquest darrer raonament, és lògic que si l'animal no presenta una reducció en els nivells d'α-tocoferol tampoc presenti diferències en els valors d'oxidació. De fet, quan després d'alimentar un pollastre amb un oli oxidat no s'han trobat diferències en el contingut en α-tocoferol de la carn tampoc s'ha trobat diferències en els valors d'oxidació (mesurant l'índex d'ATB i el contingut en HPL pel mètode del taronja de xilenol) de la carn de pollastre (Grau et al., 2001a). També sembla concordar el fet que quan s'avalua la ranciesa d'aquestes carns tampoc es trobessin diferències entre els anteriors pollastres que havien estat alimentats amb l'oli oxidat i els alimentats amb l'oli sense oxidar (Bou et al., 2001).

En relació a la composició en àcids grassos, ja s'ha esmentat que diversos autors han descrit una disminució d'AGPI en la carn de pollastre com a resultat de la pèrdua d'aquests deguda a processos oxidatius (Sheehy et al., 1993; Jensen et al., 1997). Tot i això, altres autors no han trobat diferències en la carn procedent de pollastres que han rebut pinsos que contenien olis oxidats (Sheehy et al., 1994; Lin et al., 1989b). Així doncs, sembla que aquests efectes dependran en bona mesura del grau d'oxidació dels olis afegits.

Per tant, degut a la oxidació dels olis incorporats en els pinsos, és possible que es doni una disminució del valor nutritiu de la carn, degut sobretot a la disminució d'AGPI per una banda i, per l'altra, a la disminució en l'α-tocoferol. Aquest darrer fet, per ell mateix, pot provocar una major susceptibilitat cap a l'oxidació i, en conseqüència, provocar canvis sensorials en la carn. El que no queda tant clar és si

l'estat oxidatiu del greix i, per tant, els productes d'oxidació que són afegits al pinso poden provocar una disminució en el contingut de tocoferol de la carn de pollastre.

2.1.3 Incorporació d'Antioxidants

Les carns de pollastre que tenen un major contingut en AGPI o simplement tenen un menor contingut en antioxidants, per exemple en α-tocoferol com a resultat d'afegir un oli escalfat, fa que aquestes carns siguin més susceptibles a l'oxidació.

Per tal de protegir aquesta carn podem afegir-hi additius antioxidants "naturals" i característics de la carn (tocoferol, carotenoids, àcid ascòrbic...), altres additius antioxidants "naturals" (per exemple extractes de fenols), nitrits o quelants (polifosfats). Com ja hem vist, també es pot protegir la carn suplementant la dieta de l'animal amb antioxidants per tal d'afavorir el balanç oxidatiu de la carn.

Tanmateix, la suplementació de la dieta amb antioxidants sembla quasi només factible en el cas d'afegir tocoferols (Decker i Xu, 1998), doncs l'addició d'antioxidants com l'àcid ascòrbic (King et al., 1995; Lauridsen et al., 1997b; Grau et al., 2001a, b; Morrissey et al., 1998) i els carotenoids (Jensen et al., 1998b; King et al., 1995; Maraschiello et al., 1998) presenten una eficàcia dubtosa en la prevenció de l'oxidació lipídica. L'avantatge d'afegir tocoferols en la dieta és que, d'aquesta manera, són més efectius que addicionats sobre la carn, doncs aquests s'incorporen directament en les membranes que és on s'inicia l'oxidació (Jensen et al., 1998a; Lauridsen et al., 1997a). A més, s'ha de tenir en compte que de vegades la addició d'antioxidants liposolubles sobre la carn és poc viable com, per exemple, en el cas de comercialitzar peces de carn senceres.

Respecte al α-tocoferol i els seus anàlegs, els animals no els poden sintetitzar raó per la qual la seva presència en els teixits depèn de la dieta (Jensen et al., 1998a). Les recomanacions nutritives respecte a la suplementació en vitamina E dels pinsos de pollastre per tal de cobrir les seves necessitats en condicions normals es situen en 10 mg d'acetat de dl-α-tocoferol/kg (National Research Council, 1994). Tanmateix, la suplementació a dosis més elevades és efectiva en la millora del valor nutritiu i de

l'estabilitat oxidativa (Lin et al., 1989a; Sheehy et al., 1994; Morrissey et al., 1998; Jensen et al., 1995) i és efectiva en la prevenció de l'aparició d'olors desagradables en carn de pollastre (O'Neill et al., 1998a; Bou et al., 2001), la qual està relacionada amb el contingut en compostos volàtils totals i més concretament amb la formació d'aldehids (Ajuyah et al., 1993a, b; De Winne i Dirinck, 1996, Morrissey et al., 2003).

La suplementació amb tocoferols a la dieta de la majoria d'aquests treballs s'ha fet en la forma d'acetat d'α-tocoferol essent l'acetat de dl-α-tocoferol la forma més habitual (Jensen et al., 1998a), doncs l'α-tocoferol, un cop acetilat, és més estable enfront l'oxidació assegurant una major aportació d'aquests compost a través del pinso. L'acetat de tocoferol, un cop arriba a l'intestí de l'animal, s'hidrolitza i és absorbit (Jensen et al., 1998a).

L'eficàcia de la suplementació amb acetat d'α-tocoferol per protegir els àcids grassos (Jensen et al., 1998a; Mielche i Bertelsen 1994; Grau et al., 2001a; Wood i Enser, 1997; Lauridsen et al., 1997a) i el colesterol (Grau et al., 2001b; Morrissey et al., 1998) enfront de l'oxidació ha estat demostrada en carn de pollastre, tant crua com cuita, després d'emmagatzemar, tant a temperatures de refrigeració com de congelació. A més, l'efecte protector de l'α-tocoferol sembla estar relacionat amb la menor aparició de compostos d'oxidació (De Winne i Dirinck, 1996; Jensen et al., 1998a; Grau et al., 2001a, b; Ajuyah et al., 1993a, b) que afectaran a la percepció d'aromes i flavors desagradables i/o la ranciesa en carn d'aviram (De Winne i Dirinck, 1996; Bou et al., 2001; O'Neill et al., 1998a; Lyon et al., 1988). La suplementació amb α-tocoferol també sembla tenir efectes protectors en l'oxidació de la mioglobina, afectant per tant al color de les carns, i també podria tenir un lleuger efecte en la retenció d'aigua, ja que està involucrat en l'estabilització de les membranes lipídiques de diferents animals (Jensen et al., 1998a; Morrissey et al., 2003).

De totes maneres, la protecció de l'α-tocoferol enfront de l'oxidació i les seves repercussions a nivell sensorial dependran de varis factors com són el nivell de tocoferol afegit a la dieta, el grau de insaturació i d'oxidació dels olis o greixos afegits al pinso, la duració de la suplementació, l'estat fisiològic de l'animal i en definitiva del balanç oxidatiu de la carn (Morrissey et al., 1998; Grau et al., 2000; Grau et al., 2001a; Bou et al., 2001; Jensen et al., 1997; 1998a).

2.2. Modificació de la Composició Mineral Mitjançant la Dieta

En general s'ha parat poca atenció a la possible modificació a través de la dieta animal de la composició mineral de les carns amb l'objectiu d'incrementar el seu valor nutritiu, l'estabilitat oxidativa o la seva qualitat sensorial.

Els suplements minerals de les dietes contenen una gran diversitat de minerals entre els quals s'hi troben el ferro i el coure. Aquests metalls de transició són uns grans catalitzadors de l'oxidació lipídica (veure apartat 1.1), i en molts casos només s'afegeixen amb el propòsit d'evitar les possibles deficiències d'aquests metalls en la dieta. És per aquesta raó que alguns autors van estudiar que passava quan les dietes, que no presentava deficiències en minerals, no eren suplementades. En alguns casos, l'eliminació d'aquesta suplementació en minerals va resultar en una millora de l'estabilitat oxidativa de la carn d'aviram crua i cuita (Morrissey et al., 1998; Ruiz et al., 2000; Kanner et al., 1990), però en altres és va observar una major susceptibilitat cap a l'oxidació en la carn cuita (Maraschiello et al., 2000).

Si bé l'eliminació de ferro del suplement mineral no provoca una disminució del contingut en ferro de l'animal (Morrissey et al., 1998; Kanner et al., 1990), l'eliminació de ferro i/o coure del suplement provoca una disminució en el contingut en coure de la carn (Ruiz et al., 2000). D'altra banda la suplementació de la dieta amb dosis elevades de coure sembla tenir efectes positius en els paràmetres productius (Koh et al., 1996) i disminuir el contingut de colesterol en sang i muscle de pollastre (Bakalli et al., 1995). En el cas del ferro, l'addició a dosis elevades en el pinso no sembla tenir efectes negatius en l'estabilitat de la carn de gall d'indi (Morrissey et al., 1998).

Per altra banda, s'ha de tenir en compte que el ferro i el coure, tot i que afavoreixen l'oxidació lipídica com a catalitzadors, també són components essencials en el sistema d'enzims antioxidants. De fet, hi ha diversos minerals que són necessaris pel funcionament dels enzims antioxidants. Concretament la catalasa és un enzim amb un grup hemo, mentre que la SOD present en el citoplasma conté coure i zinc. Un altre

cas és la GPx que conté seleni, aquest en forma de selenocisteïna (Arthur, 2000). Per tant, nivells molt baixos d'aquests elements poden ser contraproduents.

L'acció contrària a la reducció de certs minerals en la dieta seria la seva suplementació a dosis elevades, per tal d'estudiar els possibles canvis en la composició i en l'estabilitat oxidativa de la carn. Aquesta darrera opció ha estat poc estudiada, tot i que fa temps que es va veure que la suplementació amb seleni en les dietes de pollastre incrementava l'activitat de la GPx i disminuïa el contingut en malondialdehid en pit i cuixa de pollastre (De Vore et al., 1983). Aquest fet vindria explicat per un major contingut en selenoproteïnes com l'enzim intracel·lular GPx. Sembla clar que diferents teixits que provenen d'aviram alimentada amb dietes deficients en seleni presenten una menor activitat de la GPx i una major susceptibilitat cap a l'oxidació (Avanzo et al., 2001; Óztürk-Ürek et al., 2001; Arzu Bozkaya, 2001; Sahin et al., 2002). A més, les deficiències en seleni donen lloc a patologies similars a les causades per una deficiència en tocoferol i, a la vegada, és coneix, de fa temps, la relació de sinèrgia entre el tocoferol i el seleni enfront l'oxidació (National Research Council, 1983; Bristish Nutrition Foundation, 2001). Aquesta sinèrgia vindria donada per l'acció combinada del tocoferol i la GPx ja que mentre el tocoferol prevé la formació de peròxids segrestant radicals lliures, la GPx redueix els peròxids ja formats a alcohols.

Per altra banda, s'ha observat que l'activitat de la GPx en cèl·lules de rates és major quan aquestes han estat alimentades amb olis escalfats (Saka et al., 2002). Així doncs, pot ser que en el cas d'afegir olis oxidats faci falta un suplement en seleni per mantenir el bon funcionament de la GPx.

Amb tot, el seleni pot ser absorbit en formes tant inorgàniques com orgàniques (Foster i Sumar, 1997; British Nutrition Foundation, 2001; Food Nutrition Board, 2000) i aquestes ser transformades en selenocisteïna, la forma biològicament activa del Se que forma part de la GPx i d'altres selenoproteïnes (Holben i Smith, 1999). Per tant formes orgàniques com la selenometionina poden restaurar l'activitat de la GPx (Foster i Sumar, 1997; Schrauzer, 2000) però, a més, la suplementació en aquesta forma és preferida respecte a les formes inorgàniques doncs està associada amb una major retenció d'aigua en el muscle, amb l'increment de les reserves en seleni i amb diferents característiques reproductives i productives de l'aviram (Surai, 2002).

L'addició de seleni en el pinso és necessària, sobretot en llocs amb sòls pobres en aquest mineral, doncs segurament els pinsos també ho seran. Així s'assegura el correcte funcionament del metabolisme de l'animal. Per aquesta raó s'afegeix seleni en el suplement mineral de les dietes de pollastres. No obstant, cal atendre al fet que dosis massa elevades de seleni poden resultar tòxiques per l'animal (National Research Council, 1983).

De manera similar, l'aportació de zinc a través de la dieta afavoreix el correcte funcionament del sistema enzimàtic antioxidant per mitjà de la SOD (Virgili et al., 1999). A la vegada, s'han descrit altres possibles mecanismes pels quals el zinc té propietats antioxidants. Aquests mecanismes es basen, bé en la protecció dels grups sulfidril de les proteïnes, o bé en la reducció de la formació de radicals hidròxil a partir del peròxid d'hidrogen degut a la competència que presentaria el zinc amb altres metalls de transició (Powell, 2000; Zago i Oteiza, 2001). A més, el zinc i altres cations indueixen la síntesi de la metal·lotioneina (Nordberg, 1998; Coyle et al., 2002). Aquesta proteïna intracèl·lular està relacionada amb la aportació de zinc mitjançant la dieta i, a la vegada, permet la regulació homeostàtica d'aquest metall. Tot i això, també sembla tenir altres funcions com la protecció contra els efectes tòxics de determinats metalls pesants, l'emmagatzematge de certs metalls i com a captador de radicals lliures, entre d'altres (Maret, 2000; Nordberg, 1998; Coyle et al., 2002). Fins i tot sembla que l'activitat antioxidant del seleni pot confluir amb el metabolisme del zinc i de les reaccions redox en les que la metal·lotioneina hi pendria part (Maret, 2003).

Quan s'han afegit altes dosis de zinc als pinsos de pollastres (de 0,5 fins 20 g/kg) durant períodes curts de temps (menys de 3 setmanes) aquest metall s'acumula en diferents teixits com el fetge, els ronyons, el pàncreas i la melsa (McCormick i Cunningham, 1987; Williams et al., 1989; Emmert i Baker, 1995; Sandoval et al., 1998). Malauradament, també s'ha observat que dosis elevades (20 g/kg) redueixen el consum de pinso i el pes de l'animal (McCormick i Cunningham, 1987; Williams et al., 1989).

Així doncs, en general, el suplement mineral de qualsevol element en les dietes de pollastres ha d'assegurar el correcte desenvolupament de l'animal, assegurant uns paràmetres productius adients i el correcte funcionament del metabolisme. Tanmateix, pot ser que l'addició d'algun mineral en els pinsos en quantitats superiors a les

habituals pugui implicar un augment del seu contingut en la carn incrementant-se el seu valor nutritiu, bé millorar l'estabilitat oxidativa o bé la seva qualitat sensorial. No obstant, les addicions a la dieta de certs elements a altes dosis presenten nombroses interaccions en l'absorció de diferents minerals com, per exemple, les descrites a altes dosis de ferro que provoquen una disminució en l'absorció de zinc o bé les produïdes a altes dosis de zinc disminuint l'absorció de coure (Solomons, 1988; Lönnerdal, 2000).

III. OBJECTIUS I PLANTEJAMENT EXPERIMENTAL

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1. OBJECTIUS

Dels antecedents bibliogràfics es desprèn que, a partir de la dieta, és possible modificar la composició de la carn de pollastre i, per tant, incrementar el seu valor nutritiu. El que més s'ha estudiat és la modificació de la fracció lipídica, sobretot per augmentar el seu contingut en AGPI de la sèrie n-3. Aquest augment en AGPI pot provocar canvis sensorials en la carn de pollastre ja que el greix, en ser molt més fluid, afecta a la textura. A més, es produeix un augment de la susceptibilitat oxidativa que comporta una ràpida aparició d'aromes i flavors indesitjables, o el que és el mateix, la ranciesa. És per aquesta raó que quan s'augmenta el contingut en aquests àcids grassos, s'aconsella afegir a la dieta una quantitat superior d'antioxidants per a protegir la carn enfront de l'oxidació, essent el més usat l'α-tocoferol. Això permet, a la vegada, millorar el seu valor nutritiu, la seva estabilitat oxidativa i les seves característiques sensorials, sobretot durant emmagatzematges perllongats.

Tot i haver-hi molts estudis que avaluen les diferents possibilitats de modificar el perfil lipídic de les carns n'hi ha pocs que estiguin dissenyats per a l'obtenció d'una carn que aporti quantitats significatives, a la dieta humana, d'AGPI de la sèrie n-3. Així doncs, per tal aconseguir un producte que satisfaci una part important de les ingestes dietètiques recomanades per aquests àcids grassos, s'ha de determinar quina font de greix s'ha d'addicionar als pinsos i quant de temps. Un cop aconseguit això s'ha d'avaluar l'estabilitat oxidativa i la qualitat sensorial d'aquestes carns enriquides en aquests àcids grassos.

També, es troben a faltar estudis avaluant altres factors que permetin augmentar el valor nutritiu de la carn de pollastre a través de la seva dieta. Aquest seria el cas de les suplementacions amb diferents elements minerals que, en cas d'afectar la composició de les carns, poden ser interessants per a certs col·lectius de persones. La gent d'edat avançada seria un d'aquests col·lectius. Aquests tenen menys requeriments

en energia i generalment consumeixen menys carn, la qual cosa provoca que la ingesta en certs elements minerals sigui a voltes baixa (Tucker i Buranapin, 2001; Young, 1992; Russell, 1992; Mitchell et al., 2003).

El fet que els productes carnis siguin una font natural molt important de diferents elements, a més de presentar una bona biodisponibilitat, fa que el desenvolupament de productes carnis amb una alta densitat en elements minoritaris sigui de gran interès. Entre els possibles elements que es poden veure afectats per una ingesta pobre en carn tenim el zinc i el seleni. A la vegada, ambdós tenen en comú que estan relacionats amb enzims antioxidants i, per tant, la seva modificació pot tenir repercussions sobre l'estabilitat oxidativa de les carns. Així doncs, l'enriquiment en aquests nutrients és interessant des de diferents punts de vista com per exemple: el nutritiu, l'estabilitat oxidativa i les característiques sensorials.

El problema de l'estabilitat oxidativa es pot veure també afectat negativament pel nivell oxidatiu de la fracció lipídica del pinso. No són molts els estudis referents a l'efecte que pot tenir l'addició de diferents olis oxidats en el pinso sobre la qualitat sensorial de la carn de pollastre. L'addició d'olis oxidats en els pinsos, a dosis raonables, sembla no tenir efectes tòxics encara que pot disminuir els nivells en α-tocoferol de les carns. Tanmateix, no està massa clar si aquesta disminució en els nivells de α-tocoferol ve donada per la composició pròpia d'aquests pinsos o pels efectes que tenen els compostos d'oxidació presents en els greixos addicionats.

És per totes aquestes raons que, en la present Tesi Doctoral, es van fixar els següents objectius:

objectiu 1. Optimitzar la dosi d'oli de peix afegida al pinso per tal d'aconseguir una carn de pollastre amb un contingut en àcids grassos de la sèrie n-3 (especialment en EPA i DHA) interessant des del punt de vista nutricional. A la vegada també interessa optimitzar els nivells de suplementació amb acetat d'α-tocoferol per estabilitzar la carn i que aquesta sigui acceptable pel consumidor, inclòs després de llargs períodes d'emmagatzematge.

objectiu 2. Estudiar com afecta a la composició en àcids grassos, l'estabilitat oxidativa i la qualitat sensorial de la carn de pollastre la substitució de la dosi d'oli de peix, ja optimitzada, per altres fonts lipídiques durant els darrers dies del cicle productiu.

objectiu 3. Avaluar la possible influència de la suplementació amb zinc i seleni sobre el valor nutritiu, l'estat oxidatiu i les característiques sensorials de la carn de pollastre, per tal de proposar pautes viables d'enriquiment en aquests minerals.

objectiu 4. Quantificar el possible efecte negatiu de l'addició d'olis oxidats en els pinsos, en relació amb la composició en àcids grassos i α-tocoferol, l'estabilitat oxidativa i la qualitat sensorial de la carn de pollastre. Al mateix temps, avaluar els efectes protectors de l'α-tocoferol enfront dels possibles efectes causats per l'addició d'olis oxidats.

Per aconseguir aquests objectius concrets es va veure la necessitat de posar a punt un mètode analític per a la correcte determinació dels elements minerals que es volien estudiar, especialment important pel cas de la determinació del seleni en pinso i carn de pollastre. Així doncs va sorgir la necessitat d'un darrer objectiu que va ser:

objectiu 5. Validar un mètode analític per a la determinació de Fe, Zn, Cu i Se en pinsos i carns de pollastre.

2. PLANTEJAMENT EXPERIMENTAL

Per abordar la resolució dels objectius concrets, anteriorment esmentats, es van programar tres estudis diferents descrits a continuació.

Els diferents estudis es van dur a terme en les instal·lacions de la granja experimental de l'empresa COPAGA, Societat Cooperativa (Lleida). Les pràctiques culturals i de maneig rebudes pels animals van ser les normals de l'empresa. Les condicions de temperatura, humitat, llum i ventilació estaven controlades. Els animals estaven disposats aleatòriament en gàbies (en funció de l'estudi, 6 o 7 animals per gàbia) a nivell de terra. El pinso i l'aigua van ser subministrats *ad libitum*. A diari, es feien controls de temperatura, ventilació, estat sanitari i baixes. A més, el pes i el consum de pinso dels animals es va controlar al principi, a la meitat i al final del cicle productiu.

2.1. Primer Estudi

2.1.1 Disseny Experimental

El primer estudi es va dissenyar per cobrir el primer objectiu, que era esbrinar quina és la dosi idònia d'oli de peix que permet l'obtenció d'una carn de pollastre amb un enriquiment suficient en AGPI de la sèrie n-3.

Per aconseguir aquest objectiu es van estudiar dos nivells d'addició d'oli de peix (1,25 i 2,5%), que es van completar amb greix animal fins al 5,6% de greix total afegit. Es varen seleccionar aquestes dues dosis d'addició tenint en compte la bibliografia existent i calculant que s'arribés a obtenir una carn enriquida en AGPI de cadena molt llarga (EPA i DHA). Es va prendre com a criteri considerar una carn enriquida quan aquesta aportés un mínim del 20% de les ingestes dietètiques recomanades d'aquests àcids grassos.

Tanmateix, també és coneixien els problemes d'acceptabilitat que podien presentar-se deguts a una major susceptibilitat a l'oxidació. Per reduir la susceptibilitat a l'oxidació, es van assajar també dos nivells de suplementació amb acetat d'α-tocoferol en el pinso (70 i 140 mg acetat d'α-tocoferol/kg pinso) per veure quina podia ser la dosi idònia per protegir aquestes carns enfront l'oxidació (segona part del primer objectiu).

En aquest primer estudi també es va aprofitar per a estudiar la possible influència de la suplementació del pinso amb sulfat de zinc (0 i 200 mg Zn/kg pinso) sobre la composició de la carn de pollastre i veure si tenia altres efectes sobre paràmetres oxidatius i/o sensorials (3r objectiu).

D'aquesta manera, aquest primer estudi presentà un disseny factorial que permetia l'estudi de les interaccions entre els tres factors assajats (dosi d'oli de peix, dosi d'acetat d'α-tocoferol i dosi de Zn). Es van emprar 288 pollets que es van assignar aleatòriament, en grups de 6 animals, a una gàbia, la qual corresponia a un dels tractaments. Durant les tres primeres setmanes de vida els pollets van rebre un pinso base comercial (estàndard) que contenia un 5,2% de greix animal afegit. També, a

partir del primer dia de vida, es van subministrar els suplements de Zn i d'acetat d'α-tocoferol. Per tant, tal i com es mostra en la Taula 7, el disseny de l'experiment tenia, en aquest període, 4 tractaments. A partir de la tercera setmana i fins al sacrifici dels animals (als 42 dies de vida) es va incloure el factor dosi d'oli de peix afegit al pinso. Així doncs, en aquest moment, el disseny experimental va resultar en els 8 tractaments finals (veure Taula 7), degut a la combinació dels tres factors assajats (2x2x2). Aquest estudi factorial es va replicar sis cops.

2.1.2 Preparació de les Mostres

Els animals van ser sacrificats als 42 dies de vida segons els procediments comercials de la cooperativa agrícola COPAGA. Les canals de pollastres que provenien d'una gàbia van ser tallades longitudinalment i separades en dos grups (Figura 3). Les cuixes i els pits del costat dret de cada pollastre, amb pell inclosa, es van destinar a l'estudi de la composició i valor nutritiu. Aquestes mostres es van desossar a mà i posteriorment es van picar i envasar al buit en bosses multibarrera (Cryovac BB-4L; permeabilitat al O_2 30 cm^3/m^2, 24 h, 1 bar, ASTMD-3985). Un cop envasades es van emmagatzemar a -20 ºC fins al moment de fer les determinacions.

Degut al major contingut de greix i de metalls prooxidants com el ferro a les cuixes de pollastre, només les cuixes amb pell del costat esquerra es van usar per avaluar l'acceptabilitat i l'estabilitat oxidativa d'aquestes mostres. Les cuixes es van desossar a mà i es van envasar senceres al buit en bosses multibarrera (Cryovac CN-300; permeabilitat al O_2 15 cm^3/m^2, 24 h, 1 bar, ASTMD-3985). Un cop envasades es van coure a 85 ºC en un forn (humitat relativa = 99%) fins arribar a una temperatura a l'interior de les peces de 80 ºC. Posteriorment, les mostres es van emmagatzemar a -20 ºC fins a la seva determinació als 15 dies i als 5 mesos de conservació.

2.1.3 Determinacions

Per avaluar l'efecte dels diferents factors estudiats sobre el valor nutritiu de la carn de pollastre crua amb pell es van determinar:

- El contingut en α-tocoferol.
- El contingut en zinc.
- La grassa bruta total.
- La composició en àcids grassos.

En la cuixa de pollastre cuita amb pell, es va realitzar:

- L'anàlisi de l'acceptabilitat per part dels consumidors als 15 dies i als 5 mesos d'emmagatzematge a – 20 ºC. En cada anàlisi sensorial es va emprar un panel de 27 consumidors que en cada sessió van provar les 8 mostres diferents, escalfades durant 20 minuts a 75 ºC, que corresponien a cadascun dels tractaments.
- Avaluació de l'estat oxidatiu de les mostres per mitjà de l'índex de l'ATB als 15 dies i als 5 mesos d'emmagatzematge.

En la Figura 3, s'indica un esquema del sistema de mostreig i de les determinacions fetes, en carn de pollastre.

Paral·lelament en els pinsos es va determinar:

- El contingut en α-tocoferol.
- El contingut en zinc.
- La composició en àcids grassos.

Taula 7.- Disseny factorial de l'experiment 1.

Les 3 primeres setmanes de vida		
Tractaments	**Suplement d'acetat d'α-tocoferol (mg/kg)**	**Suplement de Zn (mg/kg)**
1 o 5	70	0
2 o 6	140	0
3 o 7	70	200
4 o 8	140	200

A partir de la 3a setmana de vida			
Tractaments	**Percentatge d'oli de peix afegit**	**Suplement d'acetat d'α-tocoferol (mg/kg)**	**Suplement de Zn (mg/kg)**
1	1,25	70	0
2	1,25	140	0
3	1,25	70	200
4	1,25	140	200
5	2,5	70	0
6	2,5	140	0
7	2,5	70	200
8	2,5	140	200

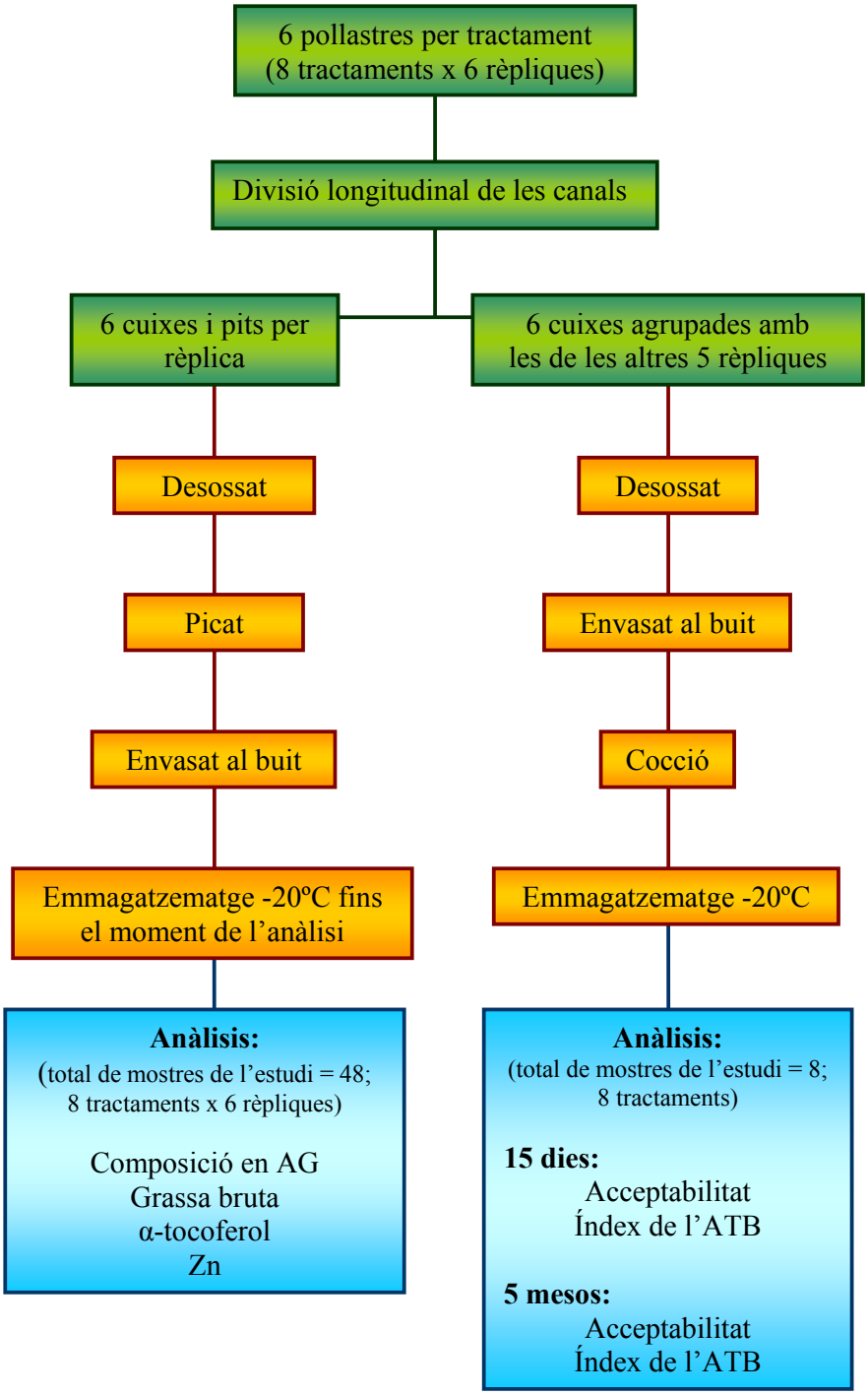

Figura 3.- Processat i determinacions realitzades en carn de pollastre procedent d'un tractament del primer estudi.

2.2. Segon Estudi

2.2.1 Disseny Experimental

El disseny del segon estudi va venir marcat pels resultats obtinguts en el primer. Així doncs, de manera similar al primer estudi, els pollets van ser alimentats amb un altre pinso base en què s'havia afegit un 1,25% d'oli de peix i un 5,81% de greix animal. Aquesta dosi d'oli de peix afegida al pinso va ser escollida com la dosi idònia, a partir dels resultats del primer estudi, des del punt de vista nutritiu i d'estabilitat oxidativa. Dels resultats de la prova anterior també es va veure que afegint una dosi d'acetat d'α-tocoferol d'entre 70 i 140 mg/kg era suficient per protegir les carns enfront de l'oxidació. Per aquesta raó es van suplementar els pinsos, des del primer dia, amb acetat d'α-tocoferol a una dosi de 100 mg/kg de pinso.

Un cop fixades aquestes característiques generals per tots els tractaments, un dels factors que es va assajar va ser la substitució d'aquesta dosi d'oli de peix per altres fonts lipídiques durant els últims dies de vida (dels 40 als 45 dies de vida), per comprovar el seu efecte sobre el valor nutritiu, l'estat oxidatiu i les característiques sensorials (2n objectiu). Les fonts lipídiques escollides van ser l'oli de llinosa i una barreja de greix animal (80% de llard i 20% de sèu). La llinosa té un elevat contingut en àcids grassos de la sèrie n-3 però, a diferència de l'oli de peix, aquest és ric en àcid linolènic i pobre en AGPI de cadena llarga de la sèrie n-3. El greix animal té un contingut en AGPI baix i un elevat contingut en àcid oleic i en àcids grassos saturats. Així doncs, durant els cinc últims dies de vida, es va utilitzar un pinso base que contenia un 1,25% d'oli de peix, o bé d'oli de llinosa o bé greix animal, completat amb un 5,81% de greix animal en tots els casos.

També, com a resultat de l'anterior prova, es va veure que la suplementació amb 200 mg Zn/kg de pinso no tenia cap efecte sobre la composició, l'estabilitat oxidativa i/o la qualitat sensorial, per la qual cosa es va decidir augmentar les dosis. Així, un altre factor que es va assajar va ser la suplementació amb Zn del pinso a 0, 300 i 600 mg/kg.

Al mateix temps, es va introduir la suplementació amb diferents formes de seleni per resoldre part del tercer objectiu. Com a conseqüència, el tercer factor assajat en el segon estudi va ser la suplementació amb seleni. Els suplements en seleni assajats foren selenit sòdic (1,2 mg Se/kg), o bé un extracte de llevats ric en selenometionina (0,2 mg Se/kg), o bé la no suplementació amb seleni.

D'aquesta forma, aquest segon estudi presentà un disseny factorial que permetia l'estudi de les possibles interaccions entre aquests tres factors. Es van emprar 324 pollets que es van assignar aleatòriament, en grups de 6 animals, a una gàbia, la qual corresponia a un dels tractaments. Durant els primers 19 dies de vida, els pollets van rebre un pinso base estàndard que contenia un 6% de greix animal afegit i alhora es van subministrar els suplements de Zn i les diferents fonts de seleni. Posteriorment, a partir del dia 20 i fins al dia 39 de vida, es va mantenir el mateix disseny però es va utilitzar un altre pinso base que contenia un 5,81% de greix animal més un 1,25% d'oli de peix. Tal i com es mostra en la Taula 8, el disseny de l'experiment tenia, fins aquest moment, 9 tractaments. Amb la inclusió del factor substitució de l'oli de peix, 5 dies abans del sacrifici dels animals, el disseny experimental va resultar en 27 tractaments (veure Taula 8) producte de la combinació dels tres factors assajats (3x3x3). L'experiment es va fer per duplicat.

2.2.2 Preparació de les Mostres

Els animals van ser sacrificats als 45 dies de vida segons els procediments comercials de la cooperativa agrícola COPAGA. Les canals de pollastres que provenien d'una gàbia van ser tallades longitudinalment i separades en dos grups. De dos pollastres a l'atzar es van seleccionar del seu costat dret les cuixes i els seus corresponents pits, amb pell inclosa, i es van destinar a l'estudi de la composició i valor nutritiu. Aquestes mostres es van desossar a mà i posteriorment es van picar i envasar al buit en bosses multibarrera (Cryovac BB-4L; permeabilitat al O_2 30 cm^3/m^2, 24 h, 1 bar, ASTMD-3985). Un cop envasades es van emmagatzemar a -20 ºC fins al moment de fer les determinacions.

De la mateixa forma que es va fer en el primer estudi, només les cuixes restants amb pell es van usar per avaluar l'acceptabilitat i l'estabilitat oxidativa d'aquestes mostres. Les cuixes es van desossar a mà i envasar al buit en bosses multibarrera (Cryovac CN-300; permeabilitat al O_2 15 cm^3/m^2, 24 h, 1 bar, ASTMD-3985). Un cop envasades es van coure a 85 ºC en un forn (humitat relativa = 99%) fins arribar a una temperatura de 78 ºC a l'interior de les peces. Posteriorment, les mostres es van emmagatzemar a -20 ºC fins a la seva determinació als 74 dies i als 18 mesos de conservació.

2.2.3 Determinacions

Per avaluar l'efecte dels diferents factors estudiats sobre el valor nutritiu de la carn de pollastre crua amb pell es van determinar:

- El contingut en α-tocoferol.
- El contingut en minerals (zinc, seleni, ferro, i coure).
- La grassa bruta total.
- La composició en àcids grassos.

Per les mateixes raons que les exposades en el primer estudi, en la cuixa de pollastre cuita amb pell es van realitzar els següents anàlisis:

- L'anàlisi de l'acceptabilitat. Aquesta es va determinar després de 74 dies i 18 mesos d'emmagatzemar les mostres a – 20 ºC, doncs, en l'estudi anterior es va poder observar l'elevada estabilitat de mostres similars. En cada anàlisi sensorial es va emprar un panel de 31 i 33 consumidors, respectivament. Degut a l'elevat nombre de mostres els tractaments en què s'havien afegit 300 mg de Zn/kg no es van analitzar. Per tant en l'anàlisi sensorial només es van estudiar 18 tractaments. Els tastadors de cada panel van avaluar les diferents mostres,

presentades a l'atzar, en tres sessions diferents. Totes les mostres abans de servir-se van ser escalfades durant 20 minuts a 75 ºC.

- Avaluació de l'estat oxidatiu de les mostres per mitjà de l'índex de l'ATB als 74 dies i als 18 mesos d'emmagatzemar.

En la Figura 4, s'exposa un esquema del sistema de mostreig i de les determinacions fetes en la carn de pollastre.

També, en els pinsos, es va determinar:

- El contingut en α-tocoferol.
- El contingut en zinc, seleni, ferro i coure.
- La composició en àcids grassos.

Taula 8.- Disseny factorial de l'experiment 2[1].

Del primer fins al 19è dia de vida[2]		
Tractaments	**Suplement de Zn (mg/kg)**	**Font de suplement de Se[3]**
1, 10 o 19	0	0
2, 11 o 20	0	inorgànica
3, 12 o 21	0	orgànica
4, 13 o 22	300	0
5, 14 o 23	300	inorgànica
6, 15 o 24	300	orgànica
7, 16 o 25	600	0
8, 17 o 26	600	inorgànica
9, 18 o 27	600	orgànica

Del 20è fins als 39è dia de vida			
Tractaments	**Greix afegit[4]**	**Suplement de Zn (mg/kg)**	**Font de suplement de Se**
1, 10 o 19	Oli de peix	0	0
2, 11 o 20	Oli de peix	0	inorgànica
3, 12 o 21	Oli de peix	0	orgànica
4, 13 o 22	Oli de peix	300	0
5, 14 o 23	Oli de peix	300	inorgànica
6, 15 o 24	Oli de peix	300	orgànica
7, 16 o 25	Oli de peix	600	0
8, 17 o 26	Oli de peix	600	inorgànica
9, 18 o 27	Oli de peix	600	orgànica

Els 5 últims dies de vida			
Tractaments	**Greix afegit[4]**	**Suplement de Zn (mg/kg)**	**Font de suplement de Se**
1	Oli de peix	0	0
2	Oli de peix	0	inorgànica
3	Oli de peix	0	orgànica
4	Oli de peix	300	0
5	Oli de peix	300	inorgànica
6	Oli de peix	300	orgànica
7	Oli de peix	600	0
8	Oli de peix	600	inorgànica
9	Oli de peix	600	orgànica
10	Oli de llinosa	0	0
11	Oli de llinosa	0	inorgànica
12	Oli de llinosa	0	orgànica
13	Oli de llinosa	300	0
14	Oli de llinosa	300	inorgànica
15	Oli de llinosa	300	orgànica
16	Oli de llinosa	600	0
17	Oli de llinosa	600	inorgànica
18	Oli de llinosa	600	orgànica
19	Greix animal	0	0
20	Greix animal	0	inorgànica
21	Greix animal	0	orgànica
22	Greix animal	300	0
23	Greix animal	300	inorgànica
24	Greix animal	300	orgànica
25	Greix animal	600	0
26	Greix animal	600	inorgànica
27	Greix animal	600	orgànica

[1] Tots els pinsos estaven suplementats amb 100 mg/kg d'acetat d'α-tocoferol.
[2] El greix afegit al pinso els primers 19 dies va ser d'origen animal.
[3] La font inorgànica consisteix en 1,2 mg Se/kg en forma de selenit sòdic mentre que la font orgànica consisteix en 0,2 mg Se/kg afegit en forma d'extracte de llevat ric en selenometionina.
[4] En el període de 20 a 39 dies és va afegir un 5,81% de greix animal més un 1,25% d'oli de peix. Durant els últims 5 dies es va afegir un 5,81% de greix animal més un 1,25% del greix corresponent (oli de peix, oli de llinosa o greix animal). El greix animal afegit en tots els pinsos era una barreja (80% llard i 20% de sèu).

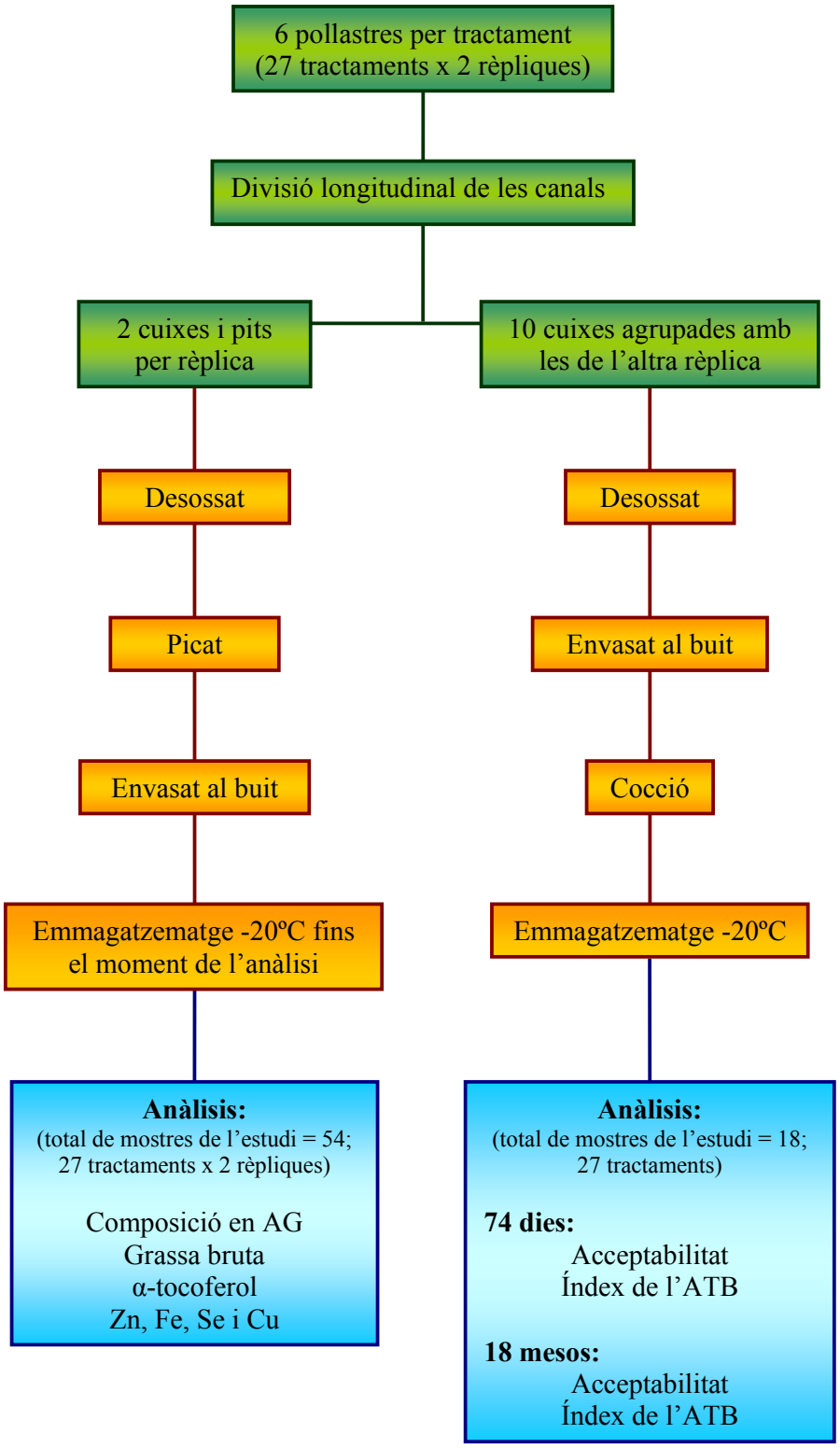

Figura 4. Processat i determinacions realitzades en carn de pollastre procedent d'un tractament del segon estudi.

2.3. Tercer Estudi

2.3.1 Disseny Experimental

El tercer estudi es va dissenyar per intentar assolir l'objectiu de quantificar l'efecte de l'addició en el pinso d'olis oxidats sobre la qualitat sensorial de la carn de pollastre (objectiu 4). Per fer-ho, a partir del 11è dia de vida i fins el 42è dia en què es van sacrificar els pollastres, es van addicionar diferents olis oxidats al 6% en un pinso base. Aquests pinsos es van preparar cada 10 dies per evitar la progressió de l'oxidació amb el transcurs del temps.

La preparació d'aquests olis oxidats, amb diferent grau i tipus d'oxidació, es va fer a partir d'un mateix oli de gira-sol. Aquest oli de gira-sol consistia en un oli refinat sense oxidar que és va anomenar "oli fresc". El segon oli a assajar es va preparar escalfant l'anterior oli, en una bany amb agitació i calefacció indirecta, a una temperatura que oscil·lava entre 50 i 60 ºC i durant un període de 12 dies. Després del tractament tèrmic aquest oli contenia un contingut elevat de compostos d'oxidació primària i el vam anomenar "oli peroxidat". En una fregidora industrial amb un sistema d'escalfament directe i sota agitació permanent es va escalfar l'oli de gira-sol fresc durant 28 h a una temperatura de 190-195 ºC. Com a resultat, es va obtenir un oli de gira-sol ric en compostos d'oxidació secundària i és va anomenar "oli molt oxidat". De la barreja al 50% de l' "oli fresc" i l' "oli molt oxidat" es va obtenir un quart oli a assajar, l'anomenat "oli oxidat". En la Taula 9 es mostren els valors d'oxidació obtinguts en els quatre tipus d'oli i el contingut en α-tocoferol. Els olis es van guardar congelats en bidons i, quan s'havien de preparar els pinsos, se'n descongelava la quantitat requerida.

Abans de preparar els pinsos, es van determinar les pèrdues en α-tocoferol en els diferents olis. Les pèrdues en α-tocoferol trobades en els olis, degudes als tractaments tèrmics, es van rectificar afegint acetat d'α-tocoferol en els pinsos. D'aquesta manera tots els pinsos aportaven la mateixa quantitat d'α-tocoferol que el pinso preparat a partir d'oli de gira-sol fresc. Això va permetre eliminar una covariable

de l'estudi i, així, poder avaluar els efectes provocats per l'addició dels diferents olis independentment de la pèrdua d'α-tocoferol que provoquen els tractaments utilitzats per oxidar-los (4t objectiu).

Un segon factor a assajar va ser la dosi d'α-tocoferol, per avaluar el seu efecte protector front als olis oxidats (2a part del 4t objectiu). Per aquesta raó es van assajar dos nivells de suplementació amb acetat d'α-tocoferol (0 i 100 mg/kg).

Finalment, com a tercer factor a assajar, i com a conseqüència dels resultats obtinguts al segon estudi, es va decidir seguir estudiant l'efecte de la suplementació amb Zn (0 i 600 mg/kg) en combinació amb la suplementació de seleni. El suplement en seleni es va fixar, per a tots els pinsos, a una dosi de 0,6 mg Se/kg pinso, en forma d'extracte de llevats ric en seleni.

En aquest estudi, els pollets, fins al 10è dia de vida, van rebre un pinso base igual per tots ells en què s'havia afegit una barreja de greix animal al 6%. En acabat, a partir del 11è dia de vida dels pollets, per mitjà d'un disseny factorial de 16 tractaments (4x2x2) es van poder estudiar els tres factors anteriorment esmentats (Taula 10). Es van emprar 336 pollets que es van assignar aleatòriament, en grups de 7 animals, a una gàbia, la qual corresponia a un dels tractaments. L'estudi es va dur a terme per triplicat.

Taula 9. Valors de les variables d'oxidació, i contingut en α-tocoferol, dels olis de gira-sol afegits als pinsos.

Oli gira-sol	**Índex de peròxids[1]**	**Índex *p*-anisidina[2]**	**K^3_{232}**	**K^4_{270}**	**Polímers[5] [%]**	**α-tocoferol [mg/l]**
Fresc	2,1	6,4	3,58	1,74	0,7	683
Peroxidat	91,9	8,5	13,66	1,78	0,7	480
Oxidat	7,0	83,1	10,28	3,25	4,9	655
Molt oxidat	17,2	153,0	17,67	4,82	9,4	619

[1] Índex de peròxids en meq peròxids/kg. AOCS Official Method (Cd 8-53).
[2] Índex de *p*-anisidina. AOCS Official Method (Cd 8-90).
[3] K_{232} i K_{270} són les absorbàncies específiques a 232 i 270 nm (Grau et al., 2001b).
[4] Polímers expressats com a percentatge de triacilglicerols polimeritzats. Modificació del IUPAC Standard Method 2.508.

Taula 10.- Disseny factorial de l'experiment 3.

Tractaments[1]	Oli afegit	Suplement d'acetat d'α-tocoferol (mg/kg)	Suplement de Zn (mg/kg)
1	Fresc	0	0
2	Fresc	100	0
3	Fresc	0	600
4	Fresc	100	600
5	Peroxidat	0	0
6	Peroxidat	100	0
7	Peroxidat	0	600
8	Peroxidat	100	600
9	Oxidat	0	0
10	Oxidat	100	0
11	Oxidat	0	600
12	Oxidat	100	600
13	Molt oxidat	0	0
14	Molt oxidat	100	0
15	Molt oxidat	0	600
16	Molt oxidat	100	600

[1] Els diferents tractaments també contenien un suplement en seleni (0,6 mg Se/kg) en forma d'extracte de llevats. Els diferents tractaments es van aplicar des de l'11è dia de vida fins el dia del sacrifici.

2.3.2 Preparació de les Mostres

Els animals van ser sacrificats als 42 dies de vida segons els procediments comercials de la cooperativa agrícola COPAGA. De cada gàbia dues cuixes amb pell van ser seleccionades a l'atzar per estudiar la composició i valor nutritiu. Aquestes mostres es van desossar a mà i posteriorment es van picar i envasar al buit en bosses multibarrera (Cryovac BB-4L; permeabilitat al O_2 30 cm^3/m^2, 24 h, 1 bar, ASTMD-3985). Un cop envasades es van emmagatzemar a -20 ºC fins al moment de fer les determinacions.

Les cuixes restants es van usar per avaluar l'acceptabilitat i l'estabilitat oxidativa. Les cuixes es van desossar a mà i envasar al buit en bosses multibarrera (Cryovac CN-300; permeabilitat al O_2 15 cm^3/m^2, 24 h, 1 bar, ASTMD-3985). Un cop envasades es van coure a 85 ºC en un forn (humitat relativa = 99%) fins arribar a una temperatura de 78 ºC a l'interior de les peces. Posteriorment, les mostres es van

emmagatzemar a -20 ºC fins a la seva determinació de l'acceptabilitat als 4 mesos de conservació. A més, transcorreguts 30 mesos, es van descongelar, es van deixar 9 dies a refrigeració a 4 ºC, i es va avaluar la ranciesa.

2.2.3 Determinacions

L'estudi es va realitzar en les cuixes de pollastre crues amb pell, doncs, pel seu major contingut en greix i metalls com el ferro, s'esperava que tinguessin una major oxidabilitat. Les determinacions que es van fer en aquestes mostres van ser:

- El contingut en α-tocoferol i altres tocoferols.
- El contingut en elements minerals (zinc, seleni, ferro i coure).
- La grassa bruta total.
- La composició en àcids grassos.
- L'avaluació de l'estat oxidatiu per mitjà de l'índex de l'ATB i el contingut d'hidroperòxids (mètode del taronja de xilenol). L'elecció d'aquests dos mètodes va ser per que mentre l'índex de l'ATB indica la presència de compostos d'oxidació secundària, el mètode del taronja de xilenol, després d'un període d'incubació, indica el nivell d'hidroperòxids presents en la mostra i, per tant, dóna idea de la susceptibilitat cap a l'oxidació d'aquesta.

En les cuixes de pollastre cuites amb pell, envasades al buit, i posteriorment emmagatzemades a -20 ºC, es van realitzar les següents determinacions:

- Anàlisi de l'acceptabilitat per part dels consumidors. Aquest es va fer després de 4 mesos d'emmagatzematge. Les mostres es van escalfar durant 20 minuts a 75 ºC i es van presentar a 32 consumidors en un disseny de blocs incomplets balancejats (Cochran i Cox, 1957) que es va portar a terme per duplicat. El disseny tenia 16 blocs, sis mostres per bloc i sis replicats per cada mostra.

- Avaluació de l'estat oxidatiu de les mostres per mitjà de l'índex de l'ATB als 4 mesos de congelació.
- Anàlisi sensorial de l'aroma i el flavor a ranci. Un cop passats 30 mesos d'emmagatzematge, les bosses envasades al buit es van obrir i es van emmagatzemar 9 dies a 4-5 ºC abans de fer l'avaluació sensorial. Per aquesta avaluació es van seleccionar 5 panelistes entrenats que van rebre totes les mostres, després d'ésser escalfades durant 20 minuts a 75 ºC, en 4 sessions. Els panelistes puntuaren l'aroma i el flavor a ranci en una línia recta de 15 cm sense marques.
- Determinació de l'estat oxidatiu per mitjà de l'índex de l'ATB de les mostres emmagatzemades 30 mesos i també de les mostres emmagatzemades 30 mesos i posteriorment deixades a temperatures de refrigeració durant 9 dies.

En la Figura 5, s'exposa un esquema del sistema de mostreig i de les determinacions fetes en carn de pollastre.

En els pinsos, es va determinar:

- El contingut en α-tocoferol i els altres tocoferols.
- El contingut en elements minerals (zinc, seleni, ferro i coure).
- La composició en àcids grassos dels diferents pinsos subministrats als animals.

En els olis, es van realitzar els següents anàlisis per determinar el seu grau d'oxidació:

- L'índex de peròxids.
- L'absorbància específica a 232 i 270 nm (K_{232} i K_{270}).
- El contingut en polímers.
- El contingut en α-tocoferol (emprat per corregir les pèrdues a posteriori).

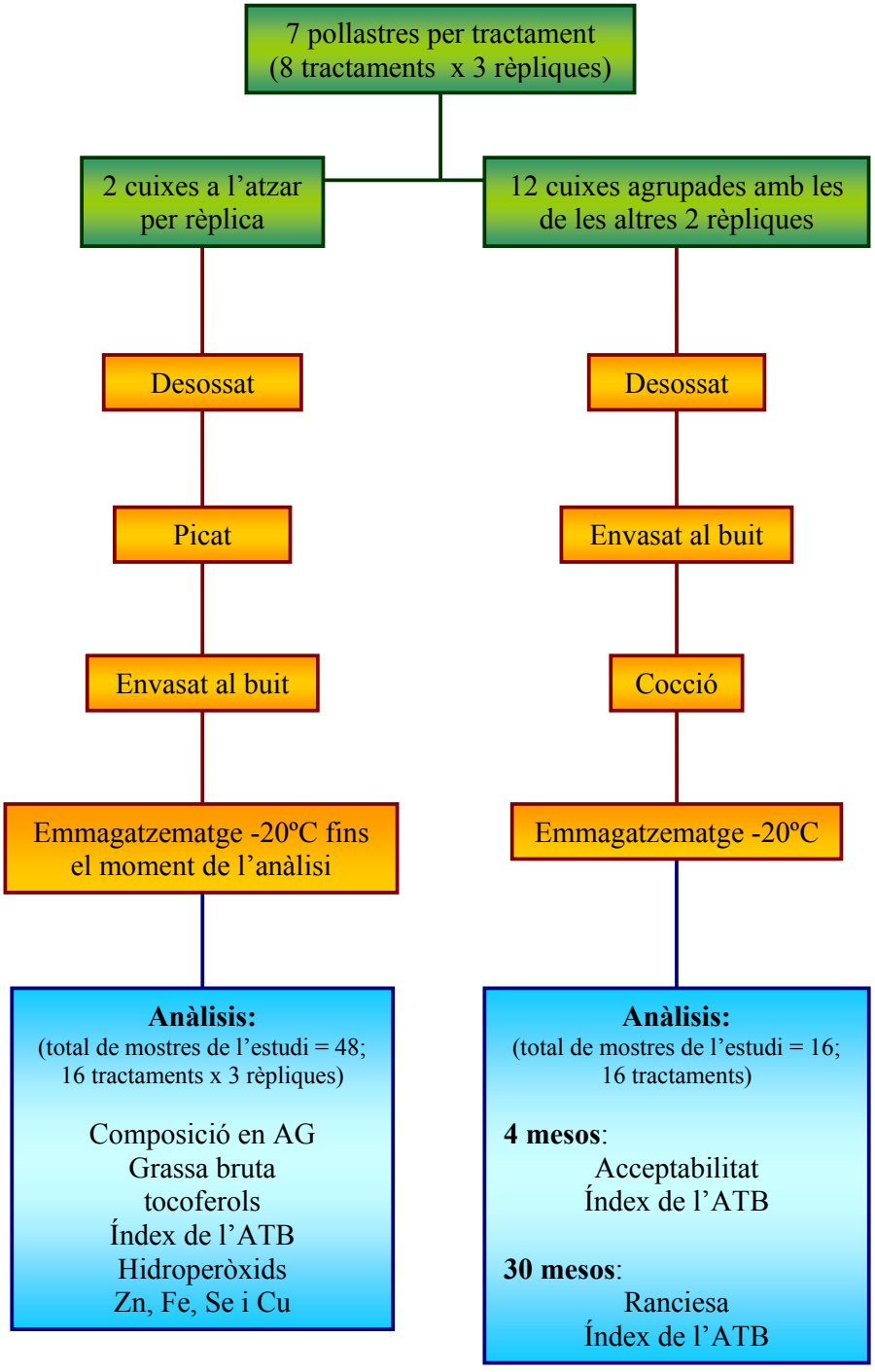

Figura 5. Processat i determinacions realitzades en carn de pollastre procedent d'un tractament del tercer estudi.

IV. PART EXPERIMENTAL

PART EXPERIMENTAL

1. PUBLICACIONS

Per a poder dur a terme tots els anàlisis que s'havien de fer en els diferents estudis era necessari posar a punt un mètode analític per a la determinació de la composició en alguns minerals en carns i pinsos. Això va donar lloc a la següent publicació.

- "Validation of mineralisation procedures for the determination of selenium, zinc, iron and copper in chicken meat and feed samples by ICP-AES and ICP-MS"
  R. Bou, F. Guardiola, A. Padró, E. Pelfort; R. Codony. 2004.
  Journal of Analytical Atomic Spectrometry 19:1361-1369.
  Factor d'impacte (2003): 3.200

Finalment, de l'estudi de tots els resultats obtinguts, van sorgir 4 articles més, recollits a continuació:

- "Effect of dietary fish oil, α-tocopheryl acetate, and zinc supplementation on the composition and consumer acceptability of chicken meat"
  R. Bou, F. Guardiola, A. Tres, A. C. Barroeta, R. Codony. 2004.
  Poultry Science 83:282-292.
  Factor d'impacte (2003): 1.253

- "Effect of dietray fat sources and Zinc and Selenium supplements on the composition and consumer acceptability of chicken meat"
  R. Bou, F. Guardiola, A. C. Barroeta, R. Codony. 2005.

Poultry Science (En premsa).
Factor d'impacte (2003): 1.253

- "Increase of geometrical and positional fatty acid isomers in dark meat from broilers fed heated oils"
  R. Bou, A. Tres, M. D. Baucells, R. Codony, F. Guardiola.
  Poultry Science (Enviat).
  Factor d'impacte (2003): 1.253

- "Effect of heated sunflower oil and different dietary supplements on the composition, oxidative stability and sensory quality of dark chicken meat"
  R. Bou, M. D. Baucells, R. Codony, F. Guardiola.
  Journal of Agricultural and Food Chemistry (Enviat).
  Factor d'impacte (2003): 2.102

1.1. Validation of Mineralisation Procedures for the Determination of Selenium, Zinc, Iron and Copper in Chicken Meat and Feed Samples by ICP-AES and ICP-MS

Títol: Validació de diferents procediments de mineralització per a la determinació de seleni, zinc, ferro i coure en mostres de carn de pollastre i pinso per ICP-AES i ICP-MS.

Resum: Es van estudiar diferents procediments de mineralització per a la determinació de Fe, Zn, Cu i Se en carn de pollastre i mostres de pinso. Finalment, es van estudiar tres procediments de preparació de mostres diferents per determinar aquests elements per tècniques de espectroscòpia d'emissió atòmica i per espectroscòpia de masses acoblades a cremadors de plasma.

Un procediment de mineralització humida en tub obert es va provar en l'anàlisi de la carn de pollastre. Usant aquest procediment és necessari concentrar els elements traça com el Cu i el Se, i també, reduir els efectes de la matriu àcida, raó per la qual les mostres van ser portades a sequedat. Al dur les mostres a sequedat es van produir pèrdues de Se per volatilització donant lloc a baixes recuperacions i una alta variabilitat.

Per tant, es va desenvolupar un procediment de mineralització amb microones en tub tancat per les mostres de carn de pollastre. Els resultats obtinguts per aquest procediment mostraren una variabilitat molt menor (2,5; 2,0; i 3,1% respectivament pel Zn, Fe i Se) i concordaven amb els valors certificats del material de referència assajat. En relació al contingut en Cu, tot i mostrar una variabilitat relativament alta (CV = 11%), també concorda amb el valor certificat del material de referència. Les recuperacions en les addicions estàndard de carn de pollastre van ser de 103-105% pel Zn, 107-108% pel Fe, 97-100% pel Se i el 89-94% pel Cu.

Malgrat això, usant aquest procediment en pinsos, algunes mostres mineralitzades presentaven un precipitat silícic el qual provocava unes baixes

recuperacions del Zn en comparació amb un altre procediment de mineralització en microones a on aquest precipitat va ser dissolt amb àcid fluorhídric. Aquest procediment alternatiu de mineralització per pinsos va mostrar, per cada element determinat, una bona precisió, recuperació i sensibilitat.

VALIDATION OF MINERALISATION PROCEDURES FOR THE DETERMINATION OF SELENIUM, ZINC, IRON AND COPPER IN CHICKEN MEAT AND FEED SAMPLES BY ICP-AES AND ICP-MS

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ABSTRACT

Mineralisation procedures for determining Fe, Zn, Cu and Se in chicken meat and feed samples were studied. We employed three different sample preparation procedures to determine these elements by ICP-AES and ICP-MS. An open vessel wet mineralisation procedure was used for chicken meat analysis. Here, in the case of the trace elements, Cu and Se, it was necessary to concentrate and reduce the acid matrix effects so samples were conducted to dryness. However, this procedure causes Se volatilisation losses leading to low recoveries and high variability. Therefore, a closed vessel microwave mineralisation procedure was developed for chicken meat samples. The results obtained using this procedure presented a much lower variability (2.5, 2.0 and 3.1 % of RSD for Zn, Fe and Se, respectively) and were consistent with the certified values for the reference material being assessed. Cu content, despite presenting a relatively high variability (RSD = 11 %) also agreed with the certified value for the reference material. Recoveries in spiked chicken meat were 103-105 % for Zn, 107-108 % for Fe, 97-100 % for Se and 89-94 % for Cu. However, when this procedure was used with feeds, some mineralised samples presented a siliceous particles which lead to a lower recovery of Zn compared with that recorded when using another microwave mineralisation procedure in which these particles had been dissolved with hydrofluoric acid. This alternative mineralisation procedure for feeds was validated by assessing the precision, recovery and sensitivity when determining each element.

1.- INTRODUCTION

The determination of certain elements in foodstuffs is important in various fields including nutrition and toxicology. Thus, for example, in addition to the biological functions of Fe, it is widely known that Fe and other elements can act in reduction-oxidation processes and participate in the spoilage of various foodstuffs, including meat and meat products. Other elements such as Se have been described as having biochemical functions, most noticeably acting as a component in the antioxidant enzyme glutathione peroxidase, which plays an important role protecting against the oxidation damage[1,2]. In addition, superoxide dismutase, of which Cu and Zn are components, has a synergistic biochemical function against oxidation damage.

Meat products are one of the main sources of dietary Se, Zn, Fe and Cu[3,4]. Many dietary factors influence the bioavailability of these elements, though the bioavailability of Fe, Zn and Cu is generally high in meat products[5]. In the case of Se, inorganic and organic forms are rapidly absorbed, although selenomethionine, the predominant form of dietary selenium, is more easily incorporated than inorganic forms[4]. In addition, many nutrient interactions, for example Fe-Zn and Zn-Cu, have been described between these and other elements, especially during absorption[6]. This means that a reliable and accurate quantification of these elements in animal feeds and meat products enables us to study the effect of these dietary elements on meat composition.

Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) are useful methods for conducting multi-element analyses, particularly when these elements are present only at trace levels. In addition, hydride generation can be used in atomic spectrometry as a sample introduction system for hydride-forming elements thereby reducing matrix effects and interferences and improving detection limits.

These methods require the complete mineralisation of samples, for which wet digestion procedures are most widely used. This mineralisation step is essential when dealing with foodstuff samples as they contain high concentrations of organic substances and, in addition, contamination and the loss of elements should be avoided

to prevent any sample preparation errors. In this regard, selenium determination is particularly difficult as many selenium compounds are volatile and can be lost, while organoselenium compounds are highly resistant to acid[7].

Wet digestion in open vessels does not require the application of any special equipment and a number of some methods have been described for the accurate measurement of selenium in foodstuff and feed samples[8-11]. However, the use of microwaves to accelerate the acid digestion of samples, in closed vessels subjected to high temperatures and pressures is currently the most frequently used method. This has the additional advantage of preventing Se losses due to volatilisation[12,13].

One major drawback in the analysis of plant and feed samples is caused by the fact that many elements are bound to silicates present. This means that the former are not fully released from the matrix which results in poor recoveries. To overcome this, hydrofluoric acid is widely used in the acid mixture to dissolve these silicates[14].

The aim of the present study is to develop suitable methods for the determination of Zn, Fe, Se and Cu in chicken meat and feed samples by comparing the precision, accuracy and sensitivity of various mineralisation methods.

2.- MATERIAL AND METHODS

Apparatus

Water-wash-down hood was used to perform open vessel digestions with perchloric acid. In these open vessel digestions a digestion block model S-508 (JP Selecta, Barcelona, Spain) was used to perform sample mineralisations. Two microwave ovens were employed for closed vessel sample digestions: the model Mars X (CEM, Matthews, NC) equipped with quartz vessels and the model Ethos Plus (Milestone, Monroe, CT) equipped with PTFE vessels.

For the elemental determination of Fe, Zn and Cu an Optima 3200RL model ICP-AES (Perkin-Elmer, Norwalk, CT) was used, while for the determinations of Se and Cu an Elan 6000 model ICP-MS (Perkin-Elmer, Norwalk, CT) was used. In addition, to determine Se content the selenium hydrides were formed and were

introduced into the ICP-MS through a FIAS 400 model flow injection system (Perkin-Elmer, Norwalk, CT).

Reagents and Standards

Milli-Q water was obtained through a Millipore purification water system model Milli-Q ZFMQ 230 04 (Millipore, Medford, MA) and was used throughout. Nitric acid 65 % ("Instra" grade), hydrochloric acid 37 % (analytical reagent grade), perchloric acid 70-72 % (analytical reagent grade) and hydrofluoric acid 38 % (analytical reagent grade), sodium hydroxide (analytical reagent grade) were from J.T. Baker (Phillipsburg, NJ). Hydrogen peroxide 33 % ("Suprapur" grade) and sodium borohydride 98 % (analytical reagent grade) were from Merck (Darmstadt, Germany). Sodium borohydride solution contained 0.2 % $NaBH_4$ and 0.05 % NaOH.

Selenium, iron, zinc and copper standard solutions (SCP Science, Montreal) were traceable to the Standard Reference Materials of the NIST and were used to perform calibration curves and several additions on samples. Seleno-L-methionine was from Sigma (St. Louis, MO) and sodium selenite was from Aldrich (Milwaukee, WI). Certified reference material (CRM 184, bovine muscle) was from the BCR of the European Commission (Brussels, Luxembourg).

Samples

Five samples of mixed raw chicken meat including its skin and two feed samples were used to optimise the mineralisation procedures (Table 1). Meat and feed samples were homogenized using a Robot Coupe mixer model BX3 (Jackson, MS) and a SPEX mill model 8000 (Edison, NJ), respectively.

Mineralization of samples

Digestion procedure I *(using open vessels and digestion block; adapted from Verlinden's method*[15]*).*

Two similar procedures were compared. In **Procedure Ia**, 1.5 g (wet weight) of chicken meat sample were introduced into a pyrex digestion tube (250 mL) and then

25 mL of nitric acid (65 %) was added. In **Procedure Ib**, 2 g (wet weight) of chicken meat sample were introduced into a pyrex digestion tube (250 mL) and then 30 mL of nitric acid (65 %) was added. Then, the pyrex digestion tubes used in procedures Ia and Ib were connected to condensers, placed into a digestion block and left for 14 h at 60 ºC under reflux. Sample digestion continued by heating for 1 h at 120 ºC. Later, condensers were removed and the mixtures evaporated to 5 mL by heating at 140 ºC. Each digestion tube was then cooled and 5 mL of perchloric acid was added and then heated to 210 ºC for 30 min with the condenser. Finally, the condenser was removed again and the solution was evaporated at 240 ºC to dryness. The digested samples were completely redissolved after 10 min of ultrasonication and gentle agitation with 10 mL of aqueous 2 % HNO_3.

The precision of element determination after mineralisation following procedure Ia was assessed by analysing nine aliquots of a sample of mixed raw dark and white chicken meat with skin (see Table 1 for sample description). A further nine aliquots of another sample of mixed raw dark and white chicken meat with skin were used to assess the precision of element determination following digestion procedure Ib. Aliquots of a third sample of mixed raw dark and white chicken meat were used to assess the precision of Se determination following procedure Ib at different concentration levels. The different concentration levels were obtained by adding 1.5 mL of aqueous solutions with different amounts of selenomethionine or sodium selenite to the sample aliquots. Aliquots of 1.5 mL of aqueous solutions of selenomethionine or sodium selenite were also treated as samples to assess Se losses during mineralisation analysis.

In addition, procedures Ia and Ib were adapted for microwave mineralisation using PTFE vessels and the Ethos Plus microwave described above. This mineralisation method was used to check Se recovery by digesting 1.5 mL of a standard Se (SCP Science) solution (2 mg / L) in closed vessels (n = 5). The procedure was carried out as follows: 5 mL of nitric acid and 1 mL of perchloric acid were added to each vessel, which were then closed. Digestion was conducted by applying the following program: 5 min with power at 250 W, 5 min without energy, then 5 min with power at 250 W, then 5 min at 400 W, 5 min at 600 W, and finally a further 5 min

without energy. Then, digested solutions were transferred into 20 mL volumetric flasks and diluted to volume with Milli-Q water.

Digestion procedure II *(using closed quartz vessels and microwave oven)***.**

0.6 g (wet weight) of mixed raw chicken meat or 0.3 g (dry weight) of the certified reference material were accurately weighed into quartz digestion vessels. Five mL of nitric acid and 2 mL of hydrogen peroxide were added to each tube, which were then closed. Digestion was conducted by applying a 4-step program described as follows: firstly by heating at a rate of 10 ºC/min up to 120 ºC and hold 5 min, then at 10 ºC/min up to 150 ºC and hold 5 min, and again at 10 ºC/min up to 180 ºC and hold 5 min, finally at 10 ºC/min up to 200 ºC and hold 10 min. Once the samples had been allowed to cool, the digested solutions were transferred into 20 mL volumetric flasks and diluted to volume with Milli-Q water.

The precision of element determination after mineralisation by procedure II was assessed by analysing 10 aliquots of a sample of mixed raw dark chicken meat with skin (see Table 1 for sample description). The accuracy of element determination following procedure II was assessed by analysing 10 aliquots of a certified reference material (CRM 184, bovine muscle).

Aliquots of another sample of a mixed raw dark chicken meat with skin were analysed following procedure II to assess element recoveries at different concentration levels. The different concentration levels were obtained by adding 0.5 mL of aqueous solutions prepared with different amounts of the standard solutions of Zn, Fe, Se and Cu purchased from SCP Science.

Digestion procedure III *(using closed PTFE vessels and microwave oven)*

Feed samples (0.25 g, wet weight) were accurately weighed into digestion PTFE vessels. Two procedures were compared. In **Procedure IIIa,** 5 mL of nitric acid, 2 mL of hydrogen peroxide and 1 mL of Milli-Q water were added to each tube and then closed. Digestion was completed according to the heating program described in procedure II. After samples had been allowed to cool, the digested solutions were transferred into 25 mL volumetric flasks and diluted to volume with Milli-Q water. By contrast, **Procedure IIIb** was carried out as follows: 4 mL of nitric acid, 2 mL of hydrogen peroxide and 2 mL of Milli-Q water were added to each tube, which were then closed. Digestion was completed according to the following program: heating at a

rate of 5 ºC/min up to 100 ºC and hold 5 min, then at 10 ºC/min up to 180 ºC and hold 5 min, then at 10 ºC/min up to 200 ºC and hold 5 min, and finally at 10 ºC/min up to 210 ºC and hold 10 min. After the samples had been allowed to cool, 1 mL of hydrofluoric acid was added and the samples were heated at a rate of 10 ºC/min up to 120 ºC and hold 10 min. After the samples had cooled, 20 mL of Milli-Q water were added and these solutions were weighed. Three 1-mL aliquots of each mineralised solution were also weighed to determine the density, which was used for the calculation of the final solution volume.

The respective precision of the element determinations after mineralisation by procedures IIIa and IIIb were compared by analysing 8 aliquots of a feed sample (see Table 1 for sample description).

Aliquots of another feed sample (supplemented with organic selenium from Se enriched yeast) were analysed following procedure IIIb to assess the element recoveries at different concentration levels. The different concentration levels were obtained by adding 0.5 mL of aqueous solution prepared with different amounts of the standard solutions of Zn, Fe, Se and Cu purchased from SCP Science.

Reagent blanks of each procedure were treated in the same way as samples.

Fe, Zn and Cu determination by ICP-AES

Digested samples were diluted to an adequate element concentration and final acid matrix (approximately 2% or above) and were analysed using the ICP-AES. Two wavelengths were measured for each element (238.204 and 259.939 nm, 213.857 and 206.200 nm, 324.752 and 327.393 for Fe, Zn and Cu, respectively). Instrumental measurement conditions are described in Table 2. Aqueous (1-2% HNO_3) calibration curves (intercept equal to 0) were used for quantification and the selected wavelengths were 259.939 nm for Fe, 213.857 nm for Zn, and 324.752 for Cu.

Se determination by hydride generation ICP-MS

One mL of digested solution and 1 mL of concentrated HCl were added into 14 mL capacity poliestyrene tubes and, after closing, they were heated for 1 h at 60ºC in an oven to allow the reduction of Se (VI) to Se (IV). After cooling, 8 mL of Milli-Q

water was added to obtain the optimal HCl concentration (prereduced Se solution) for Se quantification by Hydride Generation Inductively Coupled Plasma Mass Spectrometer (HG-ICP-MS). Prereduced Se (IV) reacted with sodium borohydride solution to form hydrogen selenide using a flow injection system and introduced into the ICP-MS. Instrumental measurement conditions are described in Table 2. Calibration curves (intercept equal to 0) were performed by addition of different amounts of Se (^{82}Se was measured) on 1 mL of Milli-Q water, reagent blank or mineralised sample. These calibration solutions were prereduced as described above.

Cu determination by ICP-MS

Digested samples were diluted (usually 1/5 to 1/10) before being analysed using an ICP-MS. Instrument measurement conditions are described in Table 2. ^{103}Rh was used as the internal standard in aqueous (1% HNO_3) calibration curves (intercept equal to 0) of ^{63}Cu and ^{65}Cu isotopes although only the first of these was selected for quantification.

Statistical Analysis

The *F* test was carried out to assess whether the slopes of the Se calibration curves were affected by the solvent media (Milli-Q water compared to a reagent blank or a mineralised sample)[16]. ANOVA tests were carried out to determine whether there were any difference between the recoveries at different levels of additions in dark chicken meat and feed samples. Student-Fisher's *t* test was conducted to compare the element concentrations obtained using the two feed mineralisation procedures. In all cases, $P \leq 0.05$ was considered significant.

3.- RESULTS AND DISCUSSION

Procedures Ia and Ib

The analysis of nine aliquots of a mixed raw dark and white chicken meat sample following digestion procedure Ia showed very good precision (Table 3) for

both Fe (RSD = 2.0 %) and Zn (RSD = 4.2 %). However, greater variability was found for Cu and Se, both of which were present at concentrations below 100 µg/kg expressed as wet basis. Although Se is present in similar amounts to Cu, Se content showed a much greater variability (RSD = 78.1 %) with values ranging from 3 to 43 µg/kg.

In order to assess, whether this degree of variability could be attributed to a lack of sample homogeneity, the sample weight was increased from 1.5 g (digestion procedure Ia) to 2 g (digestion procedure Ib). Thus, nine aliquots of another sample (mixed raw dark and white chicken meat) were analysed following procedure Ib and the results are shown in Table 3. The Fe and Zn content mean values were quite similar to those obtained for the chicken samples in procedure Ia. Despite this, our results showed a lower degree of variability for Zn but a higher degree for Fe. The variability in Cu contents obtained following procedures Ia and Ib are not comparable because the chicken meat sample analysed by procedure Ib shows a much higher Cu content, which may have led to a lower rate of variability despite the sample weight. As Cu content in these samples was quite low, a more sensitive technique, such as ICP-MS, is recommended for determining this element.

The results obtained following digestion procedure Ib showed a very low Se content as well as a very high variability (Table 3). A critical point in Se determination using HG is the prereduction step with hydrochloric acid, which allows the complete reduction of Se (VI) to Se (IV). The reduction conditions described in the material and methods section were similar to those described elsewhere[11] for meat product samples. Furthermore, the reliability of the analysis following the application of the prereduction step was verified by carrying out the selenium prereduction with HCl on sodium selenite or standard Se solutions in various media (Milli-Q water, a reagent blank and a mineralised sample) and no volatilisation losses were recorded during this step. In addition, the *F* test showed that there were non-significant differences between the slopes of the calibration curves obtained when adding different amounts of Se to the various media ($F_{0.05,5,3}$ = 1.177 and $F_{0.05,5,3}$ = 1.004 for mineralised sample and reagent blank, respectively). Therefore, the sample or reagent matrices would appear not to affect the slope of the calibration curves, which agrees with results reported elsewhere[17,18].

According to these results, a number of potential problems need to be taken into account in relation to Se quantification: i) ineffective sample digestion resulting from an incomplete breakdown of organoselenium compounds such as selenomethionine and selenocysteine and ii) volatilisation losses, during mineralisation, of organic and reduced forms of Se, which are known to be more volatile than oxidised inorganic forms of Se.

Despite these problems, Verlinden[15] proposed an HG Atomic Absorption Spectrometry (AAS) method for the determination of Se in human blood and plasma that was quite similar to procedure Ia described here, and, moreover, he reported good recoveries for Se. The main difference between procedure Ia and Verlinden's digestion procedures is that in the former the digested samples were evaporated to dryness at 240 ºC (rather than reducing the volume to 2 mL at 210 ºC) in order to concentrate and avoid the acid matrix effect because samples had very low Cu and Se contents. Therefore, procedures Ia and Ib were expected to have similar recoveries to those reported by Verlinden, which were about 85% when assessed with standard additions of selenomethionine. However, Nève et al.[7], working with a similar nitric and perchloric acid mixture and following heating for 30 min on the appearance of white perchloric fumes, reported an incomplete mineralisation of diphenyl selenide, whereas other organoselenium derivatives such as selenomethionine were much more recovered after this digestion procedure. On the other hand, these authors when combining nitric, perchloric and sulphuric acids found a complete mineralisation for all these organoselenium derivatives, even for diphenyl selenide, determined by graphite furnace AAS. This acid mixture is widely used in open vessel mineralisation although many combinations of these acids together with hydrochloric, hydrofluoric and hydrogen peroxide have been used. However, Nève et al. [7] also found that a digestion with a nitric and perchloric acid mixture allowed the complete recovery of spiked inorganic selenium in plasma. Furthermore, Tinggi et al.[10] reported similar recoveries (above 96 %) when comparing a nitric and perchloric acid mixture to a nitric, sulphuric and perchloric acid mixture in open vessel mineralization of foodstuff samples analysed by HG-AAS. In addition, Plessi et al.[19] reported a good accuracy in seafood samples, analysed by HG-AAS, using nitric acid and heating at 80 ºC until digestion was complete in open vessels. Similar results have been reported[20] in urine samples,

analysed by HG-AAS, using a nitric and perchloric acid mixture and heating at the same temperature for only 1 h. However, in such samples, Se is mainly present as trimethylated Se, its main excretion form[4]. In addition to these results, in a fish meat sample that is more similar in its characteristics to our chicken samples, Lambert and Turoczy[21] determined Se content by cathodic stripping voltammetry in a comparative study of different digestion methods. The methods they compared were: the AOAC method[22] performed in sealed vessels, the same method but performed in open vessels, a nitric acid with $Mg(NO_3)_2$ open vessel mineralisation, a calorimeter bomb method, a sample digestion using UV irradiation, and a pressure aided digestion method. The authors reported low and variable recovery methods for some of the methods assessed because organic matter was not completely destroyed or losses were suffered due to the volatilisation of Se.

Hence, in order to assess the nature of this incomplete breakdown and these volatilisation losses, two studies were carried out. First, aliquots from a new sample of raw dark and white chicken meat with skin (Table 4) were spiked with sodium selenite (inorganic source of Se) or with selenomethionine (the main source of organic Se in meat samples) and were analysed following digestion procedure Ib. The results from the analysis of these samples showed that variability was high and similar (Table 4) for both sources of selenium at each level of addition (20 and 200 μg Se/Kg). Afterwards, we analysed aqueous standard solutions of 100 μg of Se/L, made up with sodium selenite or selenomethionine (Table 4). Our results showed that recoveries were very poor and similar between Se sources. In addition, the variability of results was higher for the selenomethionine solution.

These results can be explained by volatilisation losses during digestion procedure Ib. In fact, several authors[14,23-26] have reported that the use of magnesium oxide or magnesium nitrate with or without other chemical modifiers as an ashing aid is required in order to obtain good recoveries when dry ashing procedures are used to determine Se, enabling the complete destruction of organic matter and volatilisation losses to be minimised. In addition, a number of authors have found that the analysis of a certified reference material by combining wet digestion in closed vessels with dry ashing digestion with ashing aids give results that are in agreement with the Se certified value[21,27]. However, Vassileva et al.[28], working with terrestrial plant samples,

described a dry ashing procedure in which the addition of magnesium nitrate was not necessary for Se determination by AAS. However, these authors found that aquatic plants analysed by this method cause losses of Se and As, due to the presence of more volatile species in these plants. However, a wet digestion procedure, using a nitric and perchloric acid mixture and conducting samples to dryness after mineralisation, was reported to be accurate for Se determination in a medicated shampoo sample[29]. Similar results were described in shellfish tissue samples using a nitric, hydrofluoric and perchloric acid mixture digestion and conducting samples to virtual dryness at 180 ºC[30]. However, in a comparative study[31] of four digestion methods determining Se in bovine livers by HG-AAS, it was reported that a nitric-perchloric acid digestion method, in which the volume of perchloric acid was reduced to about 1 mL showed lower mean values and higher variability than other wet digestion methods in which the acid volumes were not concentrated. In this particular nitric-perchloric acid mixture method, in which the volume was reduced to about 1 mL, the temperature was raised in a nine-step programme, up to 225 ºC. Drabek and Kalauskova[32] observed losses of ^{75}Se in nitric-perchloric acid mixture digestion of human blood or a mixed food sample at the end of the procedure when an excess of acid was evaporated. The addition of $MgCl_2$ to the digestion mix prevented the escape of Se and thus permitted the total evaporation without any loss. In addition, Sanz-Alaejos and Díaz-Romero[33], in a comprehensive review, reported that the reaction times and temperatures used to digest the samples with nitric-perchloric acid mixtures are critical when seeking to avoid Se losses. Moreover, these authors also showed that appreciable Se losses occur when samples are digested in various acid mixtures and the mineralised samples are conducted to dryness.

In order to verify the influence of these factors on Se volatilisation in the experimental conditions described here, 10 aliquots of an aqueous sodium selenite standard solution (96 μg/L) were analysed following procedure Ib, evaporating the digested samples to dryness or to 2 mL. The results obtained were respectively 49 ± 13 μg/L and 70.1 ± 6.3 μg/L, which indicates that Se losses mainly occur when samples are conducted to dryness. Thus, by just avoiding the complete evaporation of the digested samples, the Se recovery (73%) and the variability of the results were improved. In fact, when samples are conducted to dryness, reduced forms of selenium

alone (SeH_2, SeO_2) or combined with chloride (Se_2Cl_2, $SeCl_4$, $SeOCl_2$ or SeO_2.2HCl) can be formed and lost by volatilisation[15,25,31].

This finding is supported by the results obtained when 5 aliquots of an aqueous Se standard solution were mineralised with an acid mixture of HNO_3 (5 mL) and $HClO_4$ (1 mL) in PTFE closed vessels (see Material and Methods). In this study, the Se recovery was a 86 % (RSD = 0.9 %). Therefore, a sample mineralisation in closed vessels, without conducting samples to dryness, is recommended for the determination of Se, as volatilisation losses can be avoided.

Procedure II

Precision.

The aim of this second method was to avoid Se volatilisation losses, obtain a complete mineralisation, and minimise sample contamination. The use of strong oxidising and dangerous reagents such as $HClO_4$, which are not recommended in microwave assisted digestions, can be avoided in the mineralisation of samples in closed vessels because of the higher pressure and temperatures reached. In these conditions, a complete mineralisation with nitric acid alone has been reported in shellfish tissue samples[30] and different foodstuffs[33-35], or with a mixture of nitric acid with H_2O_2 in autopsy tissues[36], human milk[37] and foodstuffs[13,38,39]. However, Lan et al.[40] reported that not all microwave assisted digestion procedures, assessed in the same conditions of time and power, lead to a complete destruction of some of the organic selenium compounds present in samples. Despite this, by changing various program parameters such as temperature and time the complete digestion can be achieved.

Digestion procedure II, using nitric acid and H_2O_2 in closed vessels, brings about a complete sample mineralisation and presented a low and similar variability for Zn and Fe content (Table 5), while the values recorded were in close agreement with chicken meat content values contained in the food composition tables[41]. Se also showed a fairly low variability (Table 5) than in the previously assayed open vessel digestion procedures (Table 3). As for Cu content, since the ^{65}Cu isotope seemed to suffer slight interference from the S content, we chose the ^{63}Cu isotope for

quantification. However, even though Cu content was determined by ICP-MS, which is much more sensitive than ICP-AES, the variability for this element determination was still relatively high (Tables 3 and 5). This might have been due to the low signal-to-noise ratio observed for this element. Nevertheless, this variability does not exceed the maximum RSD recommended by the AOAC for this analyte concentration[42].

Accuracy.

The accuracy of the method was assessed by using the bovine muscle certified reference material (CRM 184). Our results, shown in Table 5, were in close agreement with certified values. To assess the performance of the method we checked that the standard error for each element was, as recommended by the BCR, less than the standard deviation of the certified values and that the means found lay within the limits of the certified values (certified ± 2 standard deviation), thereby showing good element recoveries that ranged from 92 to 107 %.

Given these results for the certified reference material it is worth highlighting that the ^{63}Cu isotope fits the certified value better for the certified value than the ^{65}Cu, which had a higher signal indicating some interference for this isotope (most probably the sample S content). On the other hand the relatively high variability found for Cu content in the certified reference material and in the chicken meat (Table 5) can be attributed to the low signal-to-noise ratio for this element. However, these variabilities do not exceed the maximum RSD recommended by the AOAC for these analyte concentrations[42].

Spiked samples.

The results of the spiked chicken samples are shown in Table 6. Zn recoveries ranged from between 103 and 105 %, which agrees with the recovery (104 %) found for the certified reference material. Fe recoveries ranged from 107 to 108 %, while the recovery was 100 % for the certified reference material. Se recoveries ranged from 97 to 100 %, while for the certified reference material the recovery was 92 %. Recoveries for Cu ranged from 89 to 94 %. These recoveries were a little lower than the recovery (107 %) reported for the certified reference material. Provided that the Cu signal-to-noise ratios are increased at higher addition levels, this would apparently explain the lower RSD at higher addition levels (Table 6). Furthermore, for each element, the ANOVA test showed that there were no differences between the recoveries at the

addition levels assayed so a global mean recovery can be assumed through the assessed range. Furthermore, the recoveries for each element at the different concentrations assayed lay within the ranges of values recommended by the AOAC[42].

Detection and quantification limits.

The detection limits were determined following the IUPAC recommendations[43], namely by assessing the standard deviation of 10 reagent blanks carried out on five different days during the analytical procedure. The limit of detection was equal to the standard deviation multiplied by a figure that was two times the Student's *t* value at the 95 % confidence level. Quantification limits were calculated by multiplying the detection limits by 3.04[43]. Both limits are expressed in μg of each element per L of final diluted mineralised sample solution and per kg of sample weight (Table 7). When Se was determined in various foodstuffs by means of ICP-AES, Dolan and Capar[34] reported much higher quantification limits for Se than ours (HG-ICP-MS determination). However, these authors[34] determining Zn and Fe by ICP-AES reported much lower (33- and 20-fold, respectively) quantification limits for these two elements, expressed as element content in sample weight, than ours (ICP-AES determination). These results can be explained by the higher sample weight used by Dolan and Capar[34] because their limits of quantification, expressed as element content in final mineralised sample solution, were only lower (5- and 3-fold, respectively) than ours. In addition, these authors[34] determining Cu by ICP-AES reported a slightly higher (1.6-fold) quantification limit for this element, expressed as element content in final mineralised sample solution, than ours (ICP-MS determination). Moreover, the quantification limits for Cu and Fe were very similar to the Fe and Cu content present in mineralised samples, which explains the relatively high RSD found in Table 6 when there were no standard additions.

Procedure IIIa and IIIb.

Precision.

Mineralisation of feeds using procedure II saw the development of siliceous particles. These particles, probably formed by SiO_2 and/or silicates (1-2% of hydrated magnesium silicate named sepiolite is often added to feeds), might influence the

precision of the method since they can adsorb some elements in their structure, thereby decreasing the atomisation yield. In order to overcome this problem, HF is often used to destroy SiO_2 and silicates[44,45], although some elements such as Ca form insoluble fluorides that easily precipitate and result in poor recoveries for these elements[46].

Given that the use of quartz vessels is not appropriate when HF has to be added to dissolve SiO_2 and silicates, we studied whether the HF addition was necessary by carrying out a comparison in PTFE vessels. Therefore, procedure IIIa was adapted from procedure II, while in procedure IIIb, 1mL of HF was added to dissolve the siliceous particles. As can be observed in Table 8, low variability was observed in both methods, although a smaller amount of Zn was found in the procedure where the siliceous material was not dissolved. This would seem to indicate an adverse effect of the siliceous particles on this element, because the Student-Fisher's *t* test showed significant differences for the Zn results obtained by these two procedures, suggesting that this might be due to an adsorption phenomenon. However, the other elements, including Fe and Cu, which are often affected by this phenomenon, failed to show any significant differences. As for Cu, both isotopes gave similar responses showing that there were no spectral interferences. In addition, both procedures showed similar means although procedure IIIb showed a higher variability for Cu content. This difference was confirmed following Cu measurement by ICP-AES. However, the relatively high variability for Cu content determined by method IIIb (RSD = 7.7 %), is only slightly higher than the maximum recommended by the AOAC (7.6 %) for this analyte concentration.

Spiked samples.

Recoveries for procedure IIIb with HF were assessed (Table 9) using a second experimental feed sample supplemented with organic selenium (approximately 600 μg/kg) derived from enriched yeast. Element recoveries were between 87-108 % and lay within the ranges of values recommended by the AOAC for the analyte concentration assayed[42]. Moreover, there were no differences between the addition levels, as assessed by ANOVA tests, in the recoveries of each element so a global mean recovery can be assumed for each element through the assayed range. In addition, Se content agreed with the expected values in spiked and non-spiked samples so a complete mineralisation was achieved with no volatilisation losses.

Detection and quantification limits.

The limits of detection were determined following the IUPAC recommendations[43], namely by assessing the standard deviation of 10 reagent blanks carried out on five different days during the analytical procedure. The limit of detection was equal to two times the Student's *t* value at the 95 % confidence level of the standard deviation. Quantification limits were calculated by multiplying the detection limits by 3.04[43]. Both limits are expressed in μg of each element per L of final diluted mineralised sample solution and per kg of sample weight (Table 7).

Few studies have been carried out on Se determination in feeds. In fact, in one such study[8], feeds and mineral mixes were analysed using an open vessel procedure and recoveries from these different matrices ranged from 74 to 120 %. Therefore, the method proposed here describes a rapid and suitable mineralisation procedure in closed vessels for Zn, Fe, Se and Cu determination in feeds. Furthermore, it overcomes the drawbacks caused by siliceous particles and volatilisation losses.

Conclusions

Our results show that methods Ia and Ib are suitable for Zn and Fe determinations, but that these mineralisation methods are not appropriate for Se quantification because of the volatilisation losses that occur.

For this reason, we proposed method II which is suitable for Zn, Fe and Se determinations. However, while the Cu contents determined with this method presented a relatively high RSD, this variability does not exceed the AOAC recommendation for the analyte concentrations determined. In addition, the results obtained for this analyte in the certified reference material are in close agreement with the certified values and showed good recovery for this element.

However, in the feed analyses described here, the presence of siliceous materials caused a decrease in measured Zn content. Thus, procedure IIIb, in which HF is added, is recommended when mineralised samples presented this precipitate.

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Table 1. Samples used for the validation of the methods

Meat and feed samples	Mineralisation procedure	Spiked	Instrumental analysis
Mixed raw dark and white chicken meat with skin 1	Ia	No	ICP-AES (Zn, Fe, Cu), HG-ICP-MS (Se)
Mixed raw dark and white chicken meat with skin 2	Ib	No	ICP-AES (Zn, Fe, Cu), HG-ICP-MS (Se)
Mixed raw dark and white chicken meat with skin 3	Ib	Na_2SeO_3 and selenomethionine	HG-ICP-MS (Se)
Mixed raw dark chicken meat with skin 1	II	No	ICP-AES (Zn, Fe), HG-ICP-MS (Se), ICP-MS (Cu)
CRM 184 (bovine muscle)	II	No	ICP-AES (Zn, Fe), HG-ICP-MS (Se), ICP-MS (Cu)
Mixed raw dark chicken meat with skin 2	II	Zn, Fe, Se, Cu standard solutions	ICP-AES (Zn, Fe), HG-ICP-MS (Se), ICP-MS (Cu)
Feed 1	IIIa	No	ICP-AES (Zn, Fe), HG-ICP-MS (Se), ICP-MS (Cu)
Feed 1	IIIb	No	ICP-AES (Zn, Fe), HG-ICP-MS (Se), ICP-MS (Cu)
Feed 2 (supplemented with organic Se from Se enriched yeast)	IIIb	Zn, Fe, Se, Cu standard solutions	ICP-AES (Zn, Fe), HG-ICP-MS (Se), ICP-MS (Cu)

Table 2. ICP-AES, ICP-MS and HG-ICP-MS operating conditions and measurement parameters

ICP-AES Perkin-Elmer model Optima 3200RL (Fe, Zn, Cu analysis)		
Sample introduction		
	"Cross flow" nebulizer	
	Auxiliary Ar flow (L/min):	0.5
	Sample flow (mL/min):	1
	Nebulizer gas flow (L/min):	0.8
Plasma		
	Alumina Injector	
	Frequency (MHz):	40
	RF power (W):	1150
	Plasma gas flow (L/min):	15
Measures		
	Background (nm):	-0.04
	Plasma height (mm):	15
	Read delay (s):	15
	Integration time (s):	2-5
	Replicates:	3
	Emission lines (nm):	
	Fe:	259.939 and 238.204
	Zn:	213.857 and 206.200
	Cu:	324.752 and 327.393
ICP-MS Perkin-Elmer model Elan 6000 (Cu analysis)		
Sample introduction		
	"Cross flow" nebulizer	
	Sample flow (mL/min):	1
	Nebulizer gas flow (L/min):	0.6
Plasma		
	Alumina Injector	
	Frequency (MHz):	40
	RF power (W):	1100
	Carrier Ar flow (L/min):	0.57
Measures		
	Scan mode:	peak hopping
	Resolution (amu):	0.7
	Replicate time (s):	1
	Dwell time (ms):	20
	Sweeps per reading:	50
	Measurements per peak:	1
	Replicates:	3
	Isotopes:	^{63}Cu ^{65}Cu ^{103}Rh
HG-ICP-MS Perkin-Elmer model Elan 6000 coupled on FIAS 400 (Se hydrides analysis)		
Hydride generation:		
	Carrier flow (mL/min):	10 (10% HCl)
	Sample volume:	200 µl
	$NaBH_4$ solution flow (mL/min):	6 (0.2% $NaBH_4$, 0.05% NaOH)
Plasma		
	Alumina Injector	
	Frequency (MHz):	40
	RF power (W):	1100
	Carrier Ar flow (L/min):	0.57
Measures		
	Scan mode:	peak hopping
	Resolution (amu):	0.7
	Replicate time (s):	12
	Dwell time (ms):	400
	Sweeps per reading:	1
	Measurements per peak:	1
	FIAS peak measurement:	area
	Reading per replicate:	30
	Replicates:	3
	Isotopes:	^{82}Se

Table 3**.** Precision of element determination (n=9) in samples of mixed dark and white chicken meat with skin after open vessel mineralisation following procedures Ia and Ib[1]

Element[2]	Procedure Ia		Procedure Ib	
	Concentration[3]	**RSD %**	**Concentration[2]**	**RSD %**
Zn (mg/kg)[4]	9.35 ± 0.39	4.2	10.28 ± 0.21	2.0
Fe (mg/kg)[4]	5.44 ± 0.11	2.0	5.02 ± 0.29	5.8
Se (µg/kg)[5]	18 ± 14	78.1	0.64 ± 0.43	67.3
Cu (µg/kg)[4]	92 ± 12	13.2	243 ± 10	4.2

[1] Chicken samples used to determine the precision of procedures Ia and Ib were different (see Table 1).

[2] Expressed in kg of wet weight.

[3] Mean ± standard deviation.

[4] Analysed by ICP-AES.

[5] Analysed by HG-ICP-MS.

Table 4. Results obtained by digestion procedure Ib and HG-ICP-MS (n=10) for a spiked raw dark and white chicken meat with skin sample (µg Se/kg) and aqueous standard solutions (µg Se/L)

Additions	Added **Found[1] / RSD %** (Recovery)	Added **Found / RSD %** (Recovery)	Added **Found / RSD %** (Recovery)
Spiked selenomethionine	0 **15 ± 12 / 82**	20 **25 ± 17 / 69** (48 %)	201 **141 ± 102 / 72** (63 %)
Spiked selenite	0 **24 ± 13 / 54**	21 **26 ± 12 / 39** (47 %)	210 **184 ± 87 / 48** (78 %)
Aqueous selenomethionine solution		100 **36 ± 11 / 30** (36 %)	
Aqueous sodium selenite solution		105 **38 ± 5 / 12** (37 %)	

[1] Mean ± standard deviation.

Table 5. Digestion procedure II: precision of element determination in mixed raw dark chicken meat with skin (n=10); certified and found values (n=10) with its recovery in the certified reference material CRM 184 (bovine muscle)

Element[1]	Chicken meat		CRM 184			
	Concentration[2]	RSD %	Certified[2]	Found[3]	RSD %	Recovery
Zn (mg/kg)[4]	13.99 ± 0.36	2.5	166.29 ± 6.3	172.68 ± 1.0	1.7	104
Fe (mg/kg)[4]	7.28 ± 15	2.0	78.87 ± 3.0	78.87 ± 0.4	1.6	100
Se (µg/kg)[5]	305.4 ± 9.4	3.1	183 ± 18	169 ± 3	5.0	92
Cu (µg/kg)[6]	400 ± 44	11.1	2,357 ± 87	2,541 ± 66	7.3	107

[1] Expressed in kg of wet weight.

[2] Mean ± standard deviation.

[3] Mean ± standard error.

[4] Analysed by ICP-AES.

[5] Analysed by HG-ICP-MS.

[6] Analysed by ICP-MS.

Table 6**.** Recoveries (n=10) in spiked (standard solutions) raw dark chicken meat with skin samples following the digestion procedure II

Element[1]	Added **Found[2] / RSD %** (Recovery)	Added **Found / RSD %** (Recovery)	Added **Found / RSD %** (Recovery)	Added **Found / RSD %** (Recovery)
Zn (mg/kg)[3]	0 **14.23 ± 0.43 / 3.0**	14.0 **28.01 ± 0.42 / 1.5** (103%)	28.0 **42.59 ± 1.44 / 3.4** (105%)	56.0 **71.11 ± 1.03 / 1.4** (105%)
Fe (mg/kg)[3]	0 **6.88 ± 0.50 / 7.3**	8.0 **15.07 ± 1.03 / 6.8** (108%)	16,0 **23.42 ± 0.52 / 2.2** (107%)	32.0 **39.83 ± 0.72 / 1.8** (107%)
Se (µg/kg)[4]	0 **284 ± 6 / 2.2**	333 **595 ± 22 / 3.7** (97%)	667 **922 ± 28 / 3.0** (99%)	1,333 **1,575 ± 25 / 1.6** (100%)
Cu (µg/kg)[5]	0 **443 ± 57 / 13.1**	333 **736 ± 36 / 4.9** (91%)	667 **1,017 ± 43 / 4.2** (89%)	1,333 **1,657 ± 47 / 2.8** (94%)

[1] Expressed in kg of wet weight

[2] Mean ± standard deviation.

[3] Analysed by ICP-AES.

[4] Analysed by HG-ICP-MS.

[5] Analysed by ICP-MS.

Table 7**.** Limits of detection (L_D) and quantification (L_Q) for element determination, expressed as µg / L of final mineralised sample solution and µg / kg of sample amount

Element	Digestion method							
	Procedure II				Procedure IIIb			
	L_D		L_Q		L_D		L_Q	
	µg / L	µg / kg	µg / L	µg / kg	µg / L	µg / kg	µg / L	µg / kg
Zn	6	985	18	2,996	5	2,585	14	7,861
Fe	12	2,029	37	6,170	9	5,137	29	15,616
Se	0.08	27.30	0.25	82.99	0.07	70.41	0.20	214.06
Cu	0.41	67.88	1.24	206.36	0.17	183.97	0.52	559.27

Table 8. Precision of element determination (n=8) in a feed sample after digestion following procedures IIIa and IIIb[1]

Element[1]	Procedure IIIa HNO_3-H_2O_2		Procedure IIIb HNO_3-H_2O_2-HF	
	Concentration[2]	RSD %	Concentration	RSD %
Zn (mg/kg)[3,6]	258.7 ± 10.2	3.9	282.9 ± 5.2	1.8
Fe (mg/kg)[3]	272.2 ± 4.4	1.6	279.6 ± 8.2	2.9
Se (µg/kg)[4]	131.7 ± 4.0	3.0	128.5 ± 3.9	3.0
Cu (mg/kg)[5]	9.2 ± 0.4	4.6	9.3 ± 0.7	7.7

[1] Expressed in kg of wet weight.

[2] Mean ± standard deviation.

[3] Analysed by ICP-AES.

[4] Analysed by HG-ICP-MS.

[5] Analysed by HG-MS.

[6] Student-Fisher's *t* test ($P \leq 0.05$) showed significant differences between digestion methods.

Table 9. Recoveries (n=8) in spiked (standard solutions) feed samples following the digestion procedure IIIb

Element[1]	Added **Found[2] / RSD %** (Recovery)	Added **Found / RSD %** (Recovery)	Added **Found / RSD %** (Recovery)	Added **Found / RSD %** (Recovery)
	0	560	1120	1866
Zn (mg/kg)[3]	**589 ± 20 / 3.4**	**1158 ± 51 / 4.4** (100%)	**1682 ± 62 / 3.7** (98%)	**2421 ± 26 / 1.1** (99%)
	0	170	340	680
Fe (mg/kg)[3]	**175.3 ± 7.7 / 4.4**	**352.3 ± 12.2 / 3.5** (103%)	**516.1 ± 16.8 / 3.2** (101%)	**862.8 ± 35.4 / 4.1** (103%)
	0	590	1180	2360
Se (µg/kg)[4]	**580 ± 13 / 2.3**	**1047 ± 22 / 2.1** (90%)	**1550 ± 26 / 1.7** (88%)	**2502 ± 37 / 1.5** (87%)
	0	22	44	88
Cu (mg/kg)[5]	**15.6 ± 2,7 / 17.7**	**38,0 ± 4,1 / 10.8** (101%)	**64,2 ± 5,3 / 8.3** (108%)	**103,5 ± 6,8 / 6.6** (100%)

[1] Expressed in kg of wet weight.

[2] Mean ± standard deviation.

[3] Analysed by ICP-AES.

[4] Analysed by HG-ICP-MS.

[5] Analysed by ICP-MS.

1.2. Effect of Dietary Fish Oil, and α-Tocopherol Acetate, and Zinc Supplementation on the Composition and Consumer Acceptability of Chicken Meat

Títol: Efecte de l'addició d'oli de peix en els pinsos, i de la suplementació amb acetat de α-tocoferol i zinc en la composició i l'acceptabilitat sensorial de la carn de pollastre.

Resum: Emprant un disseny factorial es va estudiar l'efecte de l'addició en els pinsos d'oli de peix (1,25 o 2,5%), acetat d'α-tocoferol (70 o 140 mg/kg) i Zn (0 o 200 mg/kg) sobre la composició i l'acceptabilitat sensorial de la carn de pollastre emmagatzemada a -20 ºC durant diversos períodes de temps.

La suplementació amb acetat d'acetat d'α-tocoferol incrementa el contingut en α-tocoferol en la carn. La composició en àcids grassos de la carn només va ser afectada per la quantitat d'oli de peix de la dieta. Les dietes que aportaven un 2,5% d'oli de peix donaven lloc a carns amb un contingut en EPA i DHA que doblava el contingut d'aquelles carns que provenien de dietes amb una dosi d'oli de peix del 1,25%. Per contra, la suplementació amb Zn no va afectar el contingut d'aquest metall en la carn.

A més, l'acceptabilitat sensorial de les mostres de carn després de 5 mesos d'emmagatzematge a -20 ºC no va mostrar diferències significatives entre les diferents dietes assajades i tampoc en relació a una mostra comercial recent cuita emprada com a control cec.

EFFECT OF DIETARY FISH OIL, AND α-TOCOPHERYL ACETATE AND ZN SUPPLEMENTATION ON THE COMPOSITION AND CONSUMER ACCEPTABILITY OF CHICKEN MEAT

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Abbreviation Key: DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid; α-TA = α-tocopheryl acetate; TBA = 2-thiobarbituric acid.

ABSTRACT

A factorial design was used to study the effect of dietary fish oil (1.25% and 2.5%), dl-α-tocopheryl acetate (70 and 140 mg/kg) and Zn supplementation (0 and 200 mg/kg) on the composition and consumer acceptability of chicken meat stored at – 20ºC for 5 mo. Supplementation with dl-α-Tocopheryl acetate increased the α-tocopherol content in meat. The fatty acid composition of the meat was only affected by the amount of fish oil. Diets supplied with 2.5% fish oil produced meat with an eicosapentaenoic and docosahexaenoic acid content double that of diets supplied with 1.25% fish oil. Zn supplementation did not affect the content of this mineral in the meat. Moreover, the consumer acceptability of meat samples showed no significant differences between dietary treatments after five mo of storage at –20ºC, nor with respect to a freshly cooked commercial sample, used as a blind control.

Key Words: Dietary fish oil, α-tocopheryl acetate supplementation, zinc supplementation, chicken meat composition, consumer acceptability

1.- INTRODUCTION

Several oils are used to enrich poultry and eggs with n-3 polyunsaturated fatty acids (n-3 PUFA) because of their beneficial effects on health. Linoleic acid and linolenic acid can not be synthesized by animals and tissue concentrations respond rapidly to dietary changes (Wood and Enser, 1997). Because fish oils have a high amount of C20:5 n-3 (eicosapentaenoic acid, EPA) and C22:6 n-3 (docosahexaenoic acid, DHA), their addition to animal feed is more efficient than are vegetable fat sources in obtaining a long-chain n-3 PUFA enrichment in meat. However, a higher PUFA content of poultry meat increases the degree of unsaturation and, as a result, also increases the susceptibility to oxidation. This may then lead to off-flavours and off-odours and, consequently, lower consumer acceptability. The use of fish oils at concentrations above 2% in poultry diets may entail several sensory problems that compromise meat quality (Hargis and van Elswyck, 1993).

Supplementation with tocopherols or α-tocopheryl acetate (α-TA) increases the α-tocopherol content of chicken tissues (Cherian et al., 1996; de Winne and Dirinck, 1996; Lauridsen et al., 1997; Galvin et al., 1998; O'Neill et al., 1998; Surai and Sparks, 2000). Given the potential health effects described for α-tocopherol (Christen et al., 2000; Food and Nutrition Board, 2000; Pryor, 2000; Bron and Asmis, 2001), enriched poultry meat could be considered a useful source of this vitamin in the human diet.

Moreover, the antioxidant function of tocopherols in poultry meat prevents the formation of primary (Grau et al., 2001a) and secondary (Lin et al., 1989; Sheehy et al., 1993; Jensen et al., 1995; King et al., 1995; Cherian et al., 1996; Lauridsen et al., 1997; O'Neill et al., 1999; Grau et al., 2001a, b) oxidation products, and total volatiles (Ajuyah et al., 1993a; de Winne and Dirinck, 1996).

Meat products are also one of the main sources of dietary Zn (Subar et al., 1998; Ma and Betts, 2000; Terrés et al., 2001). Furthermore, this element shows a high bioavailability in meat products (Hortin et al., 1993; Lonnerdal, 2000). However, despite the fact that the Recommended Dietary Allowances for Zn have recently been lowered (Food and Nutrition Board, 2001), several developed and undeveloped

countries have daily intakes slightly higher or lower than these recommendations (Buss and Rose, 1992; Hussein and Bruggeman, 1997; Brown et al., 2001; Terrés et al., 2001). Recently, a meta-analysis of several randomized controlled trials carried out in both developed and undeveloped countries in order to study the effect of Zn supplementation in pre-pubertal children concluded that Zn supplementation has a significant positive influence on height and weight increments, especially for underweight children or when stunting occurs (Brown et al., 2002). In addition, several studies carried out in developed countries have shown that elderly people are at greater risk of inadequate Zn intake, which has been related to an impaired immune response in this group (Girodon et al., 1999; High, 1999; de Jong et al., 2001; Savarino et al., 2001). In fact, Zn deficit has long been known to lead to cell-mediated immunodeficiency in children and elderly people (reviewed by Lesourd, 2001). Therefore, Zn enriched meat products are of potential interest, even in developed countries.

Here, we assess the possibility of obtaining chicken meats enriched in n-3 PUFA, α-tocopherol and/or Zn, and we study the oxidative stability and consumer acceptability of these meats.

2.- MATERIAL AND METHODS

Diets and Birds

Treatments were prepared from two basal diets (Table 1) in order to study the effect of various dietary factors on α-tocopherol and Zn contents, fatty acid composition, consumer acceptability scores and TBA values of dark and white chicken meat. Up to 3 wk of age, four different treatments were supplied to the chickens, corresponding to two factors (Table 2): two doses of α-TA (70 and 140 mg/kg) and two doses of Zn supplementation (0 and 200 mg/kg). From 3 to 6 wk, the above treatments were sub-divided introducing a new factor (Table 2), the percentage of fish oil (1.25% and 2.5%), and total added fat was completed up to 5.6% with animal fat so that all the treatments were isocaloric. This experiment was repeated 6 times. Fish oil

was supplied by FF of Denmark[1]. Zinc sulfate was purchased from Andrés Pintaluba, S.A.[2] and α-TA from Hoffman-La Roche[3]. Two hundred eighty eight female broiler chicks (Ross, 1-d-old) were randomly assigned to the dietary treatments (36 chicks per treatment, 6 birds per pen) and were fed ad libitum for 42 d.

Preparation, Cooking and Storage of Samples

The chickens were slaughtered according to commercial procedures and were stored for 4 h at 4ºC. Then, carcasses from each pen were longitudinally cut and divided into two groups (left and right sides). After that, in order to study the composition and nutritional value of the whole chicken meat, the right breasts and legs with skin from each pen were used. These samples were hand-deboned, mixed, ground (in average 41% of white meat, 36% of dark meat and 23% of skin), vacuum packed in high-barrier multilayer bags (approx. 15 g per bag) and immediately stored at – 20ºC until analysis (α-tocopherol and Zn content, and fatty acid composition). On the other hand, because of the higher oxidation susceptibility of legs, due to its higher Fe and fat content, only legs from the left sides were used to study the consumer acceptability. Thus, legs with skin were hand-deboned, vacuum-packed and stored at 4ºC. After 12 h being slaughtered, samples were cooked in an oven at 85ºC (99% relative humidity) to an internal temperature of 80ºC. Samples were then stored at – 20ºC until the consumer acceptability and 2-thiobarbituric acid (TBA) values were analyzed.

Reagents and Standards

Butylated hydroxytoluene (BHT), pyrogallol and dl-α-tocopherol were supplied by Sigma[4]. The methanol and ethanol (96%) used in α-tocopherol analysis were of HPLC grade. Extraction reagents for fatty acid determination were of ACS grade. The acids used to determine Zn content were of ACS grade. The stock solution

[1] FF of Denmark, Skagen, Denmark, DK-9990
[2] Andrés Pintaluba, S.A., Reus, Spain, E-43280
[3] Hoffmann-La Roche Ltd., Basel, Switzerland, CH-4070
[4] Sigma, St. Louis, MO 63103

of Zn, which was traceable to the Standard Reference Materials of NIST, was purchased from SCP Science[5].

Determination of α-Tocopherol

Two g of sample (mix of dark and white raw chicken meat plus skin) was homogenized using a polytron PT 2000[6] for 30 s at 19,800 rpm with 5 mL of absolute ethanol containing 1% pyrogallol (wt/vol), 0.012% BHT (wt/vol) and 0.4% anhydrous citric acid (wt/vol). For feed analysis 1.5 g of milled feed was homogenized as described above with 10 mL of absolute ethanol containing 0.6% pyrogallol (wt/vol), 0.008% butylated hydroxytoluene (wt/vol) and 0.26% anhydrous citric acid (wt/vol). Ten mL of 1.6 N methanolic KOH for meat and 10 mL of 2.2 N methanolic KOH for feed was then added and saponification was carried out at 70ºC for 30 min. Non-saponifiables were then extracted with petroleum ether and filtered through a 0.45 μm teflon membrane. After solvent evaporation under a nitrogen stream at 30ºC the residue was redissolved in 96% ethanol. Chromatographic separation of this solution was performed using a Hewlett Packard liquid chromatograph Series 1100[7] equipped with a Rheodyne model 7725i injector[8], with a final loop volume of 20 μL, and a column (15 x 0.4 cm) packed with 3 μm - 80 Å Extrasil ODS2 and a pre-column (1 x 0.4 cm) packed with 5 μm - 100 Å Kromasil ODS[9]. Sample compounds were isocratically eluted with methanol and detected through a 1046A Hewlett Packard spectrofluorometric detector[10] (excitation and emission wavelengths of 288 and 330 nm, respectively). α-Tocopherol content was determined by means of an experimental calibration curve, using α-tocopherol as the external standard.

[5] SCP Science, Courtaboeuf, France, F-91965
[6] Kinematica, Lucerne, Switzerland, CH-6014
[7] HP Series 1100 liquid chromatograph, Hewlett-Packard GmbH, Waldbronn, Germany, D-76337
[8] Rheodyne manual injector, Cotati, CA 94931
[9] HPLC column and pre-column, Teknokroma, Sant Cugat del Vallés, Spain E-08190
[10] Shimadzu UV-1046 A spectrofluorometric detector, Hewlett-Packard GmbH, Waldbronn, Germany, D-76337

Determination of Fatty Acid Composition

One g of meat sample (mix of dark and white raw chicken meat plus skin) or 3 g of milled feed were weighed into 32 x 210 mm tubes. Twenty mL of chloroform/methanol (2:1, vol/vol) was then added and the mixture was homogenized for 40 s at 19,800 rpm using a Polytron PT 2000. Extracts were filtered through Whatman nº1 filter paper into 50 mL screw-capped tubes and the residues were re-extracted twice with the same solvent: first with 7 mL (30 s at 19,800 rpm) and then with 5 mL (10 s at 19,800 rpm). Ten mL of water was then added to these tubes and they were stoppered and shaken for 30 s before being centrifuged for 20 min at 500 *g*. The chloroform phase was filtered through anhydrous sodium sulfate (using a Whatman nº1 filter paper), which was then washed twice with 5 mL of chloroform. The lipid extract obtained was concentrated to 1 mL in a vacuum rotatory evaporator at 35ºC and the rest of the solvent was removed in a light nitrogen stream, and storing the flask in a vacuum desiccator (10 mm Hg overnight). Fatty acid methyl esters were prepared from the extracted lipid fraction and determined following the method described by Guardiola et al. (1994).

Determination of Zn

One and a half g of sample (mix of dark and white raw chicken meat plus skin) or 0.7 g of milled feed was weighed into a pyrex digestion tube (250 mL). Thirty mL of nitric acid (65%) was then added and the tube, which was connected to a condenser, was placed into a digestion block and left 14 h at 60ºC under reflux. Digestion continued by heating at 120ºC for 1 h. The condenser was then removed and the mixture evaporated to 5 mL by heating at 140ºC. The digestion tube was then cooled, 5 mL of perchloric acid was added and it was heated to 220ºC for 30 min with the condenser. Finally, the condenser was removed again and the solution was evaporated at 240ºC to dryness for meat, and to 2 mL for feeds. The digested samples were transferred into 10 mL and 20 mL volumetric flasks, respectively, and diluted to volume using deionized water.

A Polyscan 61E[11] inductively coupled plasma atomic emission spectrometer (ICP-AES) was used to determine Zn concentration. Instrumental measurement conditions were: wavelength, 213.856 nm; background, –0.04 nm; plasma height, 15 mm; power, 1150 W; auxiliary argon flow, 0.5 L/min; plasmogen argon flow, 16 L/min; and burgener nebulizer pressure, 42 psi. A Zn stock solution of 1001 ± 5 mg/L (from Zn metal dissolved in nitric acid, final matrix 4%) was used to provide standard calibration solutions.

Sensory Analysis

Two consumer panel tests were carried out, the first 15 d after the leg with skin samples were cooked (samples were stored at –20ºC until sensory analysis) and the second after 5 mo of storage at –20ºC. Twenty-seven consumer panelists were used in each test. They were selected from our department and all had experience in poultry meat sensory analysis. Criteria for selection were: age between 20-50 years, not allergic to chicken, consumption of chicken at least once per wk and willingness to evaluate meat from chickens fed experimental diets.

Vacuum-packed cooked chicken legs were thawed by heating for 20 min at 35ºC in a water bath. The bags were then opened and chicken pieces (20 g each), with a similar amount of skin, were placed in screw-capped flasks. They were heated at 75ºC for 20 min in a conventional air oven and served to the panelists.

Samples were identified by random 3-digit numbers and all dietary treatments were presented to the consumer panelists in one session. They were asked to rank the acceptability of the product using a 9-point scale (1 = very bad; 9 = very good). Water and unsalted crackers were provided to cleanse their palates between samples.

Determination of 2-Thiobarbituric Acid Values

Vacuum-packed cooked chicken legs with skin were thawed by heating in a water bath at 35ºC for 20 min and were then ground. Two g of ground cooked dark

[11] Thermo Jarrell Ash, Franklin, MA 02038

meat was then weighed for analysis. Values of TBA were measured through a third derivative spectrophotometry method after acid aqueous extraction (Grau et al., 2000).

Determination of fat content

Fat content of the mixtures of dark and white raw chicken meat plus skin coming from all the experimental treatments was determined by the AOAC official method 991.36.

Statistical Analysis

Multifactor ANOVA (MANOVA) was used to determine whether any significant effects were produced by the factors studied on α-tocopherol content, Zn content, fat content and fatty acid composition of the mixed dark and white raw chicken meat, as well as on the consumer acceptability and TBA values of cooked dark meat. Interactions between factors higher than an order of two were ignored. Least-squares means for the main factors which had a significant effect were separated using Scheffé's test. In all cases, $P \leq 0.05$ was considered significant.

3.- RESULTS AND DISCUSSION

α-Tocopherol content

Dietary supplementation with α-TA (70 vs 140 mg/kg of feed) increased ($P < 0.001$) the α-tocopherol content of mixed dark and white raw chicken meat from 2.06 to 2.81 mg/100 g of edible portion (Table 3). Similar results have been reported previously for raw (King et al., 1995; Cherian et al., 1996; de Winne and Dirinck, 1996; Lauridsen et al., 1997; O'Neill et al., 1998; Grau et al., 2001a) and cooked (Ahn et al., 1995; Galvin et al., 1998; Grau et al., 2001a) chicken meat.

The α-tocopherol content was not affected by the percentage of fish oil added to the feed (Table 3). Surai and Sparks (2000) reported that the α-tocopherol content of various chicken tissues decreased when animals were fed with 3% tuna oil, compared

with the control (corn oil). These authors observed a significant decrease in pancreas, kidney, spleen, lung, heart, testes and liver, but not in muscle and internal fat. Thus, the authors recommended increasing α-TA supplementation when oils rich in PUFA were added to feed in order to diminish increased tissue susceptibility to lipid oxidation. Miller and Huang (1993) also reported that breast and thigh α-tocopherol content was reduced by 1% or 2% dietary fish oil, compared with 1% soya oil, when diets were supplemented with α-TA at 250 or 450 mg/kg, but not when supplemented with α-TA at 50 mg/kg. However, the decrease in α-tocopherol content observed for the 2% fish oil diet, compared with the 1% fish oil diet, was not statistically significant.

Moreover, Lin et al. (1989) observed that the addition to feed of oils at 5.5% with a different degree of unsaturation (coconut, olive, linseed and partially hydrogenated soybean oils), but without α-TA supplementation, had little effect on the α-tocopherol content of dark and white chicken meat. However, the same research team (Asghar et al., 1990), assaying the same oils under the same conditions, subsequently reported that the α-tocopherol concentration in the microsomes from dark meat, but not from white meat, was influenced to some extent by the nature of the dietary oil, the linseed oil treatment showing the lowest values.

In addition, McGuire et al. (1997) found, when feeding rats experimental diets containing various fat sources (lard, menhaden oil, sardine oil and cod liver oil) and the same amount of α-tocopherol (150 mg/kg), that cod liver oil and menhaden oil, having a similar n-3 PUFA profile, provoked different responses in plasma and liver α-tocopherol concentrations. In relation to these and other results where different sources of oils were used, several factors can affect the α-tocopherol content in chicken meat, namely, the intrinsic tocopherol content and the oxidative quality of the dietary oils used (Sheehy et al., 1993; Sheehy et al., 1994; Jensen et al., 1997), and the levels of other natural compounds present in the feed, such as β-carotene and liposoluble vitamins, which may interact with tocopherol deposition (Abawi and Sullivan 1989; Ruiz et al., 1999).Thus, further studies monitoring the anti-oxidant and pro-oxidant content of the feed ingredients, especially the fat sources, in the experimental diets are

required in order to better understand the effect of dietary fats on the α-tocopherol content in different tissues.

Zn supplementation did not affect the α-tocopherol content.

Fatty acid composition

The fatty acid composition of feed and mixed raw chicken meat is shown in Table 4. Previous studies have described how the fatty acid composition of chicken meat depends on the fatty acid profile of the feed (Ajuyah et al., 1991; Cherian et al., 1996). Several studies report that dietary supplementation with long-chain n-3 PUFA increases the content of these fatty acids in poultry meat (Sklan et al., 1983; Scaife et al., 1994; Cherian et al., 1996; López-Ferrer et al., 1999; López-Ferrer et al., 2001). In our experiment, the doses of fish oil added to the feed (1.25% and 2.5%) three wk before slaughter modified this composition, particularly long-chain n-3 PUFA (Table 4). The total saturated fatty acid (SFA) content remained constant while the total monounsaturated fatty acid (MUFA) content was lower when chickens were fed the 2.5% fish oil diet. In relation to the MUFA content, this effect can be explained by the lower content in oleic acid in the 2.5% fish oil diet. Although the total n-6 PUFA were not affected by the fish oil doses, arachidonic acid content was clearly higher in chickens fed the 2.5% fish oil diet. This could be explained by the higher content of this fatty acid in this diet. The n-3 PUFA were the most clearly affected by the fatty acid composition of the feed. Regarding EPA and DHA content in the meat, we observed that the 2.5% fish oil diet gave close to 2-fold the value found at the 1.25% dose. Therefore, the efficiency of deposition seemed to be proportional to the diet's fish oil content at the assessed doses.

An increase in the PUFA content of feeds led to an increase of these fatty acids in meat (2.5% vs 1.25% of fish oil). However, the ratio unsaturated/saturated fatty acids did not change, since the MUFA content was lower in meat from the 2.5% fish oil treatments. These findings may support the hypothesis that there is a homeostatic mechanism in the membranal lipid fraction which maintains a relatively constant state of membrane fluidity (Asghar et al., 1990).

Neither Zn nor α-TA supplementation altered the fatty acid composition of meat. This observation regarding α-TA supplementation is consistent with that of O'Neill et al. (1998), who found that supplementation with α-TA at 200 mg/kg did not affect fatty acid composition of dark and white chicken meat after treatments with tallow or olive oil at 6%. In addition, Lin et al. (1989) observed no effect of α-TA supplementation (100 mg/kg) on the fatty acid composition of neutral lipids from dark and white meat of broilers fed hydrogenated soybean oil at 5.5%.

In contrast, in their study of chickens fed for 62 wk with 3% tuna oil diets, Surai and Sparks (2000) found an increase in DHA in thigh when chickens were supplemented with 160 mg/kg of α-TA, although this was only significant in the phospholipid fraction. These authors also reported similar results in the phospholipid fraction of testes, cerebellum and heart.

Ajuyah et al. (1993a) reported similar results in cooked chicken meat. These authors found that EPA and DHA increased in the total lipid fraction of cooked white meat from broilers fed a 15% full-fat flax seed diet supplemented with mixed tocopherols at 200 mg/kg. In addition, the inclusion of mixed tocopherols produced white and dark meat with significantly higher proportions of total n-3 PUFA in the triacylglycerol and phospholipid fractions (Ajuyah et al., 1993a).

Furthermore, Cherian et al. (1996), in laying hens fed menhaden oil (3.5%), reported an increase in EPA and DHA in adipose tissue and in white meat, but not in dark meat, when they were supplemented with mixed tocopherols (350 mg/kg). This finding could be related to the fact that the phospholipid/triacylglycerol ratio is higher in breast than in thigh. In addition, these authors proposed that these n-3 PUFA were protected from deterioration by tocopherol supplementation.

Therefore, the effect of tocopherol or α-TA supplementation on long-chain PUFA composition needs further study, since our results seem to disagree with those of some authors. However, these authors used higher doses of fish oil and compared diets supplemented with tocopherols or α-TA with non-supplemented diets, whereas in our study the comparison is made between treatments supplemented at different levels of α-TA (70 vs 140 mg/kg) and only the fatty acid composition of the total lipid fraction of mixed dark and white raw chicken meat was studied.

Zn content

Zn supplementation did not affect the content of this mineral in mixed dark and white raw meat, expressed on a wet basis (Table 3). Zn content in chicken thigh is known to be higher than in breast (Emmert and Baker, 1995; Leonhardt and Wenk, 1997; Mavromichalis et al., 2000). Our results (approx. 1 mg/100 g of edible portion) are similar to those reported by other authors (Chan et al., 1995; Scherz and Senser, 2000) when using a mixture of edible portions of whole chickens. Unlike what happens in humans, where about 90% of total body Zn content is in skeletal muscle and bone, in chickens this value is only 55% (Mavromichalis et al., 2000). In addition, these authors found that about 31% of total body Zn is present in skin and feathers.

Some food composition tables show a slight variability of Zn in distinct meat cuts (Leonhardt and Wenk, 1997; Scherz and Senser, 2000) and cooked meats (Pennington et al., 1995), which could be due to various factors (such as diet, genetics or age). Leonhardt and Wenk (1997) studied the content of various trace elements and vitamins in several meat cuts (pork, beef, veal and chicken) and observed that Zn content in chicken breast and thigh was the nutrient with the lowest coefficient of variation. The authors attributed these results to the fact that the content of this mineral in meat is mainly genetically determined and is only slightly affected by feed composition.

Although the requirements for chick growth are satisfied when chicks are fed diets containing 40 mg Zn/kg (National Research Council, 1994), the Zn concentration of egg yolk increases when high supplements of this mineral (5 to 20 g/kg) are added to the feed (Williams et al., 1989; Verheyen et al., 1990). In addition, it has been demonstrated that this mineral also accumulates in different chicken tissues, such as liver, kidney, pancreas and spleen, when short-term high doses (0.5 to 20 g/kg; 4 d to 3 wk) are added to the feed (McCormick and Cunningham, 1987; Williams et al., 1989; Emmert and Baker, 1995; Sandoval et al., 1998). Moreover, short-term high-level supplements (20 g/kg) reduced feed consumption and body weight, thereby inducing forced rest in laying hens (McCormick and Cunningham, 1987; Williams et al., 1989).

In our experiment, Zn supplements (200 mg/kg) did not lower feed consumption or body weight in 42-d-old chickens (data not shown). Similar results were found in 3-wk-old chickens fed 170 mg/kg Zn supplements from d 5 to 21

(Mohanna and Nys, 1999). Conversely, Sandoval et al. (1998) observed that chicken body weight decreased with increasing dietary Zn (0, 500, 1000 and 1500 mg/kg of feed) at all ages studied (1-, 2- and 3-wk-old chickens).

In addition, these authors (Sandoval et al., 1998) observed that increasing dietary Zn supplementation resulted in higher Zn concentrations in the bone, liver, kidney and muscle studied at 1, 2 and 3 wk of age. However, the increase was less marked in muscle than in the other samples. In addition, the concentration of this mineral in muscle, as in the majority of tissue mineral concentrations, decreased from 1 to 3 wk of age and at 3 wk a poor regression between muscle Zn content and dietary Zn supplementation was observed. In relation to these results, Mohanna and Nys (1998) found that body Zn concentration in whole chickens, including feathers, supplemented with Zn (> 100 mg/kg) changed mainly with age. The higher body Zn concentrations were observed at 4 and 11 d of age and these concentrations were lower and stable from 21 to 50 d of age.

Subsequently, Mohanna and Nys (1999) reported that the whole body Zn concentration of 21-d-old chickens was significantly lower in birds receiving 40 mg/kg of Zn supplementation than in birds receiving 170 mg/kg. These authors also found that when dietary Zn content was greater than the requirements for growth, an increase in the plasma and tibia concentrations of Zn was observed up to dietary concentrations of 75 mg/kg of feed.

In addition, Emmert and Baker (1995) reported that experimental diets with different Zn content (37 vs 1,037 mg/kg) fed to 1-d-old chicks for 7 d affected Zn concentration in samples of tibia, liver, small intestine and skin plus feathers, but not in dark and white chicken meat. These authors obtained similar results when 2-d-old chicks were fed experimental diets with a different Zn content (10.6 vs 300 mg/kg) for 8 d.

From these results it can be concluded that dietary Zn affects the content of Zn in muscles less than in other chicken tissues such as bone, liver, kidney, small intestine and skin plus feathers. This lack of effect of Zn supplementation on muscle tissue Zn content could be supported by the homeostatic control of the body metabolism of this mineral, which involves a balance between absorption of dietary zinc and endogenous

secretions regulated by adaptation of reserves, this being determined by the dietary zinc supply (Cousins, 1996).

Therefore, our results showing that Zn supplementation (200 mg/kg as $ZnSO_4$) for 41 d did not affect the content of this mineral in muscle meat coming from 42-d-old chickens are consistent with the results described above. However, to our knowledge, the present study is the first which attempts to assess the effect of long-term Zn supplementations on concentrations of this mineral in muscle tissues.

In our study, neither α-TA supplements nor the dose of added fish oil affected Zn content.

Sensory analysis and TBA values

Two consumer tests were conducted to evaluate the consumer acceptability of the cooked leg samples. The tests were done 15 d after slaughtering and again after 5 mo of storage at –20ºC. After 15 d of storage at –20ºC, consumer acceptability was not affected by the factors studied (Table 5). Despite this, the highest difference in acceptability was detected when doses of fish oil were compared. This finding indicates that fish oil dose is probably a major contributor to acceptability scores. The TBA values measured after sensory analysis are also consistent with sensory quality, which have previously been described to be inversely correlated (Salih et al., 1987; Nolan et al., 1989; Ang and Lyon, 1990; Spanier et al., 1992; Mielche, 1995; Bou et al., 2001). As with consumer acceptability, no differences were found for the TBA values between the factors studied.

Consumer acceptability of cooked leg samples was evaluated again after 5 mo of frozen storage. Similarly to the previous consumer test, carried out after 15 d of storage, none of the factors studied showed a significant effect on the consumer acceptability of samples (Table 5). However, at this storage time, the dose of fish oil seemed to have a negative effect on acceptability (almost significant, $P = 0.0993$).

Despite the higher consumer acceptability scores found after meat storage for 5 mo at – 20ºC, compared to meat stored for 15 d at – 20ºC, these differences were not statistically significant and could be explained by the lack of repeatability between consumers at different working sessions. In addition, at 5 mo of storage a freshly

cooked commercial chicken meat sample stored for 1 d at – 20ºC was added to the consumer test as a blind control. The mean for the control was 4.7, a score that did not differ from the experimental samples. The low scores given by the consumers for this sample, and for the samples from the experimental treatments, can be explained by the lack of salt and seasoning.

None of the factors studied influenced TBA values, although the higher dose of fish oil corresponded to higher TBA values. The slight relationship between consumer acceptability and TBA values followed a similar pattern to that of the first sensory test. The α-TA supplements did not reduce the TBA values during long-term frozen storage, thereby indicating that under these conditions the 70 mg/kg of α-TA supplement offers a similar protection to the 140 mg/kg dose.

For both storage times, the two α-TA supplements produced no differences for the TBA values and consumer acceptability scores, thus both supplements can be said to give similar protection against oxidation. Despite this, when chicken were fed a diet supplemented with 140 mg/kg of α-TA their meat provided, per 100 g of edible portion, 19% of the Recommended Dietary Allowances (Food and Nutrition Board, 2000) for tocopherol, while when supplemented with 70 mg/kg it provided 14% (Table 6).

The oxidative stability of our samples is in agreement with the results of other authors; studies of α-TA supplementation have shown, compared with a control, an enhanced stability in raw chicken meat (Lin et al., 1989; Bartov and Frigg, 1992; Sheehy et al., 1993; Jensen et al., 1995; Lauridsen et al., 1997; O'Neill et al., 1998; Grau et al., 2001a, b), even when fish oil was added to the diet supplemented with mixed tocopherols (Cherian et al., 1996). The protective effect of tocopherol or α-TA supplementation has also been observed in cooked chicken meat (Ajuyah et al., 1993a; Jensen et al., 1995; Galvin et al., 1998; O'Neill et al., 1998; Bou et al., 2001; Grau et al., 2001a, b). In addition, Grau et al. (2001a) observed that α-TA supplementation (225 mg/kg) reduced the oxidation values (lipid hydroperoxides, TBA and cholesterol oxidation products) of raw and cooked dark chicken meat from broilers fed different fat sources (beef tallow, sunflower oil, oxidized sunflower oil or linseed oil). In addition, Ajuyah et al. (1993b) observed that the rancid flavour of white and dark cooked meat from broilers fed a 15% full-fat flax seed diet decreased when the feed

was supplemented with mixed tocopherols at 200 mg/kg. Furthermore, α-TA supplementation (200 mg/kg) also improved the fresh flavour when cooked samples were compared with the control (de Winne and Dirinck, 1996). More recently, Bou et al. (2001) observed that the rancid aroma and flavour of cooked dark chicken meat stored for 13 mo at –20ºC were lower, and consumer acceptability was higher, for meats from α-TA-supplemented treatments (225 mg/kg vs 0 mg/kg), regardless of the dietary fat source assayed (beef tallow, sunflower oil, oxidized sunflower oil or linseed oil). However, these authors compared diets supplemented with higher doses of tocopherols or α-TA (200-225 mg/kg) with non-supplemented diets, whereas in our study the comparison is made between treatments supplemented at different α-TA levels (70 vs 140 mg/kg), that is why in our case oxidative stability is high and similar for all treatments.

As high doses of fish oil or fish meal have been shown to increase long chain n-3 PUFA and reduce the sensory quality of meat (Ajuyah et al., 1993b; de Winne and Dirinck, 1996; Sheldon et al., 1997; O'Neill et al., 1998), tocopherol supplementation was used to prevent the deterioration of flavour in poultry. However, few studies have been devoted to identifying the optimum dose of fish oil supplements which would not lower meat quality. Replacement of highly unsaturated diets by diets with a low content in unsaturated fatty acids, a few wk before slaughter, has been reported to reduce the adverse effects on sensory quality (Hargis and van Elswyk, 1993; López-Ferrer et al., 1999). However, few studies have compared the addition of two doses of fish oil during the three wk prior to slaughtering on consumer acceptability of the meat.

Despite the fact that a slight fish taste was detected by some consumers, especially in meat coming from the 2.5% fish oil diet plus 70 mg/kg of α-TA supplementation, our experimental results agree with previous studies (López-Ferrer et al., 2001) which found no sensory differences between dark chicken meat from broilers fed the following treatments: i) a diet containing 8% of tallow (treatment 1); and ii) a diet containing 4% of tallow plus 4% of fish oil, which was replaced 1 or 2 wk before slaughter (namely, treatments 4 and 5, respectively) by a diet with only 1% of fish oil plus 3% of linseed oil and 4% of tallow (all assayed diets were supplemented with tocopherol at 130 mg/kg). In this study the fatty acid composition

of treatments 4 and 5 was more unsaturated than in our experimental treatments. On the other hand, González-Esquerra and Leeson (2000) reported that the addition of menhaden oil at 0.75% to the diet of chickens (without α-TA supplementation) led to dark chicken meat with lower acceptability and higher presence of off-flavors). Thus, at the doses of fish oil and α-TA assayed, all our experimental treatments can produce meats with an increased content in long-chain PUFA, similar TBA values and consumer acceptability similar to a commercial sample.

Zn supplementation showed no effect on consumer acceptability and TBA values for either storage time.

Conclusions

α-Tocopherol content is greatly affected by the α-TA supplement. However, the fish oil dose and Zn supplementation did not affect the α-tocopherol content in mixed raw chicken meat.

The fatty acid composition of chicken meat is only affected by the fatty acid composition of the diet, and not by the assayed α-TA and Zn supplements. Dietary manipulation of the fatty acid composition of this meat is easy, particularly for the EPA and DHA content.

In relation to the recommended daily dietary intakes for fatty acids (Table 6), 100 g of edible portion from experimental treatments with the 2.5% fish oil diet provide 30% for linoleic acid, 5.5% for linolenic acid, and 39% for EPA plus DHA of the adequate intakes given by Simopoulos et al., (2000). Given that there were no differences in fat content of mixed raw chicken meat samples coming from the experimental treatments, these results were calculated by taking the fat content average (11.21%).

Long-term Zn supplementation (200 mg/kg) did not affect the content of this mineral in chicken meat.

In relation to sensory quality of cooked dark chicken meat, the highest differences in consumer acceptability, in both consumer panel tests, were found when the doses of fish oil were compared (Table 5). However, these differences were not

significant. In addition, our results show that cooked meat stored for five mo at –20ºC from broilers fed the 2.5% fish oil diet supplemented with 70 mg/kg of α-TA is not different in consumer acceptability from the meat from broilers fed the treatment with a 1.25% of fish oil and 140 mg/kg of α-TA, and no different from a freshly cooked commercial sample used as a blind control.

Therefore, our study illustrates a good approach to increasing the long-chain n-3 PUFA content of chicken meat; various doses of α-TA supplementation (70 and 140 mg/kg of feed) enabled reasonable consumer acceptability scores to be maintained, even after 5 mo under frozen storage.

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Table 1. Ingredients and composition of the basal diets.

Diet up to 3 wk of age	Percentage	Diet from 3 to 6 wk of age	Percentage
Ingredients:		**Ingredients:**	
Wheat[1]	55.1	Sorghum	41.0
Soybean meal, 48% protein	18.8	Barley	20.5
Full-fat soy	10.0	Soybean meal, 48% protein	19.1
Animal fat	5.2	Meat meal, 50% protein	4.8
Meat meal, 50% protein	4.5	Wheat	4.7
Rapeseed	2.5	Animal fat	3.1
Sepiolite	1.5	Poultry by-product meal	3.1
Calcium carbonate	0.4	Sepiolite	1.5
L-Lysine	0.3	L-Lysine	0.5
DL-methionine	0.3	DL-methionine	0.3
Salt	0.3	Salt	0.3
Choline chloride	0.1	Choline chloride	0.1
Trace mineral-vitamin mix[2]	1.0	Trace mineral-vitamin mix[4]	1.0
Composition[3]:		**Composition[3]:**	
Dry matter	88.6	Dry matter	89.4
Crude protein	22.7	Crude protein	20.2
Crude fat	9.1	Crude fat	6.0
Crude fiber	3.2	Crude fiber	3.1
Ash	6.3	Ash	5.5
Metabolizable energy (cal/g)	3,025	Metabolizable energy (cal/g)	2,775

[1] β-glucanase was added to the wheat.

[2] Supplying per kg feed: 7,500 IU of vitamin A, 2,000 IU of vitamin D_3, 30 mg of vitamin E (dl-α-tocopheryl acetate), 15 µg of vitamin B_{12}, 2 mg of vitamin B_6, 5 mg of vitamin K, 5 mg of vitamin B_2, 1 mg of vitamin B_1, 40 mg nicotinic acid, 160 µg of biotin, 12 mg of calcium pantothenate, 1 mg of folic acid, 20 mg of Fe (ferrous sulfate), 71 mg of Mn (manganese oxide), 100 µg of Se (sodium selenite), 37 mg of Zn (zinc oxide), 6 mg of Cu (copper sulfate), 1.14 mg of I (potassium iodide), 400 µg of Co (cobalt sulfate), 4 mg of butylated hydroxytoluene.

[3] Results shown are estimated values.

[4] Supplies the following per kilogram of complete feed: 5,000 IU of vitamin A, 8,000 IU of vitamin D_3, 25 mg of vitamin E (dl-α-tocopheryl acetate), 10 µg of vitamin B_{12}, 3 mg of vitamin K, 3 mg of vitamin B_2, 10 mg nicotinic acid, 100 µg of biotin, 10 mg of calcium pantothenate, 1 mg of folic acid, 20 mg of Fe (ferrous sulfate), 71 mg of Mn (manganese oxide), 100 µg of Se (sodium selenite), 37 mg of Zn (zinc oxide), 6 mg of Cu (copper sulfate), 1.14 mg of I (potassium iodide), 100 µg of Co (cobalt sulfate), 4 mg of butylated hydroxytoluene.

Table 2. Factorial design of the experiment[1].

Dietary treatments	Up to 3 wk of age		From 3 to 6 wk of age		
	α-TA supplementation (mg/kg)	**Zn supplementation (mg/kg)**	**α-TA supplementation (mg/kg)**	**Zn supplementation (mg/kg)**	**Dose of fish oil[2] (%)**
1	70	0	70	0	1.25
2	70	0	70	0	2.5
3	70	200	70	200	1.25
4	70	200	70	200	2.5
5	140	0	140	0	1.25
6	140	0	140	0	2.5
7	140	200	140	200	1.25
8	140	200	140	200	2.5

[1] The experiment was repeated 6 times.
[2] Total fat added to the feeds was completed up to 5.6% with animal fat so that all the treatments were isocaloric.

Table 3. α-Tocopherol and Zn content in mixed dark and white raw chicken meat (mg/100g edible portion)[1].

Factor studied	α-Tocopherol content	Zn content
Dose of fish oil		
1.25%	2.50	0.89
2.5%	2.37	0.91
α-TA supplementation[2]		
70 mg/kg	2.06[a]	0.88
140 mg/kg	2.81[b]	0.91
Zn supplementation[3]		
0 mg/kg	2.35	0.89
200 mg/kg	2.52	0.90

[1] Values given in this table correspond to least-squares means obtained from multifactor ANOVA.
[2] Content of α-tocopherol was 101 mg/kg in feeds supplemented with 70 mg/kg and 161 mg/kg in feeds supplemented with 140 mg/kg.
[3] Content of Zn was 195 mg/kg in feeds supplemented with 0 mg/kg and 377 mg/kg in feeds supplemented with 200 mg/kg.
[a-b] Means corresponding to a certain factor bearing distinct superscripts differ significantly ($P \leq 0.05$).

Table 4. Fatty acid composition (expressed as area normalization in %) of the experimental feeds and the effect of the dietary factors on the fatty acid composition of chicken meat[1].

Fatty acids	Feed			Mix of dark and white raw chicken meat					
	Up to 3 wk of age	From 3 to 6 wk of age		Dose of fish oil (%)		α-TA supplementation (mg/kg)		Zn supplementation (mg/kg)	
		fish oil 1.25%	fish oil 2.5%	1.25	2.5	70	140	0	200
C14:0	1.39	2.32	3.03	1.42[a]	1.68[b]	1.54	1.54	1.54	1.54
C16:0	19.82	21.60	20.95	23.52	23.42	23.42	23.52	23.47	23.48
C18:0	10.40	11.95	10.31	7.46	7.45	7.44	7.48	7.49	7.42
C20:0	0.24	0.24	0.23	0.07	0.08	0.08	0.07	0.08	0.07
Total SFA	31.85	36.11	34.51	32.47	32.63	32.48	32.61	32.58	32.51
C14:1 n-9	0.17	0.16	0.15	0.25	0.25	0.25	0.25	0.25	0.25
C16:1 n-9	0.32	0.29	0.46	0.68	0.70	0.69	0.68	0.68	0.69
C16:1 n-7	1.82	2.63	2.85	4.99	4.72	4.93	4.77	4.94	4.76
C18:1 n-9	31.58	33.70	30.56	42.08[a]	39.86[b]	40.87	41.07	40.91	41.03
C18:1 n-7	2.03	1.69	1.98	2.20[a]	2.33[b]	2.29	2.24	2.24	2.29
C20:1 n-9	0.12	1.34	2.28	0.70[a]	0.99[b]	0.85	0.85	0.85	0.85
C22:1 n-9	0.03	0.11	0.20	0.38[a]	0.49[b]	0.44	0.43	0.44	0.43
Total MUFA	36.07	39.92	38.48	51.28[a]	49.34[b]	50.32	50.30	50.32	50.30
C18:2 n-6	26.00	18.30	17.66	12.37	12.43	12.45	12.36	12.35	12.46
C18:3 n-6	0.02	0.04	0.04	0.11	0.10	0.11	0.10	0.10	0.11
C20:2 n-6	0.23	0.23	0.23	0.18	0.19	0.18	0.19	0.18	0.19
C20:3 n-6	0.08	0.08	0.07	0.17	0.16	0.17	0.16	0.17	0.16
C20:4 n-6	0.19	0.28	0.34	0.38[a]	0.49[b]	0.44	0.43	0.44	0.43
C22:4 n-6	0.06	0.17	0.28	0.09	0.08	0.09	0.08	0.09	0.08
C22:5 n-6	ND	0.06	0.10	0.03	0.04	0.04	0.04	0.04	0.04
Total n-6 PUFA	26.58	19.15	18.72	13.33	13.50	13.48	13.36	13.37	13.47
C18:3 n-3	5.28	1.69	1.79	1.08[a]	1.16[b]	1.12	1.12	1.11	1.12
C18:4 n-3	0.01	0.45	0.94	0.19[a]	0.36[b]	0.27	0.28	0.27	0.28
C20:4 n-3	ND	0.14	0.24	0.07[a]	0.12[b]	0.09	0.09	0.09	0.09
C20:5 n-3	0.06	1.00	2.11	0.48[a]	0.94[b]	0.71	0.71	0.71	0.71
C22:5 n-3	0.08	0.18	0.29	0.33[a]	0.53[b]	0.43	0.43	0.43	0.42
C22:6 n-3	0.09	1.38	2.90	0.76[a]	1.43[b]	1.10	1.09	1.11	1.08
Total n-3 PUFA	5.51	4.83	8.27	2.91[a]	4.54[b]	3.72	3.72	3.73	3.71
Total PUFA	32.08	23.98	26.99	16.24[a]	18.03[b]	17.20	17.08	17.10	17.18
Total MUFA + PUFA	68.16	63.90	65.49	67.53	67.37	67.52	67.38	67.42	67.48
(MUFA + PUFA)/SFA	2.14	1.77	1.89	2.08	2.07	2.08	2.07	2.07	2.08

[1] Values given in this table for meat samples correspond to least-squares means obtained from multifactor ANOVA.

a-b Means corresponding to a certain factor bearing distinct superscripts differ significantly ($P \leq 0.05$). ND: not detected.

Table 5. Effect of the dietary factors on consumer acceptability scores and TBA values (μg malondialdehyde/kg) of cooked dark chicken meat after 0 and 5 mo of storage at – 20ºC[1,2].

	Dose of fish oil		α-TA supplementation (mg/kg)		Zn supplementation (mg/kg)	
	1.25%	**2.5%**	**70**	**140**	**0**	**200**
0 mo of storage						
Acceptability[3]	3.9	3.6	3.8	3.8	3.7	3.9
TBA values	184	297	266	215	223	258
5 mo of storage[4]						
Acceptability	4.4	3.9	4.0	4.2	4.2	4.1
TBA values	909	1231	1055	1085	1204	936

[1] Values given in this table correspond to least-squares means obtained from multifactor ANOVA.
[2] The consumers ranked the acceptability of the meats using a 9-point scale (1 = very bad; 9 = very good).
[3] Interaction between Zn supplementation and α-TA supplementation was significant.
[4] At 5 mo of storage a freshly cooked commercial chicken meat sample stored for 1 d at – 20ºC was added to the consumer test as a blind control. The consumer acceptability mean for the control was 4.7, a score that did not differ significantly from the experimental samples. The TBA value for the control was 809.

Table 6. Comparison of the human recommended daily dietary intakes (mg/d) and those provided by 100 g of edible portion (mix of dark and white raw chicken meat).

	Linoleic Acid	**Linolenic Acid**	**EPA[3] plus DHA**	**n-3 PUFA**	**Vitamin E**
Simopoulos et al. (2000)	4,440	2,220	650		
Food Nutrition Board (1989)	3,000 – 6,000			300 – 1,500	
Food Nutrition Board (2000)					15
100-g edible portion[1]	1,310 – 1,317	114 – 123	131 – 251	308 – 480	2.06 – 2.81
% recommended[2]	21.8 – 43.9%	5.2 – 5.5%	20.1 – 38.6%	20.5 – 160.0%	13.7 – 18.7%

[1]Results for fatty acids are calculated by taking the fat content average (11.21%) found in mixes of dark and white raw chicken meat with skin. Fatty acid results come from 1.25% and 2.5% fish oil diets. Vitamin E results come from diets supplemented with α-tocopheryl acetate at 70 and 140 mg/kg.
[2]Values are the percentages of the recommended daily dietary intakes provided by 100 g of edible portion.
[3]EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; PUFA = polyunsaturated fatty acid.

1.3. Effect of Dietary Fat Sources and Zinc and Selenium Supplements on the Composition and Consumer Acceptability of Chicken Meat

Títol: Efecte de diferents fonts lipídiques en la dieta, conjuntament amb suplements de seleni i zinc, en la composició i l'acceptabilitat sensorial de la carn de pollastre

Resum: Emprant un disseny factorial es va estudiar l'efecte de diferents factors relacionats amb l'alimentació dels pollastres en la composició i l'acceptabilitat sensorial de la seva carn. Cinc dies abans del sacrifici, l'oli de peix afegit a la dieta al 1,25% va ser reemplaçat per altres fonts lipídiques (greix animal, oli de llinosa, o bé continuar amb l'oli de peix) i aquestes dietes van ser suplementades amb Zn (0, 300 o 600 mg/kg) i Se (0, 1,2 mg/kg en forma de selenit sòdic o 0,2 mg/kg en forma de llevat ric en Se).

Els canvis en el greix de la dieta van donar lloc a diferents composicions en àcids grassos en la barreja de pit i cuixa de pollastre amb pell. La dieta amb oli de peix va donar lloc a les carns amb els continguts més alts en EPA i DHA, mentre que la substitució amb oli de llinosa va donar lloc a les carns amb els continguts més alts en AGPI de la sèrie n-3, especialment en àcid linolènic. Tanmateix, les carns dels animals alimentats amb greix animal tenien encara un contingut elevat en AGPI de cadena molt llarga de la sèrie n-3. El contingut en Se de la carn de pollastre va incrementar amb el suplement de Se a la dieta, així com amb la suplementació amb Zn. Tot i això, solament la font orgànica de Se va provocar un increment significatiu d'aquest mineral en la carn en relació al tractament control.

L'acceptabilitat dels consumidors i els valors de l'índex de l'ATB en les mostres de cuixa de pollastre cuites, després d'emmagatzemar-les congelades 74 dies o 18 mesos, no van ser significativament afectats per cap dels factors estudiats.

EFFECT OF DIETARY FAT SOURCES AND ZINC AND SELENIUM SUPPLEMENTS ON THE COMPOSITION AND CONSUMER ACCEPTABILITY OF CHICKEN MEAT

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Abbreviation Key: AF = animal fat; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; FA = fatty acid; FO = fish oil; GPx = glutathione peroxidase ; LO = linseed oil; MUFA = monounsaturated fatty acid; MT = metallothionein ; PUFA = polyunsaturated fatty acid; SeMet = selenomethionine; SFA = saturated fatty acid; α-TA = α-tocopheryl acetate.

ABSTRACT

A factorial design was used to study the effect of changes in broiler feed on the composition and consumer acceptability of chicken meat. One week before slaughter, 1.25% dietary fish oil was removed from the feed and replaced by other fat sources (animal fat, linseed oil or continue with fish oil), and diets were supplemented with Zn (0, 300 or 600 mg/kg), and Se (0, 1.2 mg/kg as sodium selenite or 0.2 mg/kg as Se-enriched yeast). The changes in dietary fat led to distinct fatty acid compositions of mixed raw dark and white chicken meat with skin. The fish oil diet produced meat with the highest eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content, while the linseed oil diet led to meat with the highest content in total n-3 polyunsaturated acids (PUFA), especially linolenic acid. However, meat from animals on the animal fat diet was still rich in very long chain n-3 PUFA. Se content was affected by Se and Zn supplements. Se content increased with Zn supplementation. However, only Se from the organic source led to a significant increase in this mineral in meat compared with the control. Consumer acceptability scores and TBA values of cooked dark chicken meat after 74 d or after 18 mo of frozen storage were not affected by any of the dietary factors studied.

Key Words: fat sources, zinc supplementation, selenium supplementation, chicken meat composition, consumer acceptability.

1.- INTRODUCTION

One of the goals of food scientists is to increase the nutritional value of foods without compromising sensory quality. In this regard, n-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), have beneficial effects on human health (Food and Nutrition Board, 2002a). Many studies have explored the enrichment of chicken meat with EPA and DHA through the addition of dietary fish oil (FO) (Hargis and van Elswyk, 1993; Scaife et al., 1994; Wood and Enser, 1997; Surai and Sparks, 2000). However, these acids are prone to oxidation and consequently their use in meat enrichment may produce off-tastes and off-odours, thereby reducing consumer acceptability.

Several strategies have been studied to enrich poultry meat in n-3 PUFA while maintaining optimal sensory quality (López-Ferrer et al., 1999a, 2001a; González-Esquerra and Leeson, 2000, 2001; Bou et al., 2004a). These are based on combining distinct amounts of dietary tocopherol with a range of doses of dietary FO, blends of FO with vegetable seeds or oils rich in n-3 PUFA, or the replacement of FO by other fat sources prior to slaughter. Although these strategies reduce off-flavours they also decrease the content in EPA and DHA (Hargis and Van Elswyck, 1993). Therefore, it is essential to establish which is the best strategy leading to an enriched meat in these fatty acids, and ensuring that the consumer acceptability is not lowered.

Furthermore, α-tocopheryl acetate (α-TA) supplements increase the α-tocopherol content in chicken tissues (Cherian et al., 1996; Morrissey et al., 1997; Galvin et al., 1998; Surai and Sparks, 2000). The effects of α-tocopherol on health have also been described (Food and Nutrition Board, 2000). In addition, α-tocopherol prevents lipid oxidation in chicken meat (Lin et al., 1989, Sheehy et al., 1993; Jensen et al., 1995; Grau et al., 2001a,b), thereby increasing the sensory quality (de Winne and Dirinck, 1996; Bou et al., 2001; Mielnik et al., 2002), even when animals are fed mainly on saturated or monounsaturated fatty acids (Lin et al., 1989; O'Neill et al., 1998).

Meat products are one of the main sources of Fe, Cu, Se and Zn (Pennington and Young, 1991; Buss and Rose, 1992; Foster and Sumar, 1995; Subar et al., 1998)

showing a high bio-availability (Fairweather-Tait, 1992). In addition, these elements are involved in a wide range of biochemical functions. Besides, several communities do not achieve the recommended daily intakes of some elements, even in developed countries (Penington and Young, 1991; Bou et al., 2004a). This is the case for Zn and Se intakes in elderly people (Girodon et al., 1999; de Jong et al., 2001; Savarino et al., 2001). Therefore, meat products enriched in selected fatty acids, tocopherol and elements such as Zn and Se can be of great nutritional benefit.

Here we studied the effect of various dietary factors (supplementation with distinct levels of n-3 PUFA, Zn and Se, and a fixed amount of α-TA) on the content of α-tocopherol, Zn, Se, Fe and Cu, and fatty acid composition in raw chicken meat. Furthermore, we analysed the oxidative stability and the consumer acceptability of cooked dark chicken meat.

2.- MATERIAL AND METHODS

Animals and Housing

Three hundred twenty-four female broilers chicks (Ross 308, 1-day old) were assigned to 54 floor pens (6 birds per pen) corresponding to 27 replicated dietary treatments. Assignment of the replicated dietary treatments to the pens was made to provide a completely randomized design. Pens (0.8 m^2 with wire walls) contained wood shavings. Environmental temperature was set at 33 C at d 1 and was lowered stepwise to 21-22 C. The first 10 d lights were on 24 h/d and then lowered stepwise to 21 h/d. Relative humidity and ventilation were under standard conditions. Feed and water were provided *ad libitum*. The animals were reared and slaughtered in compliance with national regulations and the experiment received prior approval by Copaga Soc. Coop.[12] Animal Care and Use Committee.

[12] Copaga, Soc. Coop., Lleida, Spain, E-25080

Diets and Experimental Design

Treatments were prepared from two basal meal diets (Table 1). Diets were formulated according to requirements recommended by the NRC (1994) and supplemented with dl-α-TA at 100 mg/kg. Nine treatments containing 6% of animal fat (AF) were fed until 19 d of age (Table 2): three doses of Zn supplementation (0, 300 and 600 mg/kg) and three levels of Se supplements (0, 1.2 mg Se/kg from sodium selenite and 0.2 mg Se/kg from Se-enriched yeast, an organic source of Se). From 20 to 39 d, the second basal diet was used and the above treatments were maintained although the total added fat was 1.25% FO plus 5.81% of AF (Table 2). From 40 to 45 d of age, 27 dietary treatments resulted from combining the distinct levels of Zn and Se supplementation and the three types of fat source at 1.25 % (AF[13], linseed oil[14] (LO) or FO[15]) plus 5.81 % of AF (Table 2).

Zinc sulphate, sodium selenite and α-TA were purchased from Andrés Pintaluba, S.A.[16]. The organic source of Se came from selenium-enriched yeast (Sel-Plex^R) and was supplied by Probasa[17].

Preparation, Cooking and Storage of Samples

The chickens were slaughtered following commercial procedures and were stored for 4 h at 4 C. Carcasses from each pen were then longitudinally cut and divided into two groups (left and right sides). Two random right sides (legs and breasts with skin) from each pen were used to study the composition and nutritional value of the meat. These samples were hand-deboned, mixed, ground, and weighed (approximately 30 g per bag) into high-barrier multilayer bags (Cryovac[18] BB-4L; permeability to O_2 30 cm^3/m^2, 24 h, 1 bar, ASTMD-3985), vacuum packed and immediately stored at –20 C until determination of FA composition, and α-tocopherol, elements and crude fat content. Because of increased susceptibility of legs to oxidation because of their higher Fe and fat content, only this part was used to study the consumer acceptability. Thus,

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[14] Sopropeche, Boulogne sur Mer Cedex, France, F-62204
[15] FF of Denmark, Skagen, Denmark, DK-9990
[16] Andrés Pintaluba, S.A., Reus, Spain, E-43280
[17] Probasa, Sta. Perpetua de la Moguda, Spain, E-08130
[18] Cryovac Europe, Sealed Air S. L., Sant Boi de Llobregat, Spain, E-08830

the remaining legs with skin of chickens from each pen were hand-deboned and stored at 4 C. Ten hours post-slaughter, samples were vacuum-packed in high-barrier multilayer bags (Cryovac CN-300; permeability to O_2 15 cm^3/m^2, 24 h, 1 bar, ASTMD-3985) and cooked in an oven at 85 C (99% relative humidity) to an internal temperature of 78 C. They were then cooled and stored at –20 C until the consumer acceptability and TBA values were determined.

Reagents and Standards

Reagents and standards used in element analyses were as described in Bou et al. (2004b) while those used in the other analyses were as described in Bou et al. (2004a).

Determination of Fatty Acid Composition

Determination of FA composition in mixed dark and white raw meat plus skin (hereafter referred to as raw meat) and in milled feed was achieved by gas chromatography, as described by Bou et al. (2004a). The resulting 54 (27 x 2) meat samples were analysed. For feed analysis, one sample for each dietary treatment was examined. Thus, 9 samples were taken from treatments used from 0 to 19 d and from 20 to 39 d, while 27 samples were taken from the diets supplied from 40 to 45 d.

Determination of α-tocopherol

Determination of α-tocopherol in raw meat and in milled feed was achieved by liquid chromatography, as described by Bou et al. (2004a). The resulting 54 (27 x 2) meat samples were analysed. For feed analysis, we examined the 27 dietary treatments from 40 to 45 d, and the 9 treatments from 0 to 19 d and from 20 to 39 d.

Determination of Zn, Se, Fe and Cu

Element determination in raw meat and in milled feed was performed as described in Bou et al. (2004b). In this method, after mineralization of samples, Zn and

Fe were determined by means of inductively coupled plasma atomic emission spectrometry while Cu was measured by inductively coupled plasma mass spectrometry and Se by hydride generation inductively coupled plasma mass spectrometry.

The resulting 54 (27 x 2) chicken meat samples were analysed. For feed analysis, only the 27 dietary treatments given from 40 to 45 d were examined.

Sensory Analysis

Two consumer acceptability panel tests were performed on cooked dark chicken meat with skin stored at -20 C for 74 d and 18 mo. Thirty-one and thirty-three experienced consumer panellists were used in each test, respectively.

Because of the high number of dietary treatments assayed (27), the consumer acceptability of samples supplemented with 300 mg/kg of Zn was not evaluated, thereby reducing the samples for evaluation to 18. Criteria for panelist selection, sample preparation and presentation were as described in Bou et al. (2004a).

Samples were presented to all panellists; however, they were presented to each panelist in a completely randomized design in 3 working sessions. In each session, 6 randomized samples and a blind control were presented. The blind control was a vacuum-packed freshly cooked commercial chicken meat sample stored for 1 d at -20 C. Panelists were asked to rank the overall acceptability using a 9-point scale (1 = very bad; 9 = very good). A comment section was also available on the score-sheet.

Determination of TBA values

Vacuum-packed cooked chicken legs with skin were thawed, as for sensory analysis, by heating in a water bath at 35 C for 20 min and were then ground. Two g of ground cooked dark meat was then weighed for analysis. TBA values were measured through a third derivative spectrophotometry method after acid aqueous extraction (Grau et al., 2000). As in the sensory analysis, only samples from 18 treatments and the blind control were analysed.

Determination of crude fat content

Fat content of the raw meat from all the experimental treatments was measured by the AOAC Official Method 991.36 (AOAC, 2000). Thus, the resulting 54 (27 x 2) samples were analysed.

Statistical Analysis

Multifactor ANOVA was used to determine whether any significant effects were produced by the factors studied on animal performance parameters, FA composition, and α-tocopherol, Zn, Se, Fe, Cu and crude fat content of the raw meat, as well as on consumer acceptability and TBA values of cooked dark meat with skin. Interactions between factors higher than an order of two were discarded. One-way ANOVA was used to determine significant differences in consumer acceptability scores between dietary treatments and the blind control used in the sensory test.

Multifactor ANOVA was used to determine significant differences caused by the factors studied in α-tocopherol, Zn, Se, Fe and Cu content of feeds.

In all cases, least-squares means for the main factors that had a significant effect were separated using Duncan test. In all cases, $P \leq 0.05$ was considered significant.

3.- RESULTS AND DISCUSSION

Animal performance

Final BW, feed intake, feed conversion and mortality were not affected by dietary treatments. Averages for BW and feed conversion after 44 d were 2,318 g and 1.41 g/g respectively. Zn supplementation lack of effect on BW agrees with previous results in chickens fed 0 or 200 mg/kg of Zn (Bou et al., 2004a). However, chickens fed 600 mg/kg had a slightly lower BW (2,250 g) which was almost significant (P = 0.052) compared to those from 0 or 300 mg/kg of Zn (2.344 and 2.359 g, respectively). Nevertheless, this BW decrease, which has been reported to be significant in chickens fed higher Zn supplements (Sandoval et al., 1998; Williams et al., 1989), did not affect

carcass weight in our case (for 0, 300 and 600 mg Zn/kg carcass weights were 1.722, 1.715 and 1.687 g, respectively). Thus, from our BW results and those reported by Sandoval et al. (1998) the upper tolerable Zn supplementation for broiler production purposes should be set between 600 and 1000 mg/kg.

Therefore, it can be assumed that mixed dark and white raw chicken meat plus skin as well as cooked dark chicken meat with skin samples are similar and comparable between treatments.

Fatty acid composition and total fat content

Feed and raw meat FA compositions are shown in Table 3. In relation to feed FA composition, when FO was added to the feed from 20 to 39 d of age, EPA and DHA increased compared to levels in feed administered up to 19 d of age. Distinct feeds from 40 to 45 d of age also showed differences for n-3 PUFA. The LO diets showed a higher linolenic acid content while FO diets had higher EPA and DHA contents. Furthermore, feeds including AF had the lowest amount of n-3 PUFA and total PUFA and the highest percentage of total monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA).

The FA composition of meat was affected by dietary fat source (Table 3). The effect of the FA composition of feed on chicken meat composition has been widely reported (Hargis and Van Elswyk, 1993; Cherian et al., 1996; Wood and Enser, 1997; Surai and Sparks, 2000). Therefore, these differences in chicken meat FA composition were reflecting those differences in diets, which had been given to the chickens for only five d before slaughter. The effect of 8.2 % dietary FO replacement, one or two weeks before slaughter, on the FA composition of thigh and breast meat has been reported (López-Ferrer et al., 1999a). These authors found that replacing dietary FO by LO just one or two weeks before slaughter produces smaller amounts of EPA and DHA and higher amounts of linolenic acid. In our study, the removal of FO and replacement by LO led to a slight decrease in 18:4 n-3, 22:5 n-3, EPA and DHA (Table 3). In addition, the LO diet led to the highest increase in total n-3 PUFA content because of the great increase in linolenic acid. On the contrary, the lowest total n-3 PUFA content was observed in meat from AF treatments. However, AF diets still

produced high concentrations of EPA, DHA and total n-3 PUFA compared to diets using non-marine fat sources throughout the feeding period (López-Ferrer, 1999a,b; Grau et al., 2001a; López-Ferrer, 2001b). Furthermore, total MUFA content was higher in AF treatments, whereas total SFA was greater in animals on FO diets. The former observation could be explained by the amount of these FA in the feed. However, the differences observed in meat were not as marked as those observed in feeds given from 40 to 45 d. This can be explained by the short period of fat replacement, though some metabolic pathways or homeostatic mechanism may also be involved (Asghar et al., 1990; Wood and Enser, 1997). These factors could explain why the highest total SFA content was detected in meat from the FO diet while no differences in total SFA content were observed between that from the LO or AF treatments.

The FA composition of feed also explained the significant differences for 14:0, 16:1 n-9, 18:1 n-9 and 20:1 n-9 in meats. However, 22:1 n-9 content was higher in meat from AF treatments but very low in feeds containing AF. The 20:4 n-6 content of meat increased when FO was added to feed. This finding is in disagreement with other authors' results when comparing increasing amounts of FO (Phetteplace and Watkins, 1990; López-Ferrer et al., 2001a). In our study, the formation of this metabolite may not have been inhibited, through Δ^6 desaturase, by the amount of n-3 fatty acids provided at 1.25% FO or LO and an enhanced deposition of this FA may have occurred in these two dietary groups.

Furthermore, the FA composition (Table 3) and crude fat content (Table 4) of raw meat were not affected by Zn or Se supplementation. The lack of effect of Zn supplementation on raw meat FA composition and crude fat content has been previously reported in a similar study with chickens fed 200 mg/kg of Zn (Bou et al., 2004a).

In addition, in our study, dietary fat sources had no effect on crude fat content of raw meat (Table 4). Therefore, the enrichment expressed as area normalization (%) in some very long chain n-3 PUFA was comparable between treatments. The lack of effect of fat source on crude fat is consistent with the results on dark and white meat with and without skin from chickens fed different fat sources (Ajuyah et al., 1992; Scaife et al., 1994; Crespo and Esteve-Garcia, 2001).

Tocopherol content

All diets were supplemented with 100 mg/kg of α-TA, a dose that ensures good consumer acceptability for chickens fed 1.25% FO (Bou et al., 2004a). The averages for α-tocopherol content in diets up to 19 d and from 20 to 39 d of age were 131 and 129 mg/kg of feed, respectively. In diets from 40 to 45 d, the average α-tocopherol content is shown in Table 5. As there were no significant differences between fat sources for α-tocopherol, it can be assumed that the fat sources assayed did not modify the dietary α-tocopherol supply.

The α-tocopherol content of raw meat was not affected by any of the factors studied (Table 4). Thus, the replacement of 1.25% FO for 1.25% AF or LO one week before slaughter did not alter α-tocopherol content. These results are consistent with those reported elsewhere, in studies of chicken meat from animals fed on both different fat sources (saturated and unsaturated) and different α-TA supplements (Lin et al., 1989; Ruiz et al., 1999; Surai and Sparks, 2000; Grau et al., 2001a; Bou et al., 2004a).

Conversely, the α-tocopherol content of dark and white meat has been described to increase in chickens fed olive oil compared with those fed tallow supplemented at either 30 or 200 mg/kg of α-TA (O'Neill et al., 1998). Nevertheless, this effect could be explained by the endogenous content of α-tocopherol present in these feeds, which was slightly higher in diets containing olive oil.

Several factors, such as feed endogenous α-tocopherol content, could also influence the levels of this antioxidant. We did not address this factor because dietary amounts of α-tocopherol were similar in all treatments (Table 5). However, some studies that provided similar levels of α-tocopherol reported a decreased α-tocopherol content in various tissues of chickens fed highly unsaturated diets. This decrease was attributed to a higher oxidation susceptibility of these tissues (Maraschiello et al., 1999; Surai and Sparks, 2000). In addition, inefficient absorption of tocopherol, other antioxidants in feed or stressful periods of early and rapid growth can explain some differences reported in α-tocopherol levels of chicken meat, eggs and other tissues (Galobart et al., 2002; Cherian and Sim, 2003; Zanini et al., 2003).

Furthermore, Zn may act as an antioxidant (Bray and Bettger, 1990; Oteiza et al., 1995; Zago and Oteiza, 2001) and animal deficiencies are alleviated by tocopherols and other antioxidants (Kraus et al., 1997). In addition, laying hens reared at low temperature (6.8 C) and supplemented with Zn (30 mg/kg) have higher serum tocopherol contents than those not receiving supplements (Onderci et al., 2003). Likewise, Japanese quails supplemented with Zn (0, 30 and 60 mg/kg) and reared under heat stress (34 C) have higher serum tocopherol contents (Sahin and Kucuk, 2003). However, these authors did not find differences in α-tocopherol content between animals on distinct levels of Zn supplements and reared at 22 C. Thus, these former results agreed with our study, in which the meat of chickens on Zn supplements (300 or 600 mg/kg) did not show a higher α-tocopherol content in raw meat (Table 4). This is consistent with a previous study (Bou et al., 2004a).

Deficiencies in Se combined with low dietary tocopherol supply have been related to various chicken diseases, which are exacerbated by an excess of dietary PUFA and can be minimised by Se or tocopherol supplements (NRC, 1983). This can be explained by the fact that both take part in the antioxidant system. In fact, Se supplements can increase glutathione peroxidase (GPx), which converts hydroperoxides into non-prooxidant molecules (Diplock et al., 1998; Surai, 2002a). Therefore, those reported increases in α-tocopherol levels in different animal tissues as a result of Se supplementation could be related with the role of Se in the antioxidant system (Surai, 2002b).

This explanation is in agreement with the increased liver and serum tocopherol content reported in Japanese quails fed Se supplements (0.1 vs. 0.2 mg/kg) and reared under heat stress (34 C) (Sahin et al., 2002). However, in our study the levels of Se supplementation did not affect α-tocopherol content (Table 4). Similarly, hamsters fed FO did not show differences in tocopherol content of heart and liver tissues when fed adequate amounts of tocopherol (27 mg/kg α-TA) and a Se supplement (3.4 mg/kg as sodium selenite) (Poirier et al., 2002). Therefore, under non-stressing conditions, α-tocopherol levels did not appear to be affected by Se supplements.

Element content

The element composition of feed is shown in Table 5. Differences in Zn and Se content reflect the supplements of these elements. In addition, the fat source led to significant differences for Cu content. Feeds containing FO had a higher Cu content while those with LO had a lower content of this metal, although the AF treatment did not differ from the FO or LO treatments.

Results for the content of each element in chicken meat, depending on each dietary factor, are shown in Table 4.

The dietary fat source factor did not affect raw meat content of elements, although Fe content was affected by an interaction between Se supplement and dietary fat source ($P = 0.027$).

Zn supplement had no effect on meat Zn content which is consistent with a previous study in which animals were supplemented at 200 mg/kg of Zn (Bou et al. 2004a). Chicken muscle Zn content decreases mainly with age (Sandoval et al., 1998; Mohanna and Nys, 1998). In this regard, body Zn concentrations are lower and stable from 21 to 50 d of age compared to earlier periods (Mohanna and Nys 1998). However, increasing dietary Zn supplementation results in higher concentrations in distinct tissues, although at 3 weeks of age a poor linear regression is observed between muscle Zn concentration and dietary Zn supplementation (Sandoval et al., 1998). These results support the Zn homeostatic control metabolism (Cousins, 1996; Food and Nutrition Board, 2002b).

Furthermore, we observed that Zn supplementation led to a significant increase in meat Se content (Table 4). The greater the Zn supplementation, the higher the Se content in raw chicken meat. Yin et al. (1991) also reported that rats supplemented with Zn showed higher Se concentrations in plasma, erythrocytes, muscle, heart and liver tissues.

This increase in chicken meat Se content induced by Zn supplementation is difficult to explain since several factors may be involved. Se, like As, is excreted through biomethylation (Foster and Sumar, 1997; Schrauzer, 2000; Gailer, 2002; Jiang et al., 2003) and many forms of Se have been reported in chicken meat (Daun et al., 2004). Furthermore, metallothionein (MT) is a cysteine-rich protein that controls the Zn pool (Cousins, 1996, Coyle et al., 2002) and its synthesis is induced by Zn and

other cations (Nordberg, 1998). This protein, through thiol groups, acts as a chelating agent for divalent cations (Nordberg, 1998; Maret et al., 1999; Coyle et al., 2002), as a reductant of biological oxidants (Klotz et al., 2003; Maret, 2003) and reacts with various forms of Se (Jacob et al., 1999; Chen and Maret, 2001), reduced glutathione (Maret, 2000) and methylated species of As (Jiang et al., 2003). Thus, MT acts in detoxifying and antioxidant systems (Schwarz et al., 1994; Maret, 2000, 2003; Coyle et al., 2002; Klotz et al., 2003;)

On the other hand, the equimolar binding of Hg and Se with selenoprotein P has been described in plasma as a detoxification mechanism (Yoneda and Suzuki, 1997). Therefore, this and the MT detoxification mechanism could explain the intertwined toxicity of Se, As and Hg (Yoneda and Suzuki, 1997; Goyer, 1997; NRC, 1999; Gailer, 2002)

Given these relationships, some forms of Se present in chicken meat (Daun et al., 2004), which does not depend on the type of dietary Se source, may be bound to MT or other selenoproteins. Thus, this MT or other Se forms could present an altered metabolism under high doses of Zn supplementation leading to an increased Se content. However, further studies are required to determine the Se content and the different Se forms present in different chicken tissues under Zn supplementation.

Conversely to this effect, the contents of Zn, Fe and Cu in chicken meat were not affected by Zn supplementation.

Organic Se supplementation produced an increase in meat Se content in comparison with chickens that received no Se addition (Table 4). However, when the inorganic Se source (sodium selenite) was added to feeds, at a relatively high dose (1.2 mg/kg feed), the content of this element did not differ between treatments with organic Se supplement (0.2 mg/kg feed) and those without supplement.

The organic form of Se used in this study was obtained from Se-enriched yeast. This yeast mainly contains this element in the form of selenomethionine (SeMet), which cannot be synthesised by mammals or poultry (Schrauzer, 2000). Furthermore, SeMet is actively absorbed in the intestine and follows the same pathways as methionine while selenite is absorbed passively (Thomson, 1998; British Nutrition Foundation, 2001; Surai, 2002b). In addition, SeMet is incorporated non-specifically into distinct proteins such as those in muscle (Thomson, 1998; Surai, 2002b). In

contrast, selenite and other forms of Se appear to be under homeostatic regulation and incorporated specifically as selenocysteine into functional selenoproteins (Thomson, 1998; British Nutrition Foundation, 2001).

In addition, SeMet and other organic Se forms are also converted into selenocysteine, which can be used in functional selenoproteins (Foster and Sumar, 1997; Schrauzer, 2000), explaining the maintained activities of GPx after Se depletion in animals on SeMet supplements (Surai, 2000). These observations indicate that selenomethionine can act as a Se reserve (Schrauzer, 2000; Surai, 2002b).

Thus, when animals were fed the inorganic Se supplement, if there was an increase in muscle Se content, it would be expected to be mainly due to the increase in functional selenoproteins such as GPx. In fact, after inorganic Se supplementation, increased GPx activity has been reported in chicken meat compared with that from animals on a basal diet containing 0.09 mg Se/kg (de Vore et al., 1983) and also in chicken erythrocytes coming from Se supplemented chickens compared with those coming from animals receiving 0.03 mg Se/kg (Aydemir et al., 2000). Nevertheless, liver GPx activity of chickens fed a basal diet containing 0.12 mg Se/kg was not increased compared with those of animals on inorganic and organic Se supplements (Holovská et al., 2003). Indeed, GPx activities can be used as indicators for estimating the requirements of Se. In relation to this, Holovská et al. (2003) reported that a diet containing 0.1 mg Se/kg provided the Se requirement for the synthesis of GPx in chicken liver. The same Se requirement was reported to maintain serum GPx activity in pigs (Mahan and Parret, 1996).

Moreover, Mahan and Parret (1996) found that Se content in pig loins from animals on Se-enriched yeast was higher than in animals on inorganic Se or no supplementation. These observations are consistent with our results in chicken meat, indicating that inorganic Se supplementation did not affect Se content, while chickens on organic Se supplement had higher Se content in meat, probably in the form of SeMet (Table 4).

Se supplementation did not affect Fe, Zn and Cu content of raw meat (Table 4). These results are consistent with those reported by Sahin et al. (2002) for Fe, Zn and Cu serum concentrations of Japanese quails on diets supplemented with inorganic Se (0.1 and 0.2 mg/kg of Se supplement).

Sensory analysis and TBA values

Two consumer tests were conducted to assess the consumer acceptability of cooked leg samples. The first was carried out after 74 d and the second after 18 mo of frozen storage.

After 74 d, consumer acceptability and TBA values did not show significant differences for the factors studied (Table 6). In addition, the one-way ANOVA performed for the sample factor did not show significant differences in acceptability between the blind control and the dietary treatments. Nevertheless, the former showed lower consumer acceptability than the latter. This observation can be explained because of the higher TBA values in the blind control. These values are inversely correlated with sensory scores (Ang and Lyon, 1990; Mielche, 1995; Bou et al., 2001).

Consumer acceptability of cooked samples was evaluated again after 18 mo of frozen storage. Similarly to at 5 mo of frozen storage, none of the dietary factors showed significant differences in consumer acceptability and TBA values. In addition, the one-way ANOVA for the sample factor showed no differences in acceptability between the blind control and the dietary treatments. Nevertheless, in this second sensory analysis, the differences in consumer acceptability scores between samples and the blind control were smaller, which can be also explained because TBA values were quite similar between experimental samples and the blind control.

Although TBA values increased after 18 mo of frozen storage (Table 6), these values are very low to allow to find significant differences in acceptability scores (Bou et al., 2001). In addition, these low TBA values can be explained by the protective effect of α-tocopherol (100 mg/kg α-TA supplementation) against oxidation, which has been reported in cooked chicken meat stored at 4 C and -20 C for various periods (Ajuyah et al., 1993; Jensen et al., 1995; Galvin et al., 1998, Grau et al., 2001b).

Dietary FO replacement by other fat sources rich in PUFA before slaughter may lead to a decrease in very long-chain n-3 PUFA depending on several factors and improve sensory quality (Hargis and van Elswyck, 1993; López-Ferrer et al., 1999a). López-Ferrer et al. (1999a) studied the effect of dietary FO at 8.2% during 5 weeks on the consumer acceptability of dark and white chicken meat and compared acceptability

scores of this meat with that from chickens fed diets in which FO was replaced by linseed or rapeseed oil 1 week or 2 weeks before slaughter. Through a triangular test, these authors showed a decrease in acceptability scores of dark meat from animals on FO diets in comparison with those fed on diets with rapeseed or linseed oils 2 weeks before slaughter. However, the FO dose used in that study was much higher than that applied in our work (1.25%) and diets were not supplemented with α-TA.

In another study with more comparable FO doses, López-Ferrer et al. (2001a) compared thighs from chickens fed 8% tallow during 5 weeks with those fed first with a high FO dose (4% FO + 4% tallow) and then with low FO dose (1% FO + 3% LO + 4% tallow) for 1 or 2 weeks before slaughter. A triangular test showed no significant differences between dietary treatments using a 5-point acceptability scale.

Therefore, our results are consistent with these studies because our commercial blind control (probably similar to meat from chickens fed 8% tallow from the former work) did not differ from the FO treatment (similar to the replacement with 1% FO 1 week before slaughter from the former work). In addition, it is noteworthy remarking that treatments assayed by López-Ferrer et al. (2001a) were not α-TA supplemented.

In our study, Zn supplementation at 600 mg/kg did not affect the sensory quality or TBA values, which is consistent with another study using 200 mg/kg of Zn supplement (Bou et al., 2004a).

In addition, various authors (de Vore et al., 1983; Surai, 2002b) have reported a crucial role of organic and inorganic Se supplements in enhancing oxidative meat stability in combination with tocopherol during storage. This increase in stability can be explained through the antioxidant effect of tocopherol combined with an increased GPx activity, which is related with a decrease in TBA values (de Vore et al., 1983). However, in our study, Se supplementation did not affect the sensory scores or TBA values of cooked meat, probably because of the protective effect of α-TA supplementation at an efficient dose (100 mg/kg) in all treatments.

In summary, the removal of dietary FO and its replacement by LO or AF one week before slaughter leads to distinct FA composition of raw meat. FO produces meat with a higher EPA and DHA content, whereas LO leads to meat with a higher content in total n-3 PUFA, especially linolenic acid. Nevertheless, meat from AF treatments is still rich in EPA, DHA and total n-3 PUFA, thereby providing a good source to cover

the human recommended daily dietary intakes for these FA (Table 7). In addition, chicken meat can be Se-enriched by Zn and organic Se supplementation (Table 7). Consumer acceptability scores and TBA values of cooked dark chicken meat from animals supplemented with 100 mg α-TA/kg were not affected by any of the dietary factors studied after 74 d and 18 mo of frozen storage. We therefore conclude that, in our conditions, this α-TA supplementation dose brings about oxidative stability and provides a source of vitamin E (Table 7).

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Table 1.- Ingredients and composition of the basal diets[1].

Diet up to 19 d	Percentage	Diets from 20 to 45 d	Percentage
Ingredients:		**Ingredients:**	
Barley	25.00	Wheat	31.42
Soybean meal, 48% protein	22.57	Soybean meal, 48% protein	16.31
Wheat	16.72	Sorghum	15.00
Sorghum	10.00	Barley	15.00
Full-fat soy	6.82	Added fat[6]	7.06
Meat meal, 50% protein	6.00	Rapeseed	4.00
Animal fat[2]	6.00	Poultry by-product meal	4.00
Tapioca, 62% starch	3.00	Meat meal, 50% protein	3.66
Sepiolite[3]	1.50	Sepiolite[3]	1.50
Trace mineral-vitamin mix[4]	1.00	Trace mineral-vitamin mix[7]	1.00
Sodium chloride	0.32	L-Lysine	0.50
DL-Methionine	0.32	DL-Methionine	0.24
L-Lysine	0.25	Sodium chloride	0.20
Calcium carbonate	0.22	Calcium carbonate	0.09
Calcium phosphate	0.13	Phytase[5]	0.06
Choline chloride	0.10	Choline chloride	0.01
Phytase[5]	0.06		
Calculated composition:		**Calculated composition:**	
Dry matter	90.06	Dry matter	89.35
Crude protein	22.60	Crude protein	20.64
Crude fat	9.54	Crude fat	9.54
Crude fiber	3.32	Crude fiber	3.12
Ash	6.78	Ash	5.41

[1] Both basal diets were supplemented with 100 mg/kg of dl-α-tocopheryl acetate.
[2] Animal fat contained 80% lard and 20% beef tallow.
[3] Hydrated magnesium silicate.
[4] Supplies the following per kilogram of complete feed: 10,000 IU of vitamin A, 2,000 IU of vitamin D_3, 30 mg of dl-α-tocopheryl acetate, 20 µg of vitamin B_{12}, 4 mg of vitamin B_6, 5 mg of vitamin K_3, 5 mg of vitamin B_2, 2 mg of vitamin B_1, 66 mg nicotinic acid, 200 µg of biotin, 12 mg of calcium pantothenate, 1 mg of folic acid, 20 mg of Fe (ferrous sulfate), 71 mg of Mn (manganese oxide), 100 µg of Se (sodium selenite), 37 mg of Zn (zinc oxide), 6 mg of Cu (copper sulfate), 1.14 mg of I (potassium iodide), 400 µg of Co (cobalt sulfate), 4 mg of butylated hydroxytoluene.
[5] (EC 3.1.3.8) which liberates 1000 FTU/g.
[6] From days 20 to 39 added fat contained 1.25% fish oil and 5.81% animal fat (containing 80% lard and 20% tallow). Diets from 40 to 45 added fat contained 5.81 % animal fat (containing 80% and 20% lard) and 1.25% of additional animal fat, linseed oil, or fish oil.
[7] Supplies the following per kilogram of complete feed: 7,500 IU of vitamin A, 2,000 IU of vitamin D_3, 30 mg of dl-α-tocopheryl acetate, 15 µg of vitamin B_{12}, 5 mg of vitamin K_3, 5 mg of vitamin B_2, 40 mg nicotinic acid, 200 µg of biotin, 12 mg of calcium pantothenate, 1 mg of folic acid, 20 mg of Fe (ferrous sulfate), 71 mg of Mn (manganese oxide), 100 µg of Se (sodium selenite), 37 mg of Zn (zinc oxide), 6 mg of Cu (copper sulfate), 1.14 mg of I (potassium iodide), 400 µg of Co (cobalt sulfate), 4 mg of butylated hydroxytoluene.

Table 2.- Dietary treatments.

Up to 19 days[1]		**From 20 to 45 days**		
Zn supplement (mg/kg)	**Se supplement**[2]	**Fat source**[3]	**Zn supplement (mg/kg)**	**Se supplement**[2]
0	0	FO or LO or AF	0	0
0	selenite	FO or LO or AF	0	selenite
0	Se-yeast	FO or LO or AF	0	Se-yeast
300	0	FO or LO or AF	300	0
300	selenite	FO or LO or AF	300	selenite
300	Se-yeast	FO or LO or AF	300	Se-yeast
600	0	FO or LO or AF	600	0
600	selenite	FO or LO or AF	600	selenite
600	Se-yeast	FO or LO or AF	600	Se-yeast

[1] Up to 19 days of age 6% of animal fat was added to the feed.
[2] Selenite provided 1.2 mg Se /kg of feed while Se-yeast provided 0.2 mg Se /kg of feed.
[3] From 20 to 39 days of age added fat contained 1.25% fish oil and 5.81% animal fat. From 40 to 45 days of age added fat contained 5.81% animal fat and 1.25% of additional fish oil (FO), linseed oil (LO) or animal fat (AF).

Table 3.- Fatty acid composition (expressed as area normalization in %) of the experimental feeds and the effect of the dietary fat source on chicken meat.

Fatty acid[1]	Feeds[2]					Mixed, raw, dark and white chicken meat with skin[3]			
	Up to19	From 20 to 39	From 40 to 45 days			Fat source			SEM
			FO	LO	AF	FO	LO	AF	
C14:0	1.51	2.56	2.43	1.49	1.81	1.66a	1.59b	1.59b	0.014
C16:0	21.12	23.60	21.86	19.98	22.71	22.81	22.61	22.47	0.121
C18:0	10.68	13.71	11.09	11.14	13.03	8.30	8.06	8.05	0.100
C20:0	0.25	0.25	0.24	0.22	0.22	0.09	0.08	0.09	0.002
Total SFA	33.56	40.12	35.62	32.82	37.76	32.87a	32.35b	32.21b	0.171
C14:1 n-9	0.18	0.22	0.18	0.17	0.20	0.27	0.26	0.27	0.004
C16:1 n-9	0.26	0.30	0.29	0.25	0.31	0.58ab	0.56a	0.60b	0.010
C16:1 n-7	2.41	3.17	2.91	2.19	2.59	4.77	4.79	4.86	0.066
C18:1 n-9	32.10	33.21	33.24	33.58	36.01	40.69a	40.82a	41.51b	0.140
C18:1 n-7	1.81	1.98	2.10	1.82	2.03	2.07	2.06	2.03	0.021
C20:1 n-9	0.48	1.25	1.50	0.53	0.59	0.77a	0.71b	0.71b	0.005
C22:1 n-9	0.03	0.13	0.14	0.06	0.04	0.64ab	0.60b	0.66a	0.016
Total MUFA	37.26	40.26	40.36	38.60	41.77	49.79a	49.77a	50.68b	0.179
C18:2 n-6	25.41	14.54	18.04	18.96	17.90	12.88	13.04	13.02	0.114
C18:3 n-6	0.00	0.00	0.00	0.00	0.00	0.12	0.12	0.12	0.003
C20:2 n-6	0.18	0.21	0.24	0.21	0.24	0.23	0.23	0.23	0.003
C20:3 n-6	0.21	0.16	0.17	0.16	0.16	0.17a	0.17a	0.18b	0.004
C20:4 n-6	0.06	0.07	0.08	0.07	0.22	0.30a	0.25b	0.24b	0.004
C22:4 n-6	0.05	0.07	0.07	0.06	0.07	0.10	0.10	0.10	0.002
C22:5 n-6	0.00	0.05	0.06	0.01	0.01	0.03a	0.02b	0.02b	0.001
Total n-6 PUFA	25.91	15.05	18.53	19.46	18.44	13.83	13.93	13.94	0.121
C18:3 n-3	2.98	1.69	1.84	8.80	1.72	1.50a	2.29b	1.51a	0.037
C18:4 n-3	0.01	0.39	0.48	0.02	0.01	0.21a	0.18b	0.18b	0.005
C20:4 n-3	0.01	0.11	0.13	0.03	0.02	0.06a	0.06a	0.05b	0.001
C20:5 n-3	0.07	0.90	1.13	0.06	0.07	0.50a	0.40b	0.38b	0.008
C22:5 n-3	0.07	0.18	0.20	0.08	0.09	0.39a	0.35b	0.35b	0.007
C22:6 n-3	0.12	1.25	1.58	0.11	0.11	0.86a	0.67b	0.71b	0.014
Total n-3 PUFA	3.27	4.52	5.38	9.10	2.03	3.51a	3.94b	3.18c	0.043
Total PUFA	29.18	19.57	23.97	28.57	20.46	17.34a	17.87b	17.11a	0.151

[1] SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid. See Table 2 for more abbreviations.
[2] Values for feed samples in this table correspond to means (n = 9).
[3] Values for meat samples in this table correspond to least-squares means (n = 18) obtained from multifactor ANOVA (n = 54).
a-c Least-squares means for fatty acids of mixed raw dark and white chicken meat with skin with different letters differ significantly ($P \leq 0.05$). Statistical results for feeds are not stated.

Table 4.- Crude fat, α-tocopherol, Zn, Se, Fe and Cu content in mixed raw dark and white chicken meat with skin expressed per 100 g of edible portion[1].

Factor studied	crude fat[2]	α-tocopherol	Zn content	Se content	Fe content[3]	Cu content
	%	mg	mg	µg	mg	µg
Fat source						
Fish oil	10.5	2.18	0.855	15.5	0.472	27
Linseed oil	11.1	2.08	0.851	15.5	0.500	24
Animal fat	10.5	2.01	0.870	14.7	0.503	27
Zn supplementation						
0 mg/kg	10.9	2.13	0.864	11.5a	0.487	25
300 mg/kg	10.5	2.00	0.854	12.9b	0.506	26
600 mg/kg	10.7	2.13	0.864	21.3c	0.487	27
Se supplementation						
0	10.6	2.19	0.867	14.8a	0.487	23
Selenite, 1.2 mg/kg	10.7	2.04	0.862	15.1ab	0.494	29
Se-yeast, 0.2 mg/kg	10.9	2.03	0.847	15.8b	0.493	29
Pooled SEM	0.12	0.041	0.0058	0.17	0.0056	1.4

[1] Values given in this table correspond to least-squares means (n = 18) obtained from multifactor ANOVA (n = 54).
[2] Significant interactions between fat source x Zn supplementation and between Zn x Se supplementation
[3] Significant interaction between fat source x Se supplementation.
a-c Values corresponding to a certain factor with different letters differ significantly ($P \leq 0.05$).

Table 5.- α-Tocopherol and Zn, Se, Fe and Cu content in feeds given from 40 to 45 d[1].

Factor studied	α-tocopherol mg/kg	Zn content mg/kg	Se content µg/kg	Fe content mg/kg	Cu content mg/kg
Fat source					
Fish oil	122	460	581	465	17a
Linseed oil	114	425	507	400	13b
Animal fat	119	378	493	399	15ab
Zn supplementation					
0 mg/kg	115	131a	560	406	16
300 mg/kg	120	419b	504	430	15
600 mg/kg	120	712c	517	428	13
Se supplementation					
0	117	388	136a	435	14
Selenite, 1.2 mg/kg	120	457	1069b	404	15
Se-yeast, 0.2 mg/kg	118	412	376c	425	15
Pooled SEM	1.4	11	20	21	1.7

[1] Values given in this table correspond to least-squares means (n = 9) obtained from multifactor ANOVA (n = 27).
a-c Values corresponding to a certain factor with different letters differ significantly ($P \leq 0.05$).

Table 6.- Effect of the dietary factors on consumer acceptability[1] scores and TBA values (µg of malondialdehyde/kg) of cooked dark chicken meat after 74 d and 18 mo of storage at –20 C[2].

Factor studied	After 74 d of frozen storage		After 18 mo of frozen storage	
	Acceptability	**TBA**	**Acceptability**	**TBA[3]**
Fat source[4]				
Fish oil	4.9	86	5.1	137
Linseed oil	5.0	84	5.1	139
Animal fat	5.1	74	5.3	139
Zn supplementation[5]				
0 mg/kg	4.9	93	5.1	137
600 mg/kg	5.1	71	5.2	140
Se supplementation[4]				
0	5.0	91	5.3	141
Selenite, 1.2 mg/kg	5.2	73	5.1	135
Se-yeast, 0.2 mg/kg	4.8	83	5.0	139
Pooled SEM	0.11	4.4	0.10	1.2
Blind control[6]				
mean	4.2	267	4.9	202
SE	0.26	2.7	0.22	1.4

[1] Overall acceptability was ranked using a 9-point scale (1 = very bad; 9 = very good).
[2] Values given in this table correspond to least-squares means obtained from multifactor ANOVA, except for mean values of the blind control.
[3] Significant interaction between Zn x Se supplements.
[4] n values are 186, 198 and 6 for acceptability after 74 d, acceptability after 18 mo and TBA values, respectively.
[5] n values are 279, 297 and 9 for acceptability after 74 d, acceptability after 18 mo and TBA values, respectively.
[6] n values are 93, 99 and 3 for acceptability after 74 d, acceptability after 18 mo and TBA values, respectively.

Table 7.- Nutrients provided by 100 g of edible portion (mix of raw dark and white chicken meat with skin) depending on different dietary factors and comparison with the human recommended daily dietary intakes (mg/d).

	Linolenic acid			**EPA plus DHA[1]**			**Vitamin E**	**Selenium**					
	Fat source			**Fat source**			**All**	**Zn supplements (mg/kg)**			**Se supplements[2]**		
	FO	**LO**	**AF**	**FO**	**LO**	**AF**	**treatments**	**0**	**300**	**600**	**0**	**selenite**	**Se-yeast**
100-g edible portion[2]	186	889	174	137	108	110	2.00-2.19	0.0115	0.0129	0.0213	0.0148	0.0151	0.0158
Recommended		1,600[4]			650[5]		15[6]				0.055[6]		
% Recommendation[7]	11.6	55.6	10.9	21.1	16.6	16.9	13.3-14.6	20.9	23.4	38.7	26.9	27.5	28.7

[1] EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.
[2] Selenite provided 1.2 mg Se/kg of feed whereas Se-yeast provided 0.2 mg Se/kg of feed.
[3] Results for fatty acids are calculated by taking the fat content average (10.7%).
[4] Adequate Intake, Food and Nutrition Board (2002a).
[5] Simopoulos et al. (2000).
[6] Recommended Dietary Allowances, Food and Nutrition Board (2000).
[7] Values are the percentages of the recommended daily dietary intakes provided by 100 g of edible portion.

1.4. Increase of Geometrical and Positional Fatty Acid Isomers in Dark Meat from Broilers Fed Heated Oils

Títol: Increment dels isòmers geomètrics i de posició d'àcids grassos en la cuixa de pollastres alimentats amb olis escalfats

Resum: L'oxidació dels AGPI dóna lloc a compostos d'oxidació primària i secundària. Els compostos i les quantitats d'aquests productes varien depenent de les condicions d'oxidació. Com que aquests productes d'oxidació presenten diferent absorció i efectes biològics, hem dut a terme, en oli de gira-sol, dos tractaments tèrmics diferents. El primer va ser escalfant l'oli a 190-195 ºC durant 28 hores (anomenat oli molt oxidat), i l'altre escalfant a 55-60 ºC durant 12 dies (anomenat oli peroxidat).

Dins d'aquest estudi, es van comparar les composicions en àcids grassos de l'oli de gira-sol sense escalfar (oli fresc), l'oli peroxidat, l'oli molt oxidat, i una barreja (1+1) de l'oli fresc i l'oli molt oxidat (anomenat oli oxidat). Les composicions en àcids grassos dels olis, i en conseqüència dels pinsos, van ser afectades pels tractaments tèrmics. A més, es van formar diferents isòmers d'àcids grassos quan l'oli es va escalfar a 190-195 ºC i, també, es van trobar diferències significatives entre la composició en àcids grassos dels pinsos addicionats amb l'oxidat i el molt oxidat.

A continuació, es va estudiar l'efecte d'alimentar pollastres amb aquests olis i la suplementació amb Zn i acetat d'α-tocoferol sobre la composició en àcids grassos de la carn de la cuixa crua. Varis isòmers d'àcids grassos *trans* van augmentar en la carn de la cuixa de pollastres alimentats amb oli oxidat i molt oxidat. A més, l'anàlisi discriminant va mostrar que el contingut en àcids *ditrans*-octadecadienoics amb els dobles enllaços no interromputs per un grup metil permetia distingir aquelles carns de cuixa de pollastre que havien estat alimentats amb olis escalfats a 190-195 ºC.

INCREASE OF GEOMETRICAL AND POSITIONAL FATTY ACID ISOMERS IN DARK MEAT FROM BROILERS FED HEATED OILS

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Abbreviation Key: BHT = butylated hydroxytoluene; CLA = Conjugated linoleic acid; DMOX = dimethyloxazoline; FAME = fatty acid methyl ester; FSO = fresh sunflower oil; LA = linoleic acid; NMIOD = non-methylene interrupted octadecadienes; OSO = oxidised sunflower oil; PSO = peroxidised sunflower oil; VOSO = very oxidised sunflower oil.

ABSTRACT.

Oxidation of polyunsaturated fatty acids leads to primary and secondary oxidation products. Compounds and amounts of these products vary, depending on the oxidative conditions. As these oxidation products have different absorption and biological effects, we performed two different heating treatments on sunflower oil. The first was heating the oil at 190-195 ºC for 28 h (i.e. very oxidised oil); and the other, heating at 60 ºC for 12 days (i.e. peroxidised oil).

In the frame of this study, we compared the fatty acid composition of a refined sunflower oil (fresh oil), the peroxidised oil, the very oxidised oil, and a mixture (1+1) of fresh and very oxidised oil (i.e. oxidised oil). Oil fatty acid compositions were affected by the heating treatments. In addition, different fatty acid isomers were formed during heating at 190-195 ºC and significant differences were found between oxidised and very oxidised oils.

We also studied the effect of feeding broilers with these oils and Zn and tocopherol supplements on the fatty acid composition of their raw dark meat. Various *trans* fatty acid isomers increased in dark meat from broilers fed oxidised and very oxidised oils. In addition, discriminant analysis showed that *ditrans*-NMIOD content was able to distinguish dark chicken meat from chickens fed sunflower oils heated at 190-195 ºC.

Key words: Heated sunflower oils, fatty acid composition, *trans* isomers, CLA, chicken meat.

1.- INTRODUCTION

Fatty acids lead to different geometrical and positional isomers after thermal treatments (Sébédio et al., 1986; Grandgirard et al., 1984; O'Keefe et al., 1993; Destaillats and Angers, 2002), though only temperatures above 180-190 ºC yield *trans* fatty acids (TFA) in fats and oils (Martin et al., 1998; Wolff, 1993a). During deep-fat frying these temperatures are often reached and sometimes surpassed, which means that TFA, even though they are minor components, increase in frying oils (Gamel et al., 1999; Sébédio et al., 1996a; Romero et al., 2000) and fried products (Sébédio et al., 1996a; Romero et al., 2000). In addition, fatty acids from foodstuffs can also undergo geometrical and positional isomerisation as a result of thermal treatments or cooking procedures (Precht et al., 1999; Daglioglu et al., 2000).

Although oil heating increased TFA, refined fresh vegetable oils also contain geometrical and positional isomers. The high temperature at the deodorisation step during refining is the main factor causing their formation (Schwarz, 2000a; Cmolík and Pokorný, 2000; Cmolík et al., 2000; Juanéda et al., 2003). In addition, geometrical and positional isomers can also be formed during partial hydrogenation of vegetable oils (Ackman and Mag, 1998; Jung et al., 2002; Ledoux et al., 2000). These are mainly monoalkenoic geometrical and positional isomers (Ledoux et al., 2000; Precht and Molkentin, 2000); whereas those formed during refining or heating are mostly *monotrans*, di and trialkenoic fatty acids derived from polyunsaturated fatty acids (Ledoux et al., 2000; Wolff et al., 1998).

Hence, conjugated linoleic acids (CLA), a specific group of positional and geometrical isomers derived from linoleic acid (LA), can also be formed by heating, refining or partial hydrogenation of oils (Sébédio et al., 1988; Destaillats and Angers, 2002; Juanéda et al., 2003; Jung et al., 2002; Fritsche et al., 1999; Jung et al., 2001).

Nevertheless, *trans* isomers also have a natural origin in the action of bacteria in the rumen of cows and other ruminants and are found in the milk and carcass of these animals: vaccenic acid (*trans*-11-octadecenoic) is the main *monotrans* isomer (Craig-Schmidt, 1992); and *cis*-9,*trans*-11-octadecadienoic, the main CLA isomer (Pariza et al., 2001). In addition, vaccenic acid is converted to *cis*-9,*trans*-11-octadecadienoic by Δ^9 desaturase action in lactating ruminants' mammary glands

(Thompson and Christie, 1991). Thus, TFA have been a part of the human diet for several centuries.

Trans and *cis* isomers do not show significant differences in their absorptions (Sébédio and Chardigny, 1998; Holmer, 1998; Emken, 1995; Hayakawa et al., 2000). Thus, TFA in feeds can increase their content in foods derived from non-ruminant animals, which is normally lower than in foods derived from ruminant animals (Boatella et al., 1993; Aro et al., 1998a; Chin et al., 1992; Fritsche and Steinhart, 1998). In relation to this, CLA also increase in meat (Szymczyk et al., 2001; Demaree et al., 2002; Averette-Gatlin et al., 2002; Raes et al., 2001) and eggs (Ahn et al., 1999; Du et al., 1999) when they are added to the feeds.

Nowadays, there is a tendency to lower TFA content, especially in margarines and fatty spreads (Schwarz, 2000b). This is because, even though the adverse health effect of TFA is subject to controversy, its intake has been described as a risk factor for coronary heart disease (Willett et al., 1993; Kromhout et al., 1995; Aschiero et al., 1999; Oomen et al., 2001). This view is supported by the less favourable LDL/HDL ratio (Mensink and Katan, 1990) and the greater concentration of lipoprotein(a) (Kritchevsky, 2000; Noone et al., 2002) caused by TFA than by saturated fatty acids. However, despite these undesirable effects of TFA, CLA may have anticarcinogenic activity, protect against atherosclerosis and show other positive physiological effects (Pariza et al., 2001; Kritchevsky, 2000; Noone et al., 2002; Belury, 2002). One of these effects is of major importance for animal nutritionists, in that CLA reduce fat deposition in favour of lean mass accretion (Simon et al., 2000; Evans et al., 2002).

In addition, in heated oils and fats, TFA are accompanied by several lipid oxidation products that have harmful biological effects (Esterbauer et al., 1991; Cohn, 2002; Schroepfer, 2000; Guardiola, et al., 2002). Some of these oxidation products are absorbed and then deposited in animal tissues (Cohn, 2002; Schroepfer, 2000; Guardiola et al., 2002; Wilson et al., 2002; Chow, 1992). In addition, these compounds, at high doses, affect animal growth and metabolism (Billek, 2000).

Therefore, we studied the formation of geometrical and positional fatty acid isomers in sunflower oil oxidised at various heating temperatures. By adding these heated oils to broiler diets, we studied the deposition of these isomers in dark chicken

meat, and whether their contents in meat can be used as markers of the addition of heated oils into the feeds.

2.- MATERIALS AND METHODS

Preparation of Heated Oils

Two thermal treatments were carried out. The first was to heat 40 L of refined fresh sunflower oil (FSO) in an indirect heating fryer at 55-60 ºC under agitation for 12 days. This reached a peroxide value of 90 milli-equivalents peroxide/kg oil, i.e. peroxidised sunflower oil (PSO). The second involved heating 90 L of FSO in a direct heating fryer at 190-195 ºC under agitation for 28 h until reaching a *p*-anisidine value of 150, i.e. very oxidised sunflower oil (VOSO). By mixing 50% of FSO with 50% of VOSO, we obtained oxidised sunflower oil (OSO). Various oxidation variables and the α-tocopherol content of these sunflower oils are shown in Table 1.

Diets and Animals

A 4 x 2 x 2 factorial design was planned and conducted in triplicate to study the influence of various dietary factors (4 types of sunflower oil, 2 levels of *dl*-α-tocopheryl acetate supplementation, and 2 levels of Zn supplementation) on the fatty acid composition of raw dark chicken meat.

336 female broiler chicks (Hubbard, 1-day old) received, up to 10 days of age, a typical basal diet containing 6% of animal fat. The animals were then randomly assigned to 16 dietary treatments (21 chicks per treatment, 7 birds per pen) and were fed *ad libitum* for 42 days. Dietary treatments were prepared from the experimental basal diet (Table 2) by combination of the dietary factors studied: the 4 previously described sunflower oils (FSO, PSO, OSO and VOSO), *dl*-α-tocopheryl acetate supplements (0 and 100 mg/kg) and Zn supplements (0 and 600 mg/kg as $ZnSO_4$). In addition, all dietary treatments were supplemented with 0.6 mg/kg of organic selenium. α-Tocopherol losses caused by thermal treatments (see Table 1) were

rectified by adding *dl*-α-tocopheryl acetate to feeds containing PSO, OSO and VOSO in order to provide the same α-tocopherol amounts as feeds prepared with FSO.

FSO was supplied by Moyresa (Valencia, Spain). After the 4 assayed sunflower oils were prepared (described above), they were frozen in 20-L capacity drums at -20 ºC until feed preparation, in order to reduce further oxidation. The 16 dietary treatments were prepared every 10 days. Oils were thawed at room temperature and then feeds were prepared as previously described. Finally, chickens were slaughtered according to commercial procedures. Legs with skin from all birds of each pen were hand-deboned, ground, vacuum-packed in high-barrier multilayer bags (approx. 20 g per bag) and immediately stored at -20 ºC till analysis.

Reagents and Standards

$ZnSO_4$ and *dl*-α-tocopheryl acetate added to the feeds were purchased from Andrés Pintaluba (Reus, Spain). Organic selenium (Sel-plexTH) derived from selenium-enriched yeast was supplied by Probasa (Barcelona, Spain).

For analyses, butylated hydroxytoluene (BHT), pyrogallol, and *dl*-α-tocopherol were obtained from Sigma (St. Louis, USA). 2-Amino-2-methyl-1-propanol and *p*-anisidine were obtained from Aldrich (Milwaukee, USA). n-Hexane was from Merck (Darmstadt, Germany).

A mixture of CLA methyl esters (main isomers were *cis*-9,*trans*-11, *trans*-9,*cis*-11, *trans*-10,*cis*-12 and *cis*-10,*cis*-12; also traces of *trans*-9,*trans*-11 and other isomers were present) and standards of single fatty acid methyl esters [tetradecanoate (14:0), hexadecanoate (16:0), octadecanoate (18:0), eicosanoate (20:0), docosanoate (24:0), *cis*-9-hexadecenoate (16:1 n-7), *cis*-9-octadecenoate (18:1 n-9), *cis*-11-eicosenoate (20:1 n-9), *cis*-9,*cis*-12-octadecadienoate (18:2 n-6), all-*cis*-6,9,12-octadecatrienoate (18:3 n-6), *cis*-11,*cis*-14-eicosadienoate (20:2 n-6), all-*cis*-8,11,14-eicosatrienoate (20:3 n-6), all-*cis*-5,8,11,14-eicosatetraenoate (20:4 n-6), all-*cis*-7,10,13,16-docosatetraenoate (22:4 n-6), all-*cis*-9,12,15-octadecatrienoate (18:3 n-3), all-*cis*-5,8,11,14,17-eicosapentaenoate (20:5 n-3), all-*cis*-7,10,13,16,19-docosapentaenoate (22:5 n-3), all-*cis*-4,7,10,13,16,19-docosahexaenoate (22:6 n-3), *trans*-9-octadecenoate (*trans*-9-18:1)] were from Sigma. Methyl *cis*-11-octadecenoate

(18:1 n-7) was from Fluka (Buchs, Switzerland). A mixture of the 4 geometrical isomers of LA, used as methyl esters (LA *cis/trans* isomer mixture), and a fatty acid methyl ester (FAME) mixture from menhaden fish oil were purchased from Supelco (Bellefonte, USA).

Oil Analyses

To determine the α-tocopherol content, 300 mg of oil were weighed and 5 mL of absolute ethanol containing 1% pyrogallol (wt/vol), 0.012% BHT (wt/vol) and 0.4% anhydrous citric acid (wt/vol) was added. Then, 10 mL of 1.6 N methanolic KOH was added and saponification was carried out at 70 ºC for 30 min. Non-saponifiable contents were then extracted with petroleum ether and filtered through a 0.45 µm teflon membrane. After solvent evaporation under a nitrogen stream at 30 ºC, the residue was redissolved in 96% ethanol. The solution was separated by chromatography, using a Hewlett-Packard (Waldbronn, Germany) Series 1100 model liquid chromatograph equipped with a Rheodyne (Cotati, CA) 7725i model injector with a loop volume of 20 µL, a Teknokroma (Barcelona, Spain) column (15 x 0.4 cm) packed with 3 µm - 80 Å Extrasil ODS2 and a pre-column (1 x 0.4 cm) packed with 5 µm - 100 Å Kromasil ODS. Sample compounds were isocratically eluted with methanol and detected by means of a Hewlett Packard-1046A (Waldbronn, Germany) spectrofluorometric detector (excitation and emission wavelengths of 288 and 330 nm, respectively). α-Tocopherol content was determined by means of an experimental calibration curve using *dl*-α-tocopherol as external standard.

Peroxide value was determined by the AOCS Official Method (AOCS, 1998a), as was *p*-anisidine value (AOCS, 1998b). Oil-specific absorbances at 232 and 270 nm (K_{232} and K_{270}) were determined as described by Grau et al. (2001). Finally, polymer content, expressed as the percentage of polymerised triacylglycerols, was determined using the IUPAC Standard Method (IUPAC, 1987). All analyses were conducted in triplicate.

Fat Extraction for Meat and Feed analysis

1 g of dark chicken meat sample or 3 g of milled feed were weighed into 32 x 210 mm tubes. 20 mL of chloroform/methanol (2:1, v/v) was then added and the mixture was homogenized for 40 s at 19,800 rpm using a Polytron PT 2000 (Kinematica, Lucerne, Switzerland). Extracts were filtered through a Whatman nº1 filter paper into 50 mL screw-capped tubes and the residues were re-extracted twice with the same solvent: first with 7 mL (30 s at 19,800 rpm) and then with 5 mL (10 s at 19,800 rpm). 10 mL of water was then added to these tubes, which were stoppered and shaken for 30 s before being centrifuged for 20 min at 500 *g*. The chloroform phase was filtered through anhydrous sodium sulphate (using a Whatman nº1 filter paper), which was then washed twice with 5 mL of chloroform. The lipid extract obtained was concentrated to 1 mL in a vacuum-rotatory evaporator at 35 ºC. The rest of the solvent was removed in a light nitrogen stream, and then by the flask's being kept in a vacuum desiccator (10 mm Hg, overnight).

Gas Chromatographic Analysis of FAME

FAME were prepared from sunflower oils or from extracted lipid fractions after reaction with sodium methoxide followed by boron trifluoride in methanol and finally extracted with n-hexane (Guardiola et al., 1994). This two-step procedure (base and acid-catalyzed methylation) allows the methylation of free fatty acids while the isomerisation of CLA by acid-catalyst is limited (Kramer et al., 1997; Raes et al., 2002). FAME was analysed on an Agilent (Waldbronn, Germany) 4890D model gas chromatograph, fitted with a flame-ionization detector and split-splitless injector port, set at 300 and 270 ºC, respectively. The split ratio was 1:30. Chromatographic separation of FAME was performed on a fused-silica capillary column (60 m x 0.25 mm i.d.) coated with 0.2 μm of a stationary phase of 90% biscyanopropyl- plus 10% cyanopropylphenyl-polysiloxane (Supelco SP-2380 model; Bellefonte, USA). Helium, at 30 psi, was used as carrier gas and the oven was programmed as follows: 12 min at 170 ºC, then increased at 1.4 ºC/min to 180 ºC and held for 0.5 min, thereafter increased at 7.3 ºC/min to 216 ºC and held for 2 min and, finally, increased at 5 ºC/min

to 236 ºC and held for 6 min; sample volume injected 0.5-1 µl. All samples were injected in duplicate.

Gas Chromatography/Mass Spectrometry Analysis of DMOX Derivatives

Derivatisation of FAME by reaction with 2-amino-2-methyl-1-propanol giving the 4,4-dimethyloxazoline (DMOX) derivatives was carried out in a similar way to Fay and Richli (1991). About 10 mg FAME, dissolved in n-hexane, were placed into a microreaction vial and evaporated under nitrogen stream at room temperature. Then, 500 µl of the above-mentioned derivatising reagent was added to the vial, which was immediately closed after being flushed with argon. The reaction was conducted by heating at 180 ºC for 18 h. After cooling, the reaction mixture was dissolved in 5 mL of diethyl ether/hexane (1:1) and washed first with 5 mL and then with 3 mL of water (1% NaCl). Afterwards, the organic solution was dried through anhydrous sodium sulphate and then filtered and subsequently evaporated under a nitrogen stream. Finally, the residue was dissolved again in the appropriate n-hexane volume for injection.

Mass spectra were obtained with the Thermo Finnigan (San Jose, USA) MD800 model coupled to a Thermo Finnigan 8,000 Top gas chromatograph. The same column as in FAME analysis was used. Helium was used as carrier gas and the chromatograhic conditions were as follows: oven temperature programmed for 12 min at 180 ºC, increased at 1.4 ºC/min to 190 ºC and held for 1 min, then increased at 7.3 ºC/min to 226 ºC and held for 2 min and, finally, increased at 5 ºC/min to 245 ºC and held for 8 min; injector temperature 270 ºC; split ratio 1:40; head pressure 30 psi; sample volume injected 2 µl. The mass spectrometer was operated in full scan mode (45-450 m/z). Mass spectrometry conditions were: interphase temperature 260 ºC; ion source temperature 200 ºC; electron energy 70 eV.

Identification and Quantification of Fatty Acids

Fatty acids were identified by comparison of the relative retention times with FAME standards and by addition of these standards to the samples (co-

chromatography). Fatty acids were also confirmed by gas chromatography/ mass spectrometry through DMOX derivatives. The area of each FAME peak was integrated by using ChemStation software, and total peak area was used to calculate the relative fatty acid composition.

Statistical Analyses

ANOVA (n = 16) was used to study the effect of thermal treatments on sunflower oil and feed fatty acid composition. Multifactorial ANOVA (n = 48) determined whether the dietary factors studied had any significant effects on the fatty acid composition of dark chicken meat. Interactions between factors higher than an order of two were ignored. Oil and feed means and dark chicken meat least-squares means for the main factors with a significant effect were separated by Duncan's test. A discriminant analysis, in which dark chicken meat fatty acid contents were taken as the discriminant variables and the four sunflower oils as the grouping variable, was performed. In addition, we studied the discriminating power of the variables involved in the former analysis by means of Wilks' lambdas (Wilks' lambda varies from 0 to 1; the smaller the lambda for a variable, the more that variable contributes to the discriminant function). A linear regression analysis of dark chicken meat fatty acid composition *versus* their feed content was also performed. In all cases, $P \leq 0.05$ was considered significant.

3.- RESULTS AND DISCUSSION

Identification of FAME

Identification was mainly achieved by FAME standards, and confirmed in oil and meat samples by means of DMOX derivatives analysed by mass spectrometry.

Although *trans*-9-octadecenoic acid was tentatively identified by the corresponding FAME standard, this peak was found by DMOX derivative mass spectra to be a group of positional *trans*-18:1 isomers. In addition, FAME of the *cis*-9,*trans*-12- and *trans*-9,*cis*-12-LA isomers were well resolved when an LA isomer

mixture was analysed. However, there was poor resolution between these isomers when oil, feed and meat were analysed, which made it necessary to take them together as *monotrans*-LA, while the *trans*-9,*trans*-12-LA (*ditrans*-LA) was well resolved. The identity of these isomers was confirmed by mass spectrometry of the DMOX derivatives.

Through the CLA standard, 3 possible CLA peaks' were also found in samples with the DMOX mass spectra characteristic of a fatty acid structure of 18 carbon atoms and two double bonds (m/z 333). The first eluted peak showed 3 intense typical diagnostic fragment ions (m/z 182, 262, 276) and characteristic losses of 12 amu (196, 208 and 222, 234), which led to identification of the compound as a CLA with the double bonds located at carbons 9 and 11. The second eluted peak showed 3 intense typical diagnostic fragment ions (m/z 210, 290, 304) and characteristic losses of 12 amu (210, 222 and 236, 248), which identified the compound as a CLA with the double bonds located at carbons 10 and 12. Hence, the analysis of the CLA standard and the previous literature (Kramer et al., 2001, 2002; Roach et al., 2002) led to identification of the first peak as *cis*-9,*trans*-11-octadecadienoic acid (9c,11t-CLA) and the second peak as *trans*-10,*cis*-12-octadecadienoic acid (10t,12c-CLA). However, the third eluted peak was identified as a mixture of different isomers. Therefore, in accordance with other reported descriptions of GC chromatograms (Kramer et al., 2001, 2002; Roach et al., 2002), we identified the peak as a mixture of *ditrans*-non-methylene interrupted octadecadienes (*ditrans*-NMIOD). Hence, this mixture can contain other isomers than *ditrans*-CLA.

Moreover, traces of 13-docosenoic (probably *cis*-13) and 15-tetracosenoic (probably *cis*-15) were found to elute together with 20:4 n-6 and 22:4 n-6, respectively.

Two more peaks with no suitable standards were identified. The first was identified by means of DMOX derivative mass spectra as a hexadecenoic acid (m/z 307) with 3 intense diagnostic fragment ions (m/z 180, 208, 264) and characteristic losses of 12 amu (168, 180), which led to its identification as 7-hexadecenoic (16:1 n-9), probably the *cis* isomer according to the literature (Adlof and Emken, 1986). The second peak was identified as all-*cis*-4,7,10,13,16-docosapentaenoic acid (22:5 n-6) on the basis of bibliographical data (Guardiola et al., 1994; Simopoulos and Salem, 1992), because there was no suitable standard and DMOX mass spectra did not show typical

ion fragments for any penta or hexaenoic fatty acids. This may be due to the low amounts present for these fatty acids and the harsh conditions for DMOX derivatisation.

Oil Fatty Acid Composition

Thermal treatments led to significant differences in sunflower oil fatty acid composition. As relative SFA content increases on heating, the lowest total SFA content was observed in FSO (Table 3). Although total SFA increased in all heated oils more than in FSO, the lowest increase was found when sunflower oil was heated at low temperatures. Some MUFA showed a similar trend, although the significant differences between treatments were not as marked as for SFA. Despite this, total MUFA and oleic acid in the VOSO were significantly higher than in the other oils. Unlike this, both total n-6 PUFA and total n-3 PUFA decreased on heating due to oxidation. Total losses in LA were higher than in linolenic acid. This was mainly due to the higher amount in LA, since linolenic acid is actually more prone to oxidation. On the other hand, though MUFA could also be oxidised, it was less susceptible to oxidation than PUFA and so its content did not decrease. Therefore, it can be assumed that heating led to significant losses in PUFA, which were greater in VOSO, and in consequence caused relative SFA and MUFA enrichment. A similar trend was previously reported in linseed oil (Wolff, 1993a) and in sunflower oil (Juanéda et al., 2003) after thermal treatments. Moreover, this relationship has also been described in oils after their use for deep-frying and pan-frying of potatoes (Sébédio et al., 1990; Andrikopoulos et al., 2002).

For TFA, there were less *trans*-18:1 and geometrical isomers of LA in FSO than in heated oils (Table 3). These contents corroborate previous findings for TFA content in various heated oils (Cmolík et al., 2000; Juanéda et al., 2003; Wolff, 1993b; Aro et al., 1998b). However, some of these compounds occur naturally in crude vegetable oils in small amounts (Schwarz, 2000a, b; Cmolík et al., 2000). In addition, TFA are also formed during oil refining. Their formation depends mainly on deodorisation temperature and time, and on fatty acid composition (Schwarz, 2000a; Cmolík and Pokorný, 2000; Kemény et al., 2001; León-Camacho et al., 2001). For

ditrans-LA, although Hénon et al. (2001) did not find it present after rapeseed deodorisation, a very small amount can be found in refined oils. Thus, it is important to assess these minor *trans* components in various refined and heated vegetable oils because they can provide valuable information such as the conditions of refining processes (Kemény et al., 2001; León-Camacho et al., 2001; Hénon et al., 2001) or the abuse of heating temperature and time. In accordance with this, as TFA are mainly formed in edible oils during refining processes, the European Community regulates that virgin olive oil must not contain more than 0.05% of total *trans*-18:1 isomers or total *trans*-LA and 18:3 isomers, whereas in refined olive oils this amount can be higher (Commission Regulation, 2002).

As TFA in heated oils are formed at temperatures above 180 ºC (Martin et al., 1998; Wolff, 1993a), this explains the low *trans*-18:1 and *trans*-LA content found in FSO and why their content did not increase in PSO whereas it did in oxidised oils (OSO and VOSO) due to the heating temperature. However, the increase in TFA coming from unsaturated fatty acids is not big enough to explain the relative increase in MUFA and SFA. In fact, the decrease in PUFA was mainly due to the formation of low molecular weight compounds and other fatty acid oxidation products, which was confirmed by the higher peroxide value found for PSO, and the higher *p*-anisidine values and polymerised triacylglycerol contents found for OSO and VOSO (Table 1). This fatty acid oxidation could explain the presence of some very late-eluting unknown peaks (data not shown) in the chromatograms of peroxidised and oxidised oils, which were higher for VOSO.

To show TFA formation due to heating, Aro et al. (1998b) reported the *trans* content in oils reutilised for deep-frying. Values of *trans*-18:1 and *trans*-LA, reported by these authors in sunflower oil, ranged from 0.03 to 0.11% and from 0.5 to 0.85%, respectively. Thus, though oxidation processes in food frying are different from those when only heating fats and oils, our results (Table 3) corroborated those of Aro et al. (1998b). In addition, these authors indicated that geometrical LA isomers probably reflect the effect of reutilization better than *trans*-18:1, which may be affected by the exchange of fatty acids between fried foods and the oils (Romero et al., 2000; Sébédio et al., 1990). Thus, interesting results were the significant increase in *trans*-18:1 observed in OSO and VOSO, because monoenoic fatty acids are very resistant to *trans*

isomerisation (Schwarz, 2000a). However, Sébédio et al. (1988) also reported the presence of *cis* and *trans* monoenes after heating sunflower oil above 200 ºC. Similar results have been reported for rapeseed and soybean oil (Grandgirard et al., 1984).

On geometrical isomerisation, Wolff et al. (1996) postulated that the existence of transient free radicals in the methylene group led to the formation of adjacent methylene-interrupted ethylenic bonds. Though this is likely for geometrical isomers of LA, Juanéda et al. (2003) did not find an increase in *monotrans*-LA in sunflower oil heated at 180 ºC, whereas it did increase a lot at 220 ºC. Similar results were described by Sébédio et al. (1996b) in deep-fat frying of French fries in peanut or soybean oil. These authors found that the isomers increased at 220 ºC, while no differences were observed, even after 30 5-minute frying operations, at 180 ºC and 200 ºC. Therefore, confirming these authors' findings, the present results show that LA isomerisation occurs when sunflower oil is heated at temperatures above 190 ºC for 28 h.

It is also worth commenting on the increase in *ditrans*-LA isomers in oxidised sunflower oils (Table 3), because *ditrans* isomers were only found in linolenic acid heated above 200 ºC (Grandgirard et al., 1984). Further, even after 30 frying operations at 220 ºC, Sébédio et al. (1996b) found no *ditrans*-LA in oils used in deep fat frying of potatoes. Wolff (1993a), heating linseed oil at different times and temperatures, only found *ditrans*-LA after 8 h at 245 ºC, and Martin et al. (1998) found it above 220 ºC after 30 h of heating. However, Sébédio et al. (1988) found *ditrans*-LA after heating sunflower oil at 200 ºC for 48 h in a commercial fryer and also in used frying oil.

These controversial results for *ditrans*-LA amounts could be explained by a number of factors. For example, the manner and time of heating (Grandgirard et al., 1984; Wolff, 1993a), the composition and nature of unsaturated fatty acids (Martin et al., 1998; Wolff et al., 1996), and their arrangement on the triacylglycerols (Martin et al., 1998) all affect *trans* formation.

On CLA content in edible oils, hydrogenation (Jung et al., 2001, 2002) and refining of oils can produce some positional isomerisation. Bleaching produces CLA by dehydration of hydroperoxides and other polar compounds (Cmolík et al., 2000). Nevertheless, deodorisation also increases CLA: Juanéda et al. (2003) found 0.1% of CLA after deodorisation of sunflower oil, which confirms the reported results for FSO

(Table 3). In addition, these authors also described CLA formation after heating sunflower oil at 180 ºC and 220 ºC. The CLA formation found by them at 180 ºC was confirmed by our results for the OSO and VOSO oils. Actually, CLA formation in sunflower oil heated at high temperatures has been previously described (Sébédio et al., 1988).

Unlike LA geometrical isomers, CLA isomers of both oxidised oils (OSO and VOSO) had greater *ditrans*-NMIOD than *monotrans*-CLA. These results were consistent with other authors' results (Sébédio et al., 1988; Juanéda et al., 2003).

Although more *ditrans*-CLA is present than *monotrans*-CLA, Juanéda et al. (2003) found that the relative proportions of CLA isomers are temperature-dependent, with the relative *ditrans* content higher at high temperatures. Moreover, different relative proportions between *monotrans*- and *ditrans*-CLA have been previously reported under different heating conditions (Sébédio et al., 1988).

In relation to this, we found a ratio close to 2 for *ditrans*-NMIOD/(9c,11t- and 10t,12c-CLA) in VOSO treatment, which was not very different from the ratio value of 2.6 reported by Juanéda et al. (2003) when sunflower oil was heated at 180 ºC. A ratio value of 3 (*ditrans*-NMIOD/total identified *monotrans*-NMIOD) was also found by Sébédio et al. (1988) in used frying oils, probably heated at 180-190 ºC.

In relation to this, Juanéda et al. (2003) reported that at temperatures of 180 ºC the main *monotrans* isomers found were the 9c,11t-CLA and the 10t,12c-CLA, and the main *ditrans* isomers were the 9t,11t- and 10t,12t-CLA. These results confirm our results, though we only identified the 9c,11t-CLA and the 10t,12c-CLA and a peak where some *ditrans*-NMIOD were present, whereas other isomers were not detected. This finding is supported by the fact that some isomers have closer chromatographic elution times (Kramer et al., 2001, 2002), leading to a difficult peak identification. Moreover, a concerted pericyclic isomerisation mechanism, a (1,5) sigmatropic rearrangement, of CLA has also been described, suggesting that the conjugated system is limited by this mechanism to isomerisation from *cis*,*trans* to *trans*,*cis* and *viceversa* (Destaillats and Angers, 2002). In addition, these authors did not detect the presence of 8c,10t- or 11t,13c-CLA isomers in heated oils containing LA.

Therefore, the reported results seemed to confirm the presence of different geometrical and positional TFA due to isomerisation at temperatures above 190 ºC.

However, while some TFA such as *ditrans*-NMIOD are mainly formed during heating at high temperatures (Sébédio et al., 1988; Juanéda et al., 2003), others like 9c,11t-CLA are mainly found in ruminant animal fats (Chin et al., 1992).

Feed Fatty Acid Composition

As 6% of each sunflower oil was added to the feeds (up to 70% of total fat), feed fatty acid composition was greatly affected by the fatty acid composition of the added sunflower oil (Table 3). Despite this, feeds had more linolenic acid and total SFA and moderately lower total MUFA and total n-6 PUFA than added sunflower oils did. However, as feed fatty acid composition greatly reflects sunflower oil composition, FSO feed had less LA and increased total SFA and total MUFA than VOSO feed. However, no significant differences between feeds were observed for linolenic acid because this fatty acid came mainly from feed ingredients other than sunflower oil. This finding confirms that the differences for fatty acids between feeds were less clear than for oils, due to dilution of sunflower oils in the basal feed lipid fraction.

For the content of TFA in feeds containing OSO and VOSO, compared with TFA content in oils, a dilution effect, which indicates that *trans* isomers were slightly present in the basal diet, was also found.

This meant that the contents of 9c,11t- and 10t,12c-CLA did not differ between feeds containing OSO and VOSO. However, for other *trans* isomers, there were significant differences between feeds containing OSO and VOSO. Thus, these results led to significant differences between OSO and VOSO feeds for total LA isomers, total positional (NMIOD) isomers, total octadecadienoic (18:2) acid isomers and total isomers.

Meat Fatty Acid Composition

Total SFA, MUFA and PUFA were not affected by dietary oil source in dark chicken meat (Table 3). However, the same trend present in feeds and oils was observed for many fatty acids. For instance, despite not being significant, the differences observed for LA in feeds were also observed in chicken meat. In addition,

a significant increase in 20:0 was observed in dark meat coming from chickens fed OSO and VOSO diets, which seems to indicate that this fatty acid was largely influenced by the diet. Although the influence of feed fatty acid composition on chicken meat fatty acid profile has been described before (Ajuyah et al., 1991; Cherian et al., 1996; López-Ferrer et al., 2001; O'Neill et al., 1998), the slight differences between the fatty acid compositions of assayed feeds did not lead to other significant differences in meat fatty acid composition, apart from the differences observed for isomerised fatty acids (Table 3).

The reported amounts of geometrical isomers of LA and *trans*-18:1 (isomerised fatty acids) in dark chicken meat were much lower than those previously described in chicken meat (Aro et al., 1998a) and turkey meat (Wong and Sampugna, 1993). Aro et al. (1998a), who studied various meat products from several European countries, found that chicken and turkey meats had slightly more variable proportions of TFA than other meats. In this study, total *trans* content (CLA were not included) ranged from 0.24 to 1.7%. This great variability was attributed to feed composition differences. Actually, the incorporation of TFA into most tissues is roughly in proportion to their abundance in the diet of swine (Royce et al., 1983, Pettersen and Opstevedt, 1992) and rats (Bysted et al., 1998).

Consistent with this, Emkem (1995) reported in a review that TFA are subject to the same metabolic control mechanisms that regulate the metabolism of saturated and *cis* unsaturated fatty acids. However, whereas adipose tissue seemed not to incorporate or exclude selectively any specific fatty acid, other more specific tissues like brain have a low and relatively constant TFA content (Holmer, 1998; Emken, 1995).

In addition, some *trans*-18:1 isomers are hindered from incorporation into various tissue lipid classes, whereas other *trans*-18:1 are preferred (Holmer, 1998; Bysted et al., 1998). Table 3 shows that *trans*-18:1 content in chicken meat tended to be higher when VOSO was added to the feed, though the difference was not significant. In addition, the higher relative content of these fatty acids in dark chicken meat than in feeds seems to indicate that *trans*-18:1 are incorporated into dark chicken meat more quickly than other fatty acids (Table 3).

Monotrans-LA and *ditrans*-LA of dark chicken meat were also affected by the sunflower oil source added to the feed: they reflected the differences observed in the diets. Nevertheless, the LA/*ditrans*-LA isomer ratios in chicken meats were fairly different from those in the feeds, whereas the LA/*monotrans*-LA isomer ratios were similar to those in the diets, indicating that *monotrans*-LA isomers behave similarly to LA, which confirms previously reported results (Sébedio and Chardigny, 1998). In addition, the LA/*ditrans*-LA ratios in feeds and meats suggest that the incorporation rate in chicken meat is higher for *ditrans*-LA than for LA, which is similar to what happens for *trans*-18:1 isomers and oleic acid.

CLA, present in all dietary treatments (Table 3), were absorbed and deposited, in line with previously reported results, in chicken meat (Szymczyk et al., 2001; Simon et al., 2000; Badinga et al., 2003) and eggs (Raes et al., 2001; Ahn et al., 1999; Du et al., 1999). However, as dietary treatments showed no differences between 9c,11t-CLA and 10t,12c-CLA contents, they did not affect the content of these fatty acids in dark chicken meat.

Nevertheless, the ratios for these CLA isomers in feeds and meats seem to indicate that they were incorporated into dark meat at different rates. Thus, 9c,11t-CLA seemed to be more easily incorporated in dark chicken meat than 10t,12c-CLA (see results in Table 3). These results corroborate previous findings (Szymczyk et al., 2001; Simon et al., 2000; Badinga et al., 2003). However, it is not completely understood whether these results are due to an increased incorporation of 9c,11t-CLA or to a higher enzymatic modification (elongation, desaturation or β-oxidation) of 10t,12c-CLA (Evans et al., 2002; Sébédio et al., 1997; Sergiel et al., 2001). In addition, the likely biosynthesis of 9c,11t-CLA *via* the action of Δ^9 desaturase on *trans*-11-18:1, described in humans (Adlof et al., 2000; Turpeinen et al., 2002) and in other animals (Pariza et al., 2001; Palmquist and Santora, 1999; Gläser et al., 2000), should be taken into account.

Chin et al. (1992) also detected CLA in commercial chicken meat samples (0.09% CLA), in which 9c,11t-CLA was about 84% of total CLA. The reported content was higher than the levels we found (Table 3). Therefore, results found by these authors must be due to high amounts of CLA in the feeds, because CLA in chicken meat increases linearly with feed CLA content (Szymczyk et al., 2001).

On the other hand, *ditrans*-NMIOD showed significant differences between dietary sunflower oil sources, indicating the effect of feed fatty acid composition. However, this group of isomers were incorporated into dark meat less than 9c,11t-CLA and higher than 10t,12c-CLA. On this varying incorporation of isomers, Yang et al. (2002) reported that *ditrans*-NMIOD were preferentially incorporated into rat liver rather than other NMIOD and that there was also discrimination between *monotrans* isomers. Nevertheless, these authors also reported that rat milk CLA isomeric distribution reflected the distribution in the diet. In addition, these results for rat liver isomer composition seemed to confirm those reported for hepatic fatty acid composition in chickens fed a CLA mixture (Badinga et al., 2003).

α-Tocopheryl acetate supplementation in diet brings about an increase in 20:3 n-6, 22:4 n-6 and 22:5 n-3 and a decrease in 20:1 n-9. Similar increased amounts in very long-chain PUFA were found when chickens were supplemented with tocopherols (Cherian et al., 1996; Ajuyah et al., 1993; Surai and Sparks, 2000). Cherian et al. (1996) suggested that these PUFA were protected from oxidation by tocopherol supplementation.

Zn supplementation increased the *trans*-18:1 content. This is difficult to explain, even more so when the interaction between Zn supplementation and sunflower oil source in the diet is taken into account. It is difficult to explain why Zn supplementation decreases *trans*-18:1 content in dark meat coming from chickens fed PSO diet, while the *trans*-18:1 content is increased by Zn supplementation in meat from chickens fed FOS, OSO or VOSO diets. Thus, further studies are required to confirm the effect of Zn supplementation and its interaction on *trans*-18:1 content in dark chicken meat.

In accordance with these results, in which various fatty acids were increased by different dietary oils, we performed a discriminant analysis to find which fatty acids found in dark chicken meat could act as markers of the addition of oxidised oil into feeds. Canonical discriminant analysis revealed that 3 discriminant functions (Wilks' lambda = 0.037; $P < 0.001$) including various fatty acids (*ditrans*-NMIOD, 20:2 n-6, 22:5 n-3 and 9c,11t-CLA) distinguish correctly both OSO and VOSO from the other oils in dark chicken meat fed different sunflower oils (Figure 1). As can be seen, chickens fed FSO and PSO treatments were not distinguished clearly. Isomers

probably had the main responsibility for discriminating, because the cumulative proportion of explained variance in function 1 was 98.7%. In fact, discriminant analysis predicted correctly 58.3% of chickens fed FSO and 83.3% of chickens fed PSO, whereas 100% correct allocation to their groups was achieved for chickens fed with oxidised oils (OSO and VOSO).

Moreover, regarding individual Wilks' lambda-obtained values (lambda varies from 0 to 1, with 0 meaning that group averages differ), it can be confirmed that *ditrans*-NMIOD content was the main factor responsible for predicting group memberships (Table 4). In this table, each fatty acid or sum of fatty acids was ranked by its Wilks' lambda values. As can be seen, sums of fatty acid isomers also had lower lambda values, though they were not as low as for the *ditrans*-NMIOD lambda value. Moreover, the discriminant analysis run again with only *ditrans*-NMIOD distinguished fairly well dark chicken meats to whose feeds heated (above 190 ºC) sunflower oils (OSO and VOSO) were added (Table 4). Addition of the total 18:2 isomer content to the discriminant analysis did not improve its ability to distinguish groups. It was only improved after the addition of the 9c,11t-CLA isomer. This lack of effective discrimination of groups for fatty acids with lower lambdas can be explained, apart from their high values, by the strong correlations observed between these fatty acids and *ditrans*-NMIOD (data not shown). Finally, the addition of the 20:2 n-6 to the discriminant analysis led to the previously reported values. However, some isomers (*trans*-18:1 and 10t,12c-CLA) were not suitable for predicting group memberships of chicken meats to which different sunflower oils were added. This confirms the previously reported results, in that *trans*-18:1 was significantly affected by Zn supplementation (Table 3). On the other hand, the 20:0, which showed significant differences between chicken fed different oils, did not increase the correctly predicted group membership in the discriminant analysis, probably because it correlated closely with *ditrans*-NMIOD.

Since fatty acid isomers were able to predict groups, we also ran the regression analysis of these isomers in chicken meat and its feed content (Table 5). The best linear regression analyses were for *ditrans*-NMIOD dark chicken meat content *versus* *ditrans*-NMIOD feed content ($R^2 = 0.876$) and *versus* total NMIOD feed content ($R^2 = 0.878$). However, regression analysis for chicken meat content of 9c,11t-CLA *versus*

its feed content was not significant and did not show a good R^2. However, other authors feeding chickens with much higher doses than we did found that both 9c,11t- and 10t,12c-CLA content in chicken meats increased linearly with CLA content in feed (Szymczyk et al., 2001).

Thus, the content of *ditrans*-NMIOD and total NMIOD isomers were suitable markers, even at trace levels, of the addition of heated (above 190 ºC) sunflower oil to feeds. In addition, the reported effects and interactions caused by Zn and tocopherol supplements had no significant influence on the content of the proposed markers.

The relevance of *ditrans*-NMIOD as markers is due to their being mainly formed during fat and oil heating at high temperatures, while other potential markers, like 9c,11t-CLA, have other important origins like biohydrogenation, partial hydrogenation and refining. Nevertheless, 9c,11t- and 10t,12c-CLA were not completely necessary to distinguish dark meats from chickens fed these heated oils. Despite that, further studies are required to check the suitability of these isomers as tracers of the addition of heated fats and oils to feeds.

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Table 1. Oxidation Variables and α-Tocopherol Content of the Sunflower Oils Added to the Basal Diet.

Sunflower Oil	**PV[a]**	**pAV[1]**	**K^2_{232}**	**K^3_{270}**	**Polymers[4] [%]**	**α-tocopherol[5] [mg/l]**
Fresh	2.1	6.4	3.58	1.74	0.7	683
Peroxidised	91.9	8.5	13.66	1.78	0.7	480
Oxidised	7.0	83.1	10.28	3.25	4.9	655
Very oxidised	17.2	153.0	17.67	4.82	9.4	619

[1] PV: Peroxide value in millieq. peroxide/kg. AOCS Official Method (Cd 8-53).

[2] pAV: *p*-Anisidine value. AOCS Official Method (Cd 8-90).

[3] K_{232} and K_{270} are the oil-specific absorbances at 232 and 270 nm (Grau *et al*., 2001).

[4] Polymers expressed as percentage of polymerised triacylglycerols. IUPAC standard method 2.508.

[5] Tocopherol content analysis performed as described in Material and Methods section.

Table 2. Ingredients and Composition of the Basal Diet Fed from 11 to 42 Days of Age.

Ingredients	**Percentage**	**Composition**[1]	**Percentage**
Wheat	56.61	Dry matter	90.1
Soybean meal	24.69	Crude protein	20.9
Sunflower oil[2]	6.00	Crude fat	8.2
Sunflower meal	5.03	Crude fiber	3.6
Soy grass	3.14	Ash	6.1
Calcium carbonate	1.69		
Monocalcium phosphate	1.23		
Mineral-vitamin premix[3]	0.50		
L-Lysine	0.38		
DL-Methionine	0.26		
Salt	0.20		
Sodium bicarbonate	0.14		
Enzymes	0.12		
Choline chloride	0.03		

[1] Metabolizable energy 3,000 cal/g. Results shown are estimated values.

[2] Four sources of sunflower oil were added to the feeds depending on the dietary treatments.

[3] Include *dl*-α-tocopheryl acetate (30 mg/kg of feed) and Se-enriched yeast (0.6 mg Se/kg of feed).

TABLE 3. Fatty Acid Composition (Expressed as Area Normalization in %) of the Experimental Oils and Feeds, and the Effect of Dietary Factors on the Fatty Acid Composition of Dark Chicken Meat.

Fatty acid	Sunflower oils				Feeds with added sunflower oils				Dark chicken meat							
									Sunflower oil added				Tocopherol added [mg/kg]		Zn added [mg/kg]	
	FSO	**PSO**	**OSO**	**VOSO**	**FSO**	**PSO**	**OSO**	**VOSO**	**FSO**	**PSO**	**OSO**	**VOSO**	**0**	**100**	**0**	**600**
14:0	0.068a	0.070b	0.070b	0.073c	0.114	0.118	0.111	0.114	0.370	0.373	0.369	0.364	0.368	0.370	0.370	0.368
16:0	6.148a	6.256b	6.296b	6.503c	9.114a	9.309bc	9.204ab	9.379c	16.135	16.259	16.446	16.273	16.282	16.274	16.406	16.150
18:0	3.982a	4.075b	4.087c	4.206d	3.920a	3.961b	3.952ab	4.019c	5.729	5.625	5.710	5.598	5.650	5.681	5.644	5.687
20:0	0.280a	0.286b	0.289c	0.296d	0.287a	0.291ab	0.292b	0.297c	0.206ab	0.201a	0.224b	0.226b	0.214	0.215	0.211	0.218
22:0	0.714a	0.729b	0.737c	0.764d	0.564a	0.567a	0.576b	0.585c								
24:0	0.229a	0.230ab	0.232b	0.238c	0.197b	0.186a	0.200b	0.200b								
Total SFA	11.422a	11.646b	11.710c	12.080d	14.196a	14.431b	14.334b	14.593c	22.441	22.457	22.749	22.460	22.407	22.433	22.528	22.312
16:1 n-9	0.020	0.022	0.020	0.019	0.036a	0.039b	0.039b	0.040b	0.413	0.429	0.400	0.402	0.409	0.413	0.415	0.407
16:1 n-7	0.087a	0.089ab	0.089ab	0.095b	0.154	0.158	0.149	0.153	2.371	2.527	2.586	2.713	2.563	2.535	2.597	2.502
18:1 n-9	26.673a	27.152b	27.174b	27.719c	24.276a	24.470bc	24.395ab	24.596c	29.930	30.477	30.581	30.794	30.605	30.286	30.648	30.244
18:1 n-7	0.549	0.529	0.544	0.558	0.748	0.758	0.786	0.785	1.110	1.124	1.088	1.124	1.106	1.118	1.087	1.136
20:1 n-9	0.156a	0.158bc	0.157ab	0.159c	0.237	0.245	0.239	0.241	0.259	0.263	0.259	0.262	0.264a	0.258b	0.262	0.260
Total MUFA	27.486a	27.950b	27.985b	28.551c	25.452a	25.670b	25.607b	25.815c	34.084	34.820	34.914	35.295	34.947	34.610	35.008	34.548
18:2 n-6	60.379a	59.710b	59.463c	58.400d	57.254a	56.777b	56.838b	56.244c	39.294	38.613	38.292	38.011	38.458	38.647	38.243	38.862
18:3 n-6									0.109	0.109	0.109	0.106	0.108	0.108	0.108	0.108
20:2 n-6									0.315	0.309	0.295	0.303	0.304	0.307	0.304	0.307
20:3 n-6									0.238	0.247	0.242	0.256	0.240a	0.252b	0.245	0.247
20:4 n-6					0.028	0.030	0.028	0.031	0.889	0.882	0.820	0.891	0.845	0.895	0.861	0.880
22:4 n-6									0.257	0.249	0.227	0.244	0.235a	0.254b	0.241	0.247
22:5 n-6									0.063	0.063	0.058	0.065	0.060	0.065	0.061	0.063
Total n-6 PUFA	60.379a	59.710b	59.463c	58.400d	57.282a	56.807b	56.866b	56.275c	41.165	40.469	40.043	39.878	40.356	40.641	40.173	40.824
18:3 n-3	0.171a	0.164b	0.160c	0.148d	2.660	2.693	2.679	2.740	1.784	1.753	1.768	1.787	1.768	1.778	1.758	1.788
20:5 n-3									0.035	0.031	0.035	0.037	0.033	0.036	0.035	0.034
22:5 n-3									0.096	0.093	0.084	0.093	0.087a	0.096b	0.091	0.092
22:6 n-3					0.022	0.024	0.019	0.020	0.067	0.062	0.051	0.060	0.057	0.063	0.062	0.058
Total n-3 PUFA	0.171a	0.164b	0.160c	0.148d	2.683	2.716	2.698	2.760	1.983	1.937	1.938	1.976	1.945	1.973	1.946	1.972
Total PUFA	60.550a	59.874b	59.623c	58.548d	60.025a	59.523b	59.564b	59.035c	43.148	42.407	41.981	41.854	42.301	42.614	42.119	42.795
Trans-18:1	0.011a	0.011a	0.042b	0.066c	0.034a	0.033a	0.047b	0.057c	0.116	0.114	0.122	0.127	0.118	0.122	0.110a	0.129b
Ditrans-LA	0.021a	0.021a	0.048b	0.071c	0.005a	0.000a	0.038b	0.051c	0.046a	0.045a	0.051a	0.066b	0.052	0.052	0.053	0.051
Monotrans-LA	0.404ab	0.382a	0.428b	0.471c	0.247b	0.238a	0.276c	0.285d	0.187a	0.182a	0.201b	0.207b	0.193	0.196	0.196	0.193
Total LA isomers	0.425a	0.404a	0.476b	0.543c	0.252b	0.238a	0.313c	0.337d	0.233ab	0.227a	0.253b	0.272c	0.245	0.243	0.244	0.244
9c,11t-CLA	0.025a	0.032a	0.036b	0.041c	0.027	0.032	0.034	0.039	0.050	0.045	0.048	0.051	0.049	0.048	0.048	0.049
10t,12c-CLA	0.019	0.022	0.026	0.028	0.018	0.018	0.022	0.024	0.010	0.007	0.006	0.008	0.007	0.009	0.009	0.006
Ditrans-NMIOD	0.062a	0.061a	0.102b	0.144c	0.057a	0.058a	0.078b	0.100c	0.034a	0.036a	0.047b	0.060c	0.045	0.037	0.044	0.044
Total NMIOD	0.106a	0.115a	0.164b	0.213c	0.102a	0.108a	0.134b	0.164c	0.094a	0.086a	0.101a	0.119b	0.100	0.101	0.102	0.100
Total 18:2 isomer	0.532a	0.519a	0.640b	0.756c	0.354b	0.346a	0.447c	0.500d	0.327a	0.316a	0.354b	0.391c	0.345	0.349	0.350	0.344
Total isomers	0.543a	0.530a	0.682b	0.822c	0.388b	0.379a	0.494c	0.558d	0.444a	0.430a	0.475b	0.519c	0.463	0.470	0.461	0.473

Values given in this Table for sunflower oils (n = 16) and feeds (n = 16) are means, while for meat samples values are for least-squares means obtained from MANOVA (n = 48). Values in the same row with different letters differ significantly ($P \leq 0.05$). FSO = fresh sunflower oil, PSO = peroxidised sunflower oil, OSO = oxidised sunflower oil, VOSO = very oxidised sunflower oil. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; LA = linoleic acid; CLA = conjugated linoleic acid; NMIOD = non-methylene interrupted octadecadienes.

Table 4. Wilks' Lambda and Discriminant Analysis Results for Dark Chicken Meat Fatty Acids.

	Wilks' lambda	***P***	**Correctly predicted group memberships [cumulative %]**			
			FSO	**PSO**	**OSO**	**VOSO**
Ditrans-NMIOD	0.109	<0.001	58.3	50.0	83.3	100.0
Total 18:2 isomers	0.447	<0.001	58.3	50.0	83.3	100.0
Ditrans-LA	0.467	<0.001	58.3	50.0	83.3	100.0
Geometrical LA	0.491	<0.001	58.3	50.0	83.3	100.0
Total *trans*	0.515	<0.001	58.3	50.0	83.3	100.0
Total NMIOD	0.611	<0.001	58.3	50.0	83.3	100.0
Monotrans LA	0.645	<0.001	58.3	50.0	83.3	100.0
20:0	0.834	0.044	58.3	50.0	83.3	100.0
16:1 n-7	0.836	0.046	58.3	50.0	83.3	100.0
22:6 n-3	0.846	0.059	58.3	50.0	83.3	100.0
22:5 n-3	0.868	0.098	58.3	50.0	83.3	100.0
20:5 n-3	0.880	0.127	58.3	50.0	83.3	100.0
22:4 n-6	0.881	0.131	58.3	50.0	83.3	100.0
16:1 n-9	0.886	0.144	58.3	50.0	83.3	100.0
18:1 n-9	0.887	0.150	58.3	50.0	83.3	100.0
9c,11t-CLA	0.891	0.162	58.3	83.3	91.7	100.0
18:2 n-6	0.893	0.170	58.3	83.3	91.7	100.0
20:3 n-6	0.905	0.219	58.3	83.3	91.7	100.0
20:2 n-6	0.912	0.252	58.3	83.3	100.0	100.0
20:4 n-6	0.925	0.325	58.3	83.3	100.0	100.0
22:5 n-6	0.940	0.432	58.3	83.3	100.0	100.0
18:3 n-3	0.948	0.498	58.3	83.3	100.0	100.0
Trans-18:1	0.949	0.507	58.3	83.3	100.0	100.0
18:0	0.959	0.599	58.3	83.3	100.0	100.0
14:0	0.966	0.670	58.3	83.3	100.0	100.0
16:0	0.970	0.718	58.3	83.3	100.0	100.0
18:3 n-6	0.970	0.721	58.3	83.3	100.0	100.0
10t,12c-CLA	0.976	0.784	58.3	83.3	100.0	100.0
18:1 n-7	0.979	0.815	58.3	83.3	100.0	100.0
20:1 n-9	0.986	0.890	58.3	83.3	100.0	100.0

FSO = chicken fed fresh sunflower oil diet, PSO = chicken fed peroxidised sunflower oil diet, OSO = chicken fed oxidised sunflower oil diet, VOSO = chicken fed very oxidised sunflower oil diet, NMIOD = non-methylene interrupted octadecadienes, CLA = conjugated linoleic acid, LA= linoleic acid.

Table 5. Regression Equations of Selected Dark Chicken Meat Fatty Acid Contents Versus Selected Feed Fatty Acid Contents.

Chicken meat fatty acid content (y)	**Feed fatty acid content (x)**	**Equation**	**R^2**
ditrans-NMIOD	*ditrans*-NMIOD	y = 0.003 + 0.567x	0.876
ditrans-NMIOD	total NMIOD	y = -0.008 + 0.411x	0.878
total 18:2 isomers	total 18:2 isomers	y = 0.094 + 0.652x	0.518
ditrans-LA	*ditrans*-NMIOD	y = 0.020 + 0.442x	0.480
total *trans*	total isomers	y = 0.233 + 0.287x	0.446
ditrans-LA	total *ditrans*-LA	y = 0.044 + 0.348x	0.438
9c,12t-CLA	9c,12t-CLA	y = 0.044 + 0.152x	0.008

NMIOD = non-methylene interrupted octadecadienes, CLA = conjugated linoleic acid, LA= linoleic acid.

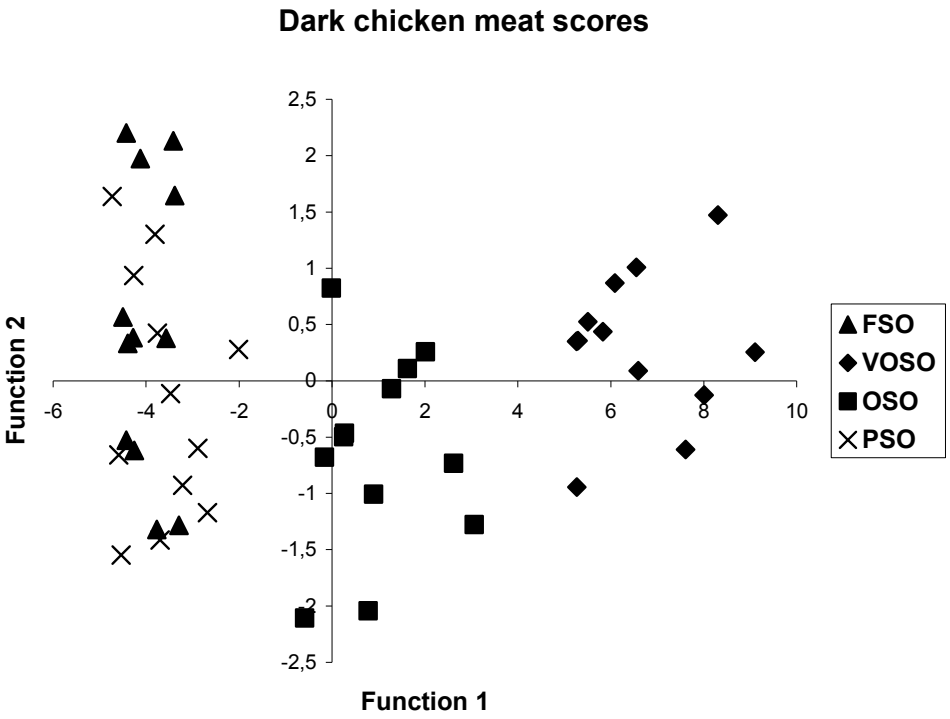

Figure 1. Plot scores of the first two canonical functions in dark chicken meat samples. Canonical discriminant functions are: function 1 = -16.17 + 83.78x(cis-9,trans-12-octadecadienoic acid) + 403.28x(ditrans-non-methylene interrupted octadecadienes) – 34x(20:2 n-6) + 56.3x(22:5 n-3); function 2 = -14.36 + 70.75x(cis-9,trans-12-octadecadienoic acid) + 4.77x(ditrans-non-methylene interrupted octadecadienes) – 16.95x(20:2 n-6) + 60.62x(22:5 n-3); function 3 = -1.30 + 120.73x(cis-9,trans-12-octadecadienoic acid) – 22.95x(ditrans-non-methylene interrupted octadecadienes) – 2.06x(20:2 n-6) – 31.98x(22:5 n-3). These functions distinguish dark chicken meat fed very oxidised sunflower oil (VOSO) and oxidised sunflower oil (OSO) from other sunflower oils (FSO = fresh and PSO = peroxidised).

1.5. Effect of Heated Sunflower Oils and Different Dietary Supplements on the Composition, Oxidative Stability and Sensory Quality of Dark Chicken Meat

Títol: Efecte de l'addició d'olis de gira-sol escalfats i d'altres suplements dietètics en la composició, estabilitat oxidativa i la qualitat sensorial de la cuixa de pollastre

Resum: Emprant un disseny factorial es va estudiar l'efecte en la dieta de diferents olis de gira-sol oxidats (oli de gira-sol fresc, el mateix oli escalfat a baixes temperatures o escalfat a altes temperatures, aquest últim afegit a dos nivells), de la suplementació amb acetat d'α-tocoferol (0 o 100 mg/kg) i de la suplementació amb Zn (0 o 600 mg/kg) sobre la composició, estabilitat oxidativa i la qualitat sensorial de la carn de la cuixa de pollastres, tots ells alimentats amb un suplement de Se que provenia de llevats rics en selenometionina (0,6 mg Se/kg).

Les carns crues de la cuixa amb pell de pollastres alimentats amb olis oxidats a altes temperatures tenen un major contingut en 20:0, i, també, un major contingut en isòmers de posició i geomètrics de l'àcid linoleic. A més, les suplementacions d'acetat d'α-tocoferol i de Zn també van modificar lleument la composició en àcids grassos. A la vegada, la suplementació amb acetat d'α-tocoferol va provocar un augment en el contingut d'α-tocoferol en la carn, mentre que els valors de l'índex de l'ATB i el contingut en hidroperòxids lipídics de la carn de la cuixa crua amb pell van disminuir, el mateix va succeir amb els valors de l'índex de l'ATB, i amb l'aroma i flavor a ranci en la carn de la cuixa cuita amb pell després d'emmagatzemar-la durant 18 mesos a congelació i posteriorment 9 dies a refrigeració. Tanmateix, cap dels factors estudiats va tenir influència en les puntuacions de l'acceptabilitat dels consumidors en carns cuites emmagatzemades 4 mesos a congelació. Per altra banda, la suplementació amb Zn va provocar un augment en el contingut en Se de la carn de la cuixa crua de pollastre amb pell.

EFFECT OF HEATED SUNFLOWER OIL AND DIETARY SUPPLEMENTS ON THE COMPOSITION, OXIDATIVE STABILITY AND SENSORY QUALITY OF DARK CHICKEN MEAT

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Abbreviation Key: α-TA = α-tocopheryl acetate, GPx = glutathione peroxidase, FSO = fresh sunflower oil, PSO = peroxidised sunflower oil, OSO = oxidised sunflower oil, VOSO = very oxidised sunflower oil, LA = linoleic acid, NMIOD = non-methylene interrupted octadecadienoic, FOX = ferrous oxidation-xylenol orange, PUFA = polyunsaturated fatty acid, MUFA = monounsaturated fatty acid, SFA = saturated fatty acid.

ABSTRACT

A factorial design was used to study the effect of dietary oxidized sunflower oils (fresh, heated at low temperatures and heated at high temperatures), dl-α-tocopheryl acetate (0 or 100 mg/kg) and Zn supplementation (0 or 600 mg/kg) on the composition, oxidative stability and sensory quality of dark chicken meat with skin from animals fed Se supplements (Se-enriched yeast, 0.6 mg Se/kg). The positional and geometrical isomers of linoleic acid were increased in raw meat from chickens fed oils oxidized at high temperatures. In addition, supplementation with α-tocopheryl acetate increased the α-tocopherol content, whereas TBA values and lipid hydroperoxide content were reduced. Likewise, TBA values, rancid aroma and rancid flavor also decreased in cooked dark meat stored for 30 mo at –20 ºC and then for 9 d at 4 ºC. However, none of the dietary factors studied affected consumer acceptability scores of cooked meat previously stored for 4 mo at –20 ºC. Furthermore, Zn supplementation increased the Se content in raw meat.

Key words: Oxidized sunflower oil, α-tocopherol supplementation, Zn supplementation, Se supplement, fatty acid isomers

1.- INTRODUCTION

Oils rich in polyunsaturated fatty acids are prone to lipid oxidation, which can occur at low or high temperatures, such as during long-term storage at room temperature or during frying. Nevertheless, a considerable number and amount of distinct oxidation products are produced depending on the oxidative conditions.

At low temperatures, fatty acid hydroperoxides are formed mainly in the initial stages of oxidation. They reach a plateau and then decompose into secondary oxidation products (Frankel, 1998), whereas at high temperatures, these hydroperoxides are immediately decomposed into secondary oxidation products (Perkins, 1996).

Several of these oxidation products have a range of biological effects, which are mainly detrimental and related to a number of chronic and degenerative diseases (Esterbauer et al., 1991; Schroepfer, 2000; Guardiola et al., 2002). In addition, lipid oxidation products, at high doses, affect animal growth and metabolism (Billek, 2000; Márquez-Ruiz and Dobarganes, 1996; Mahungu et al., 1999). Nevertheless, moderate consumption of commercially used frying fats and oils are not detrimental to experimental animals even in long-term feeding trials (Billek, 2000; Clark and Serbia, 1991; Márquez-Ruiz and Dobarganes, 1996).

However, few studies have addressed the effects of moderate levels of dietary supplements with distinct types of oxidized oils on the composition, oxidative stability, acceptability and rancidity of meat. The consumption of oxidized oil leads to reduced oxidative stability of meat, which can be attributed to oil tocopherol loss in these oils (Lin et al., 1989a; Galvin et al., 1997). Moreover, decreased oxidative stability of meat from chickens fed oxidized oils is reversed by α-tocopheryl acetate supplementation (α-TA) (Galvin et al., 1997; Sheehy et al., 1993, 1994), which is related to an improved sensory quality (Ajuyah et al., 1993a; O'Neill, 1998; de Winne and Dirinck, 1996).

In addition, Se and tocopherol are interrelated in the *in vivo* antioxidant system in which the former, through glutathione peroxidase (GPx), contributes to the decomposition of lipid hydroperoxides into non-pro-oxidant species, whereas tocopherol acts as a chain-breaking antioxidant (NRC, 1983; BNF, 2001). Furthermore, the highest susceptibility to oxidation has been observed in animals fed

diets deficient in Se and tocopherol, (Avanzo et al., 2001; Arzu Bozkaya et al., 2001; Öztürk-Ürek et al., 2001), which demonstrates that both are crucial in the antioxidant system. Although inorganic and organic sources of Se restore GPx activity, supplementation with the latter is preferred in poultry farming because it is associated with lower meat drip loss, higher increase in meat Se content, and better productive characteristics (Surai, 2002; Bou et al., 2005a).

Zn is also related to the antioxidant system through superoxide dismutase. Supplements of this mineral have no effect on the Zn content, oxidative stability or sensory quality of chicken meat; however, they do lead to an increased Se content in mixed dark and white chicken meat (Bou et al., 2004a, 2005a).

Here we studied the effect of various dietary factors (oxidation degree of the fat source, α-TA supplements and Zn supplements) on animal performance, fatty acid composition, crude fat content, α-tocopherol, Zn, Se, Fe and Cu content, consumer acceptability and rancidity scores, and TBA and ferrous oxidation-xylenol orange (FOX) values of raw and cooked dark chicken meat.

2.- MATERIAL AND METHODS

Preparation of oxidized oils

Two thermal treatments were performed. The first consisted of heating 40 L of refined fresh sunflower oil (FSO) in an indirect heating bath at 55-60 ºC under agitation for 12 d. This procedure produced a peroxide value of 90 meq O_2/kg oil, that is to say peroxidized sunflower oil (PSO). The second treatment involved heating 90 L of FSO in a direct heating fryer at 190-195 ºC under agitation for 28 h until reaching a *p*-anisidine value of 150 and 9.4% of polymerised triacylglycerols, i.e. very oxidized sunflower oil (VOSO). By mixing 50% of FSO with 50% of VOSO, we obtained oxidized sunflower oil (OSO). Further oxidation parameters, α-tocopherol content and fatty acid isomerisation of these oils are described in Bou et al. (2005b). Oils were frozen at -20 ºC until feed preparation.

Animals and Housing

Three-hundred and thirty-six female broiler chicks (Ross 308, 1-day old) were randomly assigned to 48 floor pens (7 birds per pen) corresponding to 16 triplicated dietary treatments. Further housing parameters were as described in Bou et al. (2005a). Feed and water were provided *ad libitum* for 42 d. The animals were reared and slaughtered in compliance with national regulations, and the experiment received prior approval by the Animal Care and Use Committee of Copaga Soc. Coop.[19].

Diets and Experimental Design

A factorial design was used to study the effect of various dietary factors on animal performance, α-tocopherol, crude fat, Zn, Se, Fe and Cu content, fatty acid composition, consumer acceptability and rancidity scores, and TBA and ferrous oxidation-xylenol orange (FOX) values of raw and cooked meats. Up to 10 d of age, a typical basal diet containing 6% of animal fat was supplied to the chickens (Table 1). From 11 to 42 d, chickens were given one of 16 dietary treatments, all supplemented with Se-enriched yeasts at 0.6 mg Se/kg. The treatments were prepared from a basal diet (Table 1) by combination of the dietary factors studied (Table 2): sunflower oil (FSO, PSO, OSO or VOSO), Zn supplementation (0 or 600 mg/kg) and α-TA supplementation (0 or 100 mg/kg). To provide the same α-tocopherol amounts as feeds prepared with FSO, the OSO, PSO and VOSO diets were supplemented with α-TA to rectify tocopherol loss caused by heat treatment. These 16 dietary treatments were prepared every 10 d and supplied to chickens from 11-21, 22-32 and 33-42 d of age. For feed analyses, approximately 200 g of each dietary feed were immediately vacuum packed in high-barrier multilayer bags (Cryovac[20] BB-4L; permeability to O_2 30 cm^3/m^2, 24 h, 1 bar, ASTMD-3985) and kept at -20 ºC.

Zinc sulfate and dl-α-TA were purchased from Andrés Pintaluba, S.A.[21]. The organic source of Se came from Se-enriched yeast (Sel-Plex®) and was supplied by Probasa[22] .

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[20] Cryovac Europe, Sealed Air S. L., Sant Boi de Llobregat, Spain, E-08830
[21] Andrés Pintaluba, S.A., Reus, Spain, E-43280
[22] Probasa, Sta. Perpetua de la Moguda, Spain, E-08130

Preparation, Cooking and Storage of Samples

The chickens were slaughtered and were then stored for 24 h at 4 ºC. Two random legs plus their skin were then chosen from each pen to study the composition and oxidative stability of raw meat. These samples were hand-deboned, mixed, ground, and weighed (approximately 30 g per bag), placed in high-barrier multilayer bags (Cryovac BB-4L; permeability to O_2 30 cm^3/m^2, 24 h, 1 bar, ASTMD-3985) and then vacuum packed and immediately stored at – 20 ºC until analysis of α-tocopherol, mineral content, fat content and fatty acid composition. The remaining legs (with skin) from each pen were hand-deboned and vacuum packed in high-barrier multilayer bags (Cryovac CN-300; permeability to O_2 15 cm^3/m^2, 24 h, 1 bar, ASTMD-3985). At 35 h after slaughter, these samples were cooked in an oven at 85 ºC (99% relative humidity) to an internal temperature of 78 ºC. They were then cooled and stored at – 20 ºC until the consumer acceptability and rancidity scores, and TBA values of cooked meat were determined.

Reagents and Standards

The reagents and standards used in element analysis were as described in Bou et al. (2004b) while those used in the other analyses were described in Bou et al. (2004a). Tocopherol analogs (α, β, γ, δ) were quantified using a kit from Calbiochem[23].

Determination of Fatty Acid Composition

Gas chromatography was used to measure fatty acid composition in raw dark meat and in milled feed, as described elsewhere (Bou et al., 2005b). The resulting 48 (16 treatments x 3) meat samples and the 16 feeds prepared for chickens from 22 to 32 d of age were analyzed.

[23] Calbiochem, San Diego, CA 92121

Determination of crude fat content

The fat content of the resulting 48 meat samples was measured by the AOAC Official Method 991.36 (AOAC, 2000).

Determination of tocopherols

α-tocopherol was measured in raw meat and in milled feed following the method described elsewhere (Bou et al., 2004a). In addition, using the same method, β-, γ- and δ-tocopherol analogs were also measured. The chromatographic conditions did not separate β- and γ-tocopherol. The resulting 48 meat samples were analyzed. For feed analysis, the 16 feeds prepared for each period (11-21, 22-32, 33-42 d of age) were analyzed.

Determination of Zn, Se, Fe and Cu

The method used for to measure element content was as described in Bou et al. (2004b). The resulting 48 meat samples and the 16 feeds prepared for chickens from 33 to 42 d of age were analyzed.

Determination of TBA and FOX values

The TBA values of raw meat samples were measured through third derivative spectrophotometry after acid aqueous extraction (Grau et al., 2000a). Susceptibility to oxidation was determined by the FOX method, described by Grau et al. (2000b), after 130 h of incubation at room temperature. After incubation, absorbance was measured at 560 nm and lipid hydroperoxide content was quantified as described by Navas et al. (2004).

Samples of cooked chicken legs were thawed, as for sensory analyses, by heating in a water bath at 35 ºC for 20 min, and were then ground before TBA measurement (Grau et al., 2000a). TBA values of the blind control and the maximum and minimum references used for the sensory analysis were also determined.

Sensory Analysis

A consumer acceptability panel test was done on cooked meat after 4 mo of storage at – 20 ºC. Thirty-two experienced panelists were selected from our department. Criteria for panel selection and sample preparation were as described elsewhere (Bou et al., 2004a). Samples were presented to the panelists in a balanced incomplete block design (Cochran and Cox, 1957): 16 blocks, six samples per block and six replicates for each sample. This design was duplicated. In addition, each panelist evaluated the acceptability of a blind control (total samples presented to each panelist = 7), which was a vacuum-packed freshly cooked commercial chicken sample that had been stored for 1 d at –20 ºC. Panelists were asked to rank the overall acceptability of the product using a 9-point scale (1 = very bad; 9 = very good).

In addition, a sensory analysis of the rancid aroma and flavor of the meat was carried out after 30 mo of storage at –20 ºC. Sample preparation was as follows: bags containing frozen samples were opened and kept at 4-5 ºC for 9 d. Then, as for consumer acceptability test, 20-g chicken pieces, with a similar amount of skin, were placed in screw-capped flasks. They were then heated at 75 ºC for 20 min in a conventional air oven and served to trained panelists.

Twenty-four volunteers were trained to evaluate rancid aroma and flavor using cooked meat after refrigeration for a range of periods. Afterwards, 8 pre-selected panelists received samples of this meat. After this test, 5 panelists, which showed correlation coefficients between TBA values and either descriptor ≥ 0.5, were selected.

Samples were presented to each selected panelist in a Latin square design (Cochran and Cox, 1957): Panelists received all samples in 4 working sessions in which 4 random samples were presented. They were asked to score rancid aroma and flavor, each on a 15 cm unstructured line scale anchored on the left side with the term "weak" and on the right "strong". Vertical lines marks drawn by the panelists through the horizontal line scales were converted into percentage scores. In addition to the samples to be evaluated, the panelists also received two reference samples corresponding to the minimum and the maximum of the scale. As a minimum sample, we chose meat from animals fed FSO and supplemented with α-TA that had been thawed overnight. As a maximum sample, we used meat that had been stored for 10-d at 4-5 ºC from animals fed PSO and not supplemented with α-TA.

Statistical Analysis

Multifactor ANOVA was used to determine significant effects produced by the dietary factors on productive parameters, fatty acid composition, crude fat, tocopherol, Zn, Se, Fe and Cu content, and oxidative stability (FOX and TBA values) of the raw meat stored under a range of conditions. Likewise, multifactor ANOVA was used to determine significant effects on consumer acceptability, rancidity scores and TBA values of cooked meat stored under various conditions. Interactions between factors higher than an order of two were ignored. One-way ANOVA was used to determine significant differences between dietary treatments and the blind control used in consumer acceptability test. Least-squares means for the main factors with a significant effect were separated using the Duncan test. In all cases, $P \leq 0.05$ was considered significant.

3.- RESULTS AND DISCUSSION

Animal Performance

No differences were recorded in final BW, feed conversion or mortality after 42 d of breeding. Although feed intake was significantly lower in animals on Zn supplements (3,720 *vs*. 3,801 g/bird), this reduced intake did not result in a significant decrease in BW (1,898 *vs*. 1,906 g) or feed conversion ratio (1.963 *vs*. 1.996). The lack of effect of Zn supplements on BW and feed conversion ratio is consistent with previous results (Bou et al., 2005a).

Fatty acid composition and total fat content

The fatty acid composition of feed was affected by the addition of sunflower oil (Table 3). The thermal treatment of these oils led to a significant decrease in linoleic acid (LA) and total polyunsaturated fatty acids (PUFA) and, consequently, provoked a relative increase in monounsaturated and saturated fatty acids, as discussed in Bou et

al. (2005b). Fats and oils heated at 190 ºC or above suffer *trans* isomerisation (Sébédio et al., 1988; Wolff, 1993; Juanéda et al., 2003; Bou et al., 2005b). This explains the differences recorded in feeds containing OSO and VOSO for a mixture of *trans*-octadecenoic acids (*trans*-18:1), *trans*-9,*trans*-12-octadecadienoic (*ditrans*-LA), *trans*-9,cis-12- and cis-9,trans-12-octadecadienoic acid group (*monotrans*-LA), *cis*-9,*trans*-11-octadecadienoic acid (9c,11t-CLA), and a mixture of *ditrans*-non-methylene interrupted octadecadienoic acids (*ditrans*-NMIOD). The identification, formation and deposition of these geometrical and positional isomers in dark meat have been discussed elsewhere (Bou et al., 2005b).

Dietary sunflower oil produced significant differences in the fatty acid composition of dark meat (Table 3). These differences reflected, to a certain extent, the differences in feeds and explained the increase in 20:0 in meat from animals fed OSO and VOSO. Moreover, the total LA isomer group, which is the sum of *monotrans*-LA and *ditrans*-LA, was higher in animals on VOSO, followed by animals on OSO. This result shows the effect of the fatty acid composition of feed on meat. Likewise, *ditrans*-NMIOD content showed significant differences between the OSO and VOSO groups because of heating treatments, whereas the *ditrans*-NMIOD content of meat from animals on FSO and PSO did not differ. Thus, *ditrans*-NMIOD and total LA isomer content in meat reflected the differences observed in feeds. However, taking into the account total positional isomers of LA (total NMIOD) in dark meat, this group was only different in animals fed VOSO.

In contrast, 9c,11t-CLA and 10t,12c-CLA did not differ between treatments. Nonetheless, the former was more easily incorporated than the latter. In addition, a non-significant decrease in the LA content of meat was observed, though there were significant differences in the fatty acid composition of feeds.

Significant increases in 20:3 n-6, 22:4 n-6 and 22:5 n-3 were recorded in meat from α-TA treatments (Table 3), which is consistent with other findings (Ajuyah et al., 1993b; Surai and Sparks, 2000). In this regard, several authors propose that tissue PUFA are protected from oxidation by tocopherol supplementation or that tocopherols enhance the synthesis of a number of PUFA through the Δ^6 desaturase pathway (Cherian et al., 1996; Surai and Sparks, 2000).

Conversely, α-TA supplementation has no effect on the fatty acid composition of meat from animals on hydrogenated soybean oil (Lin et al., 1989b), olive oil or tallow (O'Neill et al., 1998), and comparing animals fed 1.25% or 2.5% of fish oil either α-TA supplemented at 70 and 140 mg/kg (Bou et al., 2004a).

In addition, the increase in the content of some PUFA may cause a relative decrease in 20:1 n-9, which was recorded when animals received α-TA supplements. Nevertheless, the interaction between sunflower oil source and α-TA supplements affected the 18:1 n-9, 20:0 and 20:1 n-9 content. The animals on FSO had more 18:1 n-9 and 20:1 n-9 when supplemented with α-TA whereas those on the PSO and VOSO treatments had higher 18:1 n-9 and 20:1 n-9 content in the absence of α-TA supplements. Indeed, Lauridsen et al. (1997) also reported a significant effect, without finding a pattern, of the interaction between dietary oil source (tallow and olive oil) and α-TA supplementation (20 and 200 mg/kg) on the occurrence of fatty acids in chicken muscle membranal fractions. In relation to our findings, the distinct amounts of PUFA provided by the feeds and the effect of α-tocopherol on oxidative stability, and probably on Δ^6 desaturase, could partially explain this interaction.

Zn supplementation at 600 mg/kg caused a significant increase in *trans*-18:1 content. No explanation can be given for this effect and further studies are required to confirm this result and also the effect of interactions observed between sunflower oil source and Zn supplementation on *trans*-18:1 and between α-TA and Zn supplements on LA and 20:1 n-9.

However, Zn supplementation had no effect on any other fatty acid. Our results are consistent with a previous study on Zn supplementation (0, 300 and 600 mg/kg) which reported no effect on the fatty acid composition of meat from chickens fed various non-heated fat sources (Bou et al., 2005b).

Furthermore, dietary sunflower oil source, and Zn and α-TA supplements did not affect the crude fat content of meat (Table 4).

Tocopherol content

The average contents of α-tocopherol and other tocopherols supplied by the feeds are shown in Table 5. As tocopherol losses from sunflower oil caused by the

thermal treatments were rectified (see Material and Methods section), there were no significant differences in feed α-tocopherol content. No differences were observed for other tocopherols between treatments. Nevertheless, as expected, dietary supplements with α-TA provided distinct feed α-tocopherol supplies.

Chickens on feeds rectified with α-TA did not show significant differences in the α-tocopherol content of raw meat (Table 4). However, non-rectified diets in which added oxidized oils were heated at 80 ºC until reaching high peroxide values (400 and 189 meq O_2/ kg, respectively) led to lower α-tocopherol content in chicken meat (Lin et al., 1989a; Galvin et al., 1997). Similar results were obtained for diets including oxidized oils that had been heated at 30 ºC, until reaching 156 meq O_2/ kg (Engberg et al., 1996; Jensen et al., 1997). Therefore, these results may be attributed to either the decreased oil tocopherol content, the destruction of α-tocopherol in the feeds by oxidation products, and the use of some of the α-tocopherol in protecting tissue lipids from oxidized oil-induced free radical attack (Lin et al., 1989a; Engberg et al., 1996; Galvin et al., 1997).

Similar results have been reported when animals received diets rectified with α-tocopherol, in which the oxidized oils added were previously heated at high temperatures (Sheehy et al., 1993; 1994). Therefore, diets containing oxidized oils are rich in secondary oxidation products and are comparable to our OSO and VOSO treatments.

Sheehy et al. (1993) achieved oxidized sunflower and linseed oils by heating at 140 ºC for 24 h. These authors compared the effect of feed supplements (8%) with fresh and oxidized sunflower and linseed oils on tissue α-tocopherol content. All diets were rectified with α-TA addition in order to achieve the same amount of α-tocopherol (50 mg/kg). Dietary treatments using oxidized oils produced a lower α-tocopherol content in chicken plasma, breast and thigh tissues than diets including fresh oils.

Likewise, these authors compared the effect of feed supplements (4%) with fresh sunflower oil, containing 30 mg α-tocopherol /kg feed, with the addition of a sunflower oil heated for 11 h at 120 ºC, containing 25 mg α-tocopherol /kg feed, on the α-tocopherol content of several chicken tissues (Sheehy et al., 1994). In that study, chicken plasma, liver, thigh, breast, lung, pancreas and spleen had reduced α-

tocopherol contents whereas the brain was not affected by the addition of oxidized sunflower oil. The lowered tissue α-tocopherol content was attributed to impaired intestine hydrolysis of α-TA by lipid oxidation products, the destruction of α-tocopherol in the gastrointestinal tract by free radical attack and the effect of certain oxidation products of the heated oils, which may be absorbed and may destroy α-tocopherol in animal tissues.

Although the diets used in the abovementioned studies were rectified by adding α-TA, they provided a much lower α-tocopherol content than those tested in our study (non-supplemented diets provided 70 mg α-tocopherol/kg). Therefore, only when diets containing oxidized oils provide a low and/or different content in α-tocopherol cause a decreased α-tocopherol content in chicken meat. Nevertheless, our results are consistent with the trend reported by these authors.

In contrast, a significant increase in α-tocopherol was observed in broilers fed α-TA supplements (Table 4). This effect has been widely reported in raw chicken meat (King et al., 1995; Cherian et al., 1996, Lauridsen et al., 1997; Grau et al., 2001).

Zn supplementation had no influence on chicken meat α-tocopherol content (Table 4), which is in agreement with previous studies (Bou et al., 2004a; 2005a).

In relation to tocopherol analogs, δ-tocopherol was not detected in meat, therefore we report only the sum of β- and γ-tocopherol, which were not affected by any of the dietary factors studied (Table 4).

Element content

Feed element composition is shown in Table 5. As expected, Zn supplementation led to significant increase in this element in feeds.

The content of Zn, Se, Fe and Cu in raw meat was not affected by the sunflower oil source (Table 4). Neither do chickens fed 1.25% animal fat, linseed oil or fish oil one week before slaughter show significant differences in the content of these elements (Bou et al., 2005a).

α-TA supplementation did not have a significant effect on the element content of raw meat (Table 4). However, when poultry are reared under environmental stress, plasma and tissue concentrations of minerals are reduced (Onderci et al., 2003; Sahin

and Kucuk, 2003). In this situation, in Japanese quail, these decreases are counteracted by increasing both α-TA and Se supplementation, which causes increased Fe and Zn serum concentrations, though a decrease in Cu also occurs (Sahin et al., 2002).

The Se content of mixed dark and white raw meat is increased by Zn or organic Se supplementation (Bou et al., 2005a). In the present study, the organic Se supplementation (0.2 mg Se/kg) came from Se-enriched yeasts. These are rich in selenomethionine, which is incorporated non-specifically into distinct proteins (Surai, 2002, Bou et al., 2005a). However, Bou et al. (2005a) reported an increased Se content as a result of Zn supplementation (0, 300, 600 mg/kg).

In the present study, we confirm that Zn supplementation (600 mg/kg) provokes an increase in Se meat content when animals receive a high dose of organic Se supplementation (0.6 mg Se/kg).

Zn supplementation led to increased Se content in dark meat, thereby confirming our previous results (Table 4). Nevertheless, the Se content reported here was greater and the differences were smaller than those reported by Bou et al. (2005a) in mixed dark and white raw meat. This finding can be explained by the higher dose of organic Se added to the feeds, and the fact that dark chicken meat has a distinct metabolism, consequently this meat that is more rich in a number of elements (i.e., Fe, Zn, Se) than white meat (Chan et al. 1995).

However, Zn supplementation did not affect the Zn, Fe or Cu content of dark meat (Table 4), which is also consistent with previously reports (Bou et al., 2004a, 2005a). However, Japanese quails reared under heat stress (34 ºC), fed Zn (30 or 60 mg/kg) supplements showed increased serum Zn, ascorbic acid and α-tocopherol concentration as dietary Zn supplementation increased (Sahin and Kucuk, 2003). Moreover, laying hens, reared at low temperature (6.8 ºC) and fed a Zn supplement (30 mg/kg), have increased serum α-tocopherol, ascorbic acid, Fe, Zn, Mn and Cr concentrations (Onderci et al., 2003). Therefore, although some vitamin and element serum concentrations are increased through Zn supplements in animals reared under environmental stress, in our conditions this supplementation did not increased the Fe, Zn or Cu content of dark meat. In contrast, supplements with Zn did increase the content of Se.

Oxidative stability of raw dark chicken meat (TBA and FOX values)

The oxidative stability of dark meat was studied using the TBA and FOX methods. The former measures the malondialdehyde content, a secondary oxidation product, whereas the latter measures lipid hydroperoxide formation.

Dietary sunflower oil source did not increase TBA values (Table 6). These results are in accordance with those reported by Grau et al. (2001), who described that the addition of 6% of oxidized sunflower (160 ºC, 12h) did not increase TBA values in raw and cooked dark chicken meat compared with animals fed 6% fed beef tallow, sunflower oil, or linseed oil. Similarly, Sheehy et al. (1994) reported that the plasma TBA values of animals fed 4% FSO did not differ from those fed a heated sunflower oil (120 ºC, 11 h) when this oil contained the same amount of α-tocopherol as FSO. Nevertheless, these authors reported higher TBA values for animals fed the same heated sunflower oil without rectifying the tocopherol losses caused by oil heating. In accordance with these findings, the authors proposed that the increased TBA values in plasma of chicks fed oxidized sunflower oil without rectifying α-tocopherol losses resulted from peroxidation of plasma lipids, rather than from the absorption of malondialdehyde and other TBA-reactive substances from the gastrointestinal tract (Sheehy et al., 1994).

Galvin et al. (1997) compared the TBA values of dark and white meat from chickens on several diets containing 6% FSO or 6% of an oxidized sunflower oil. The TBA values from animals fed FSO plus 30 mg/kg of α-TA did not differ from those on oxidized sunflower oil plus 200 mg/kg of α-TA. However, the TBA values from the oxidized oil or oxidized oil plus 30 mg α-TA/kg treatments were higher than in the FSO plus 30 mg α-TA/kg group. Nevertheless, it should be taken into the account that Galvin et al. (1997) did not rectify the tocopherol losses caused by oil heating. Thus, these authors hypothesized that the lower tocopherol and the higher oxidation product content in treatments with oxidized sunflower oil leads to higher TBA values in dark and white meats. In our study, because the α-tocopherol content of the oxidized oils was rectified and the supply of tocopherol was high (non-supplemented diets contained 70 mg α-tocopherol/kg), no differences for TBA values were detected between

treatments. However, the same trend for these values was observed in animals on PSO and VOSO compared with those on FSO (Table 6).

Similarly to TBA values, lipid hydroperoxide formation, measured using the FOX method, did not show differences between sunflower oil sources (Table 6). This result is consistent with findings on raw chicken meat from oxidized sunflower oil and FSO treatments (Grau et al. 2001). Therefore, neither the consumption of oils rich in secondary nor in primary oxidation products provoked a higher oxidative status (TBA values) or susceptibility to oxidation (FOX values).

Furthermore, animals on α-TA supplements showed decreased TBA and FOX values (Table 6). These results are in agreement with the values reported in dark chicken meat from animals on α-TA (0 *vs*. 225 mg/kg) supplements (Grau et al., 2001). Similar results have also been described in meat from chickens on oxidized sunflower oil supplemented with α-TA (Galvin et al., 1997; Lin et al. 1989a). Thus, the meat from animals on α-TA supplements contained lower secondary oxidation products and were also less susceptible to oxidation.

Therefore, the α-tocopherol content in feeds and their further incorporation into tissues protects meat against oxidation. Therefore, the high α-tocopherol content supplied by the feeds assayed (Table 5) accounted for the absence of significant differences between animals fed oxidized oils.

Zn supplementation did not affect TBA or FOX values (Table 6). However, lower serum and liver TBA values have been reported in poultry, reared under environmental stress conditions of low and high temperature, on Zn supplements in comparison with untreated animals (Sahin and Kucuk, 2003; Onderci et al., 2003).

Finally, it should be taken into account that dietary Se, especially in combination with α-tocopherol, lowers TBA values in chicken tissues and increases GPx activity in several tissues (Arzu Bozkaya et al., 2001; Aydemir et al., 2000; Sahin et al., 2002) and also in chicken muscles (De Vore et al., 1983; Avanzo et al., 2001).

Sensory analysis and TBA values of cooked dark chicken meat

Two sensory analyses were carried out. First, a consumer panel performed an acceptability test of cooked meat after 4 mo of frozen storage. Second, the rancidity of the meat was evaluated by a trained panel.

Dietary sunflower oil did not have a significant effect on overall consumer acceptability though the greatest differences were observed within this dietary factor (Table 7), which is consistent with the results from a previous study (Bou et al., 2001).

Likewise, α-TA supplementation did not affect consumer acceptability scores (Table 7). However, this dietary treatment (225 mg/kg) has been reported to increase the consumer acceptability of cooked dark meat with skin from chickens fed a range of fats (beef tallow, sunflower oil, oxidized sunflower oil, linseed oil) after storage for 13 mo at –20 ºC (Bou et al., 2001). However, animals fed fats with a low degree of unsaturation, and/or receiving enough α-TA supplementation, provide acceptable meat even after long-term frozen storage (Bou et al., 2001, 2004a, 2005a).

Zn supplementation did not have a significant effect on consumer acceptability, as previously reported (Bou et al., 2004a, 2005a).

In relation to TBA values, samples stored for 4 mo at –20 ºC were affected only by α-TA supplementation (Table 7). Therefore, α-tocopherol, which is increased after this supplementation (Table 4), led to more stable meat since it delays the oxidative deterioration of meat (Wood and Enser, 1997; Jensen et al., 1998; Lauridsen et al., 1997). Thus, α-tocopherol reduces TBA values and undesirable flavors and odors in chicken meat, both of which are highly correlated (Lyon et al., 1988; O'Neill et al., 1998; De Winne and Dirinck, 1996; Igene et al., 1985; Bou et al., 2001). However, the TBA values and their differences between dietary treatments (Table 7) are too low to detect significant differences in acceptability scores (Bou et al., 2001).

The TBA values of cooked samples were analyzed again after 30 mo of frozen storage (Table 7). Neither dietary sunflower oils nor Zn supplementation affected these values. However, α-TA supplementation led to a decrease in TBA values, indicating its protective effect, and also after 4 mo of frozen storage (Table 7). Again, the TBA values and their differences between dietary treatments were not high enough to detect sensory differences between samples (Bou et al., 2001). Consequently, samples were further stored under refrigeration in order to induce oxidation. Thus, after 30 mo, the

bags containing frozen samples were opened and stored for 9 d at 4-5 ºC before rancidity evaluation.

Neither rancid aroma nor flavor scores were affected by dietary sunflower oils or Zn supplementation after further storage of the cooked meat for 9 d under refrigeration (Table 7). However, α-TA supplementation led to a decrease in these scores. In addition, α-tocopherol supplementation significantly affected the TBA values of meat stored for 30 mo at – 20 ºC and then for 9 d at 4-5 ºC. This effect was also significant after 4 and 30 mo of frozen storage. Therefore, TBA determination is a much more sensitive method to assess lipid oxidation in chicken meat than the sensory evaluation of rancidity. Moreover, from these results differences in meat rancidity can be set by the trained panel at above 700 μg malondialdehyde/kg of meat, which is in agreement with the results reported by Gray and Pearson (1987).

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Table 1. Ingredients and composition of basal diets.

Diet up to 10 d	Percentage	Diet from 11 to 42 d[1]	Percentage
Ingredients:		**Ingredients:**	
Barley	45.42	Wheat	56.61
Soybean meal, 48% protein	23.37	Soybean meal, 48% protein	24.69
Sorghum	10.00	Sunflower oil[4]	6.00
Meat meal, 50% protein	6.00	Sunflower meal	5.03
Animal fat	6.00	Soy grass	3.14
Tapioca, 62% starch	3.03	Calcium carbonate	1.69
Soy grass	1.89	Monocalcium phosphate	1.23
Sepiolite	1.49	Trace mineral-vitamin mix[5]	0.50
Trace mineral-vitamin mix[2]	1.00	L-Lysine	0.38
L-Lysine	0.44	DL-Methionine	0.26
DL-Methionine	0.34	Salt	0.20
Salt	0.31	Sodium bicarbonate	0.14
Monocalcium phosphate	0.27	Avizyme® 1300	0.12
Calcium carbonate	0.26	Choline chloride	0.03
Choline chloride	0.12		
DL-Treonine	0.05		
Phytase[3]	0.04		
Calculated composition:		**Calculated composition:**	
Dry matter	89.4	Dry matter	90.1
Crude protein	21.5	Crude protein	20.9
Crude fat	8.6	Crude fat	8.2
Crude fiber	2.5	Crude fiber	3.6
Ash	6.6	Ash	6.1
Metabolizable energy (cal/g)	3,025	Metabolizable energy (cal/g)	3,000

[1] Supplemented with 0.6 mg of Se/kg from Se-enriched yeast.
[2] Supplies the following per kilogram of complete feed: 10,000 IU of vitamin A, 2,000 IU of vitamin D_3, 30 mg of dl-α-tocopheryl acetate, 20 μg of vitamin B_{12}, 4 mg of vitamin B_6, 5 mg of vitamin K_3, 5 mg of vitamin B_2, 2 mg of vitamin B_1, 66 mg nicotinic acid, 200 μg of biotin, 12 mg of calcium pantothenate, 1 mg of folic acid, 20 mg of Fe (ferrous sulfate), 71 mg of Mn (manganese oxide), 100 μg of Se (sodium selenite), 37 mg of Zn (zinc oxide), 6 mg of Cu (copper sulfate), 1.14 mg of I (potassium iodide), 400 μg of Co (cobalt sulfate), 4 mg of butylated hydroxytoluene.
[3] (EC 3.1.3.8) which releases 1000 FTU/g.
[4] Sunflower oils with distinct degrees of oxidation were added depending on the dietary treatments.
[5] Supplies the following per kilogram of complete feed: 10,000 IU of vitamin A, 2,000 IU of vitamin D_3, 30 mg of vitamin E (dl-α-tocopheryl acetate), 5 mg of vitamin B_2, 2 mg of vitamin B_6, 2 mg of vitamin K_3, 1 mg of vitamin B_1, 10 μg of vitamin B_{12}, 30 mg nicotinic acid, 48 μg of biotin, 10 mg of calcium pantothenate, 1 mg of folic acid, 20 mg of Fe (ferrous carbonate), 100 mg of Mn (manganese oxide), 100 μg of Se (sodium selenite), 80 mg of Zn (zinc oxide), 10 mg of Cu (copper sulfate), 2 mg of I (potassium iodide), 200 μg of Co (cobalt sulfate).

Table 2. Dietary treatments

Sunflower oil	**α-Tocopheryl acetate (mg/kg)**	**Zn supplement (mg/kg)**
Fresh	0	0
Fresh	100	0
Fresh	0	600
Fresh	100	600
Peroxidized	0	0
Peroxidized	100	0
Peroxidized	0	600
Peroxidized	100	600
Oxidized	0	0
Oxidized	100	0
Oxidized	0	600
Oxidized	100	600
Very oxidized	0	0
Very oxidized	100	0
Very oxidized	0	600
Very oxidized	100	600

Table 3. Fatty acid composition (expressed as area normalization in %) of the experimental feeds and the effect of dietary factors on the fatty acid composition of raw dark chicken meat[1].

Fatty acid	Feeds with added sunflower oils				Raw dark chicken meat with skin							
					Sunflower oil added				Tocopherol added[2] [mg/kg]		Zn added[3] [mg/kg]	
	FSO	**PSO**	**OSO**	**VOSO**	**FSO**	**PSO**	**OSO**	**VOSO**	**0**	**100**	**0**	**600**
14:0	0,114	0,118	0,111	0,114	0,370	0,373	0,369	0,364	0,368	0,370	0,370	0,368
16:0	9,114a	9,309bc	9,204ab	9,379c	16,135	16,259	16,446	16,273	16,282	16.274	16,406	16,150
18:0	3,920a	3,961b	3,952ab	4,019c	5,729	5,625	5,710	5,598	5,650	5,681	5,644	5,687
20:0	0,287a	0,291ab	0,292b	0,297c	0,206ab	0,201a	0,224b	0,226b	0,214	0,215	0,211	0,218
22:0	0,564a	0,567a	0,576b	0,585c								
24:0	0,197b	0,186a	0,200b	0,200b								
Total SFA	14,196a	14,431b	14,334b	14,593c	22,441	22,457	22,749	22,460	22,407	22,433	22,528	22,312
16:1 n-9	0,036a	0,039b	0,039b	0,040b	0,413	0,429	0,400	0,402	0,409	0,413	0,415	0,407
16:1 n-7	0,154	0,158	0,149	0,153	2,371	2,527	2,586	2,713	2,563	2,535	2,597	2,502
18:1 n-9	24,276a	24,470bc	24,395ab	24,596c	29,930	30,477	30,581	30,794	30,605	30,286	30,648	30,244
18:1 n-7	0,748	0,758	0,786	0,785	1,110	1,124	1,088	1,124	1,106	1,118	1,087	1,136
20:1 n-9	0,237	0,245	0,239	0,241	0,259	0,263	0,259	0,262	0,264a	0,258b	0,262	0,260
Total MUFA	25,452a	25,670b	25,607b	25,815c	34,084	34,820	34,914	35,295	34,947	34,610	35,008	34,548
18:2 n-6	57,254a	56,777b	56,838b	56,244c	39,294	38,613	38,292	38,011	38,458	38,647	38,243	38,862
18:3 n-6					0,109	0,109	0,109	0,106	0,108	0,108	0,108	0,108
20:2 n-6					0,315	0,309	0,295	0,303	0,304	0,307	0,304	0,307
20:3 n-6					0,238	0,247	0,242	0,256	0,240a	0,252b	0,245	0,247
20:4 n-6	0,028	0,030	0,028	0,031	0,889	0,882	0,820	0,891	0,845	0,895	0,861	0,880
22:4 n-6					0,257	0,249	0,227	0,244	0,235a	0,254b	0,241	0,247
22:5 n-6					0,063	0,063	0,058	0,065	0,060	0,065	0,061	0,063
Total n-6 PUFA	57,282a	56,807b	56,866b	56,275c	41,165	40,469	40,043	39,878	40,356	40,641	40,173	40,824
18:3 n-3	2,660	2,693	2,679	2,740	1,784	1,753	1,768	1,787	1,768	1,778	1,758	1,788
20:5 n-3					0,035	0,031	0,035	0,037	0,033	0,036	0,035	0,034
22:5 n-3					0,096	0,093	0,084	0,093	0,087a	0,096b	0,091	0,092
22:6 n-3	0,022	0,024	0,019	0,020	0,067	0,062	0,051	0,060	0,057	0,063	0,062	0,058
Total n-3 PUFA	2,683	2,716	2,698	2,760	1,983	1,937	1,938	1,976	1,945	1,973	1,946	1,972
Total PUFA	60,025a	59,523b	59,564b	59,035c	43,148	42,407	41,981	41,854	42,301	42,614	42,119	42,795
Trans-18:1	0,011a	0,011a	0,042b	0,066c	0,116	0,114	0,122	0,127	0,118	0,122	0,110a	0,129b
Ditrans-LA	0,021a	0,021a	0,048b	0,071c	0,046a	0,045a	0,051a	0,066b	0,052	0,052	0,053	0,051
Monotrans-LA	0,404ab	0,382a	0,428b	0,471c	0,187a	0,182a	0,201b	0,207b	0,193	0,196	0,196	0,193
Total LA isomers	0,425a	0,404a	0,476b	0,543c	0,233ab	0,227a	0,253b	0,272c	0,245	0,243	0,244	0,244
9c,11t-CLA	0,025a	0,032a	0,036b	0,041c	0,050	0,045	0,048	0,051	0,049	0,048	0,048	0,049
10t,12c-CLA	0,019	0,022	0,026	0,028	0,010	0,007	0,006	0,008	0,007	0,009	0,009	0,006
Ditrans-NMIOD	0,062a	0,061a	0,102b	0,144c	0,034a	0,036a	0,047b	0,060c	0,045	0,037	0,044	0,044
Total NMIOD	0,102a	0,108a	0,134b	0,164c	0,094a	0,086a	0,101a	0,119b	0,100	0,101	0,102	0,100

[1]SFA = saturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid, LA = linoleic acid, CLA = conjugated linoleic acid, NMIOD = non-methylene interrupted octadecadienoic, FSO = fresh sunflower oil, PSO = peroxidised sunflower oil, OSO = oxidised sunflower oil, VOSO = very oxidised sunflower oil. Values given in this table for feed samples correspond to means (n = 4) whereas those for meat samples correspond to least-squares means obtained from multifactor ANOVA (n = 48).

[2] Supplementation with 0 or 100 mg/kg of α-tocopheryl acetate.

[3] Supplementation with 0 or 600 mg/kg of Zn as Zinc sulfate.

a-c Means and least-squares means with different letters differ significantly ($P \leq 0.05$).

Table 5. Tocopherol, Zn, Se, Fe and Cu content in feeds[1].

Factor studied	α-tocopherol (mg/kg)	β- + γ-tocopherol (mg/kg)	δ-tocopherol (mg/kg)	Zn content (mg/kg)	Se content (µg/kg)	Fe content (mg/kg)	Cu content (mg/kg)
Sunflower oil							
Fresh	110	22.2	2.8	417	633	173	25
Peroxidized	105	21.3	2.7	336	617	167	22
Oxidized	111	22.4	2.7	386	623	178	22
Very oxidized	103	21.0	2.7	370	626	174	18
SEM	3.7	0.10	0.38	28	11	2.3	3.2
α-Tocoferyl acetate supplementation							
0 mg/kg	70a	22.0	2.7	364	614	172	21
100 mg/kg	144b	21.5	2.7	390	633	174	23
SEM	2.6	0.39	0.06	20	8.1	1.6	2.3
Zn supplementation							
0 mg/kg	104	21.3	2.7	107a	631	176	21
600 mg/kg	110	22.2	2.8	647b	619	170	22
SEM	2.6	0.39	0.06	20	8.1	1.6	2.3

[1] Values given in this table correspond to least-squares means obtained from multifactor ANOVA (n = 48 and n =16 for tocopherol analogs and element content, respectively).
a-b Values corresponding to a certain factor with different letters differ significantly ($P \leq 0.05$).

Table 4. Fat, α-tocopherol, Zn, Se, Fe and Cu content in raw dark chicken meat expressed in 100 g edible portion[1].

Factor studied	Fat (g)	α-tocopherol (mg)	β- + γ-tocopherol (mg)	Zn content (mg)	Se content (µg)	Fe content (mg)	Cu content (mg)
Sunflower oil							
Fresh	14.7	2.58	0.123	1.38	32.6	0.71	35.0
Peroxidized	15.2	2.17	0.116	1.36	32.4	0.77	36.2
Oxidized	15.7	2.44	0.119	1.39	33.1	0.73	34.3
Very oxidized	15.4	2.26	0.117	1.40	31.6	0.75	33.4
SEM	0.47	0.11	0.0063	0.025	0.54	0.024	1.4
α-Tocopheryl acetate supplementation							
0 mg/kg	15.5	1.68a	0.116	1.39	32.5	0.75	34.7
100 mg/kg	15.0	3.04b	0.121	1.38	32.3	0.73	34.8
SEM	0.33	0.080	0.0044	0.018	0.38	0.017	1.0
Zn supplementation							
0 mg/kg	15.5	2.33	0.114	1.36	31.9a	0.73	35.2
600 mg/kg	15.0	2.39	0.123	1.41	33.0b	0.76	34.2
SEM	0.33	0.080	0.0044	0.018	0.38	0.017	1.0

[1] Values given in this table correspond to least-squares means obtained from multifactor ANOVA (n = 48).
a-b Values corresponding to a certain factor with different letters differ significantly ($P \leq 0.05$).

Table 6. TBA (µg of malondialdehyde/kg) and FOX (µmol cumene hydroperoxide equivalents/ kg) values of raw dark chicken meat[1].

Factor studied	TBA	FOX
Sunflower oil		
Fresh	18	713
Peroxidized	22	965
Oxidized	20	762
Very oxidized	22	699
SEM	1.6	148
α-Tocopheryl acetate supplementation		
0 mg/kg	22a	1,222a
100 mg/kg	19b	348b
SEM	1.1	105
Zn supplementation		
0 mg/kg	20	660
600 mg/kg	21	909
SEM	1.1	105

[1] Values given in this table correspond to least-squares means obtained from multifactor ANOVA (n = 48).
a-b Values corresponding to a certain factor with different letters differ significantly ($P \leq 0.05$).

Table 7.- Effect of dietary factors on acceptability, rancid aroma, rancid flavor and TBA values of cooked dark chicken meat stored for a range of periods under different conditions[1].

Factor studied	Acceptability[2]	TBA 4 mo[3]	TBA 30 mo[4]	Rancid aroma[5]	Rancid flavor[5]	TBA 30 mo + 9 d[6]
Sunflower oil						
Fresh	5.3	115	147	56	48	779
Peroxidized	5.2	119	194	57	49	768
Oxidized	4.8	104	179	40	33	850
Very oxidized	5.1	102	212	48	34	670
SEM	0.29	11	19	6.7	5.7	57
Tocopheryl acetate supplementation						
0 mg/kg	5.1	130a	210a	59a	50a	841a
100 mg/kg	5.1	90b	156b	41b	33b	692b
SEM	0.20	8.1	13	4.8	4.0	41
Zn supplementation						
0 mg/kg	5.1	108	177	52	43	719
600 mg/kg	5.1	112	189	48	39	814
SEM	0.20	8.1	13	4.8	4.0	41

[1] Values given in this table correspond to least-squares means obtained from multifactor ANOVA.
[2] Overall acceptability of samples stored for 4 mo at -20 C (n = 192) was ranked using a 9-point scale (1 = very bad; 9 = very good). Treatments did not differ from the blind control.
[3] TBA values (μg of malondialdehyde/kg, n = 32) were measured in samples stored for 4 mo at -20 C. Blind control averages 123 μg of malondialdehyde/kg.
[4] TBA values (μg of malondialdehyde/kg, n = 32) were measured in samples stored for 30 mo at -20 C.
[5] Rancid aroma and flavor scores of samples stored for 30 mo at -20 C and then for 9 d at 4-5 C (n = 80). Scores for rancid aroma and flavor ranged from 0 (minimum reference) to 100 (maximum reference).
[6] TBA values (μg of malondialdehyde/kg, n = 32) were measured in samples stored for 30 mo at -20 C and then stored for 9 d at 4 C. Among the samples evaluated for rancidity the minimum and the maximum references showed the lowest and the highest TBA values, respectively.
a-b Values corresponding to a certain factor with different letters differ significantly ($P \leq 0.05$).

2. RESULTATS I DISCUSIÓ GENERAL

- **Validació d'un mètode per a la determinació de Fe, Zn, Se i Cu en pinsos i en carn de pollastre (objectiu 5)**

Les carns són una de les principals fonts de la dieta de tota una sèrie d'elements minerals com per exemple el Fe, Zn, Cu i Se (Buss i Rose, 1992; Foster i Sumar, 1997). Tanmateix, tenint en compte que alguns d'aquest elements es troben a molt baixes concentracions i que el Se és un element relativament volàtil, la seva determinació requereixi d'un procediment i d'un instrumental analític adequat.

Per a la determinació d'aquests elements, al principi, es va adaptar un mètode descrit per a la determinació de Se en plasma (Verlinden, 1982). Concretament, el procediment d'anàlisi emprat consisteix en la mineralització de 1,5 g de mostra de carn de pollastre mitjançant l'addició de 25 ml d'àcid nítric i després de 5 ml d'àcid perclòric amb l'aplicació de calor en tub obert. La mineralització es va fer fins portar les mostres a sequedat per concentrar al màxim la mostra i reduir els efectes de la matriu àcida. Aquest darrer pas, es diferencia del mètode proposat per Verlinden (1982) en què s'evaporava fins obtenir un volum de 2 ml. Un cop s'han portat les mostres a sequedat, les mostres es redissolen i es determina el Fe, Zn i el Cu per espectroscòpia d'emissió atòmica de plasma acoblat inductivament (ICP-AES) mentre que el Se es determina, amb la generació prèvia d'hidrurs de Se, per espectrometria de masses de plasma acoblat inductivament (HG-ICP-MS).

Els coeficients de variació (CV en %) per les determinacions de Fe i Zn en carn de pollastre després d'aquest procediment de mineralització van ser del 2,0% i del 4,2%, respectivament. En canvi els coeficients de variació pel Se i Cu van ser molt alts (78,1% i 13,2%, respectivament). Per aquesta raó, i pensant en la possible falta d'homogeneïtat de la mostra, es va provar d'augmentar del pes de mostra (de 1,5 g fins a 2 g) i el volum d'àcid nítric, aquest darrer per facilitar la mineralització. L'augment de pes va resultar en una millora substancial de la precisió (CV = 4,2%) per la

determinació del Cu encara que no va millorar substancialment la del Se (CV = 67,3%).

La prereducció de Se^{6+} a Se^{4+} amb àcid clorhídric, necessària per a la determinació del Se per HG, es va comprovar que no afectava a la precisió del mètode i que per tant el problema es trobava en la mineralització de les mostres de pollastre. Les possibles causes que es van tenir en compte van ser: la digestió incompleta de compostos de Se, com per exemple la selenometionina i altres, que són molt resistents als àcids (Nève et al., 1982), i les pèrdues d'aquest element per volatilització.

Així doncs, per resoldre el dubte de quin era el factor determinant es van comparar les recuperacions i les precisions de la mineralització d'unes solucions estàndard de selenit sòdic i de selenometionina així com de les addicions d'aquestes solucions estàndard en mostres de carn. De l'estudi dels resultats es va veure que, independentment de la font de seleni o de si s'havia estat addicionat a la carn o no, la variabilitat de la determinació del seleni venia provocada per la volatilització d'aquest. Aquestes pèrdues es donarien sobretot com a conseqüència de portar les mostres a sequedat, doncs, quan aquestes es van evaporar fins aproximadament uns 2 ml es va comprovar que la recuperació i la precisió per aquest element van augmentar significativament.

Així doncs, per impedir la volatilització del Se es va decidir provar la mineralització en un tub tancat en un microones emprant àcid nítric i peròxid d'hidrogen. L'ús d'aquest procediment va donar lloc a unes precisions (2,5; 2,0; 3,1 i 11,1% per Zn; Fe; Se i Cu) i recuperacions (103-105% pel Zn, 107-108% pel Fe, 97-100% pel Se i 89-94% pel Cu) acceptables (AOAC, 1993). L'exactitud i precisió del mètode també es va comprovar amb la determinació d'aquests elements en un material de referència. Així doncs, aquest protocol de mineralització permet la correcta determinació d'aquests elements en mostres de carn de pollastre.

Quan aquest mateix procediment es va validar per ser aplicat en mostres de pinsos la variabilitat del mètode pels diferents elements va ser baixa. Tot i això, es va observar que les mostres presentaven un precipitat silícic, segurament degut a l'addició de sepiolita (silicat de magnesi) en els pinsos. Donat que la formació de precipitats silícics pot donar lloc a baixes recuperacions en certs elements (Krachler et al., 2002) es va comparar aquest procediment amb un altre exactament igual però en el qual, al

final, s'addicionava fluorhídric per redissoldre el precipitat. De la comparació, es va poder observar que els valors de Zn eren menors quan el precipitat no és redissolia. Per tant, sempre que es vulgui determinar el Zn, en presència d'aquest precipitat, és convenient redissoldre el material silícic. Les precisions (1,8; 2,9; 3,0 i 7,7% pel Zn; Fe; Se i Cu respectivament) i les recuperacions (100-98% pel Zn, 101-103% pel Fe, 87-90% pel Se i 100-108% pel Cu) de les addicions estàndard pels diferents elements en mostres de pinso també van ser acceptables (AOAC, 1993), raó per la qual es recomana l'ús d'aquest mètode amb l'addició de fluorhídric quan es vulguin analitzar pinsos.

- **Elecció de la dosi òptima d'oli de peix (objectiu 1)**

El contingut de greix de la carn de pollastre (barreja de pit i cuixa crua amb pell) no es veu afectat per les dosis d'oli de peix afegides al pinso (1,25% o 2,5%). Malgrat això, la composició en àcids grassos de la carn de pollastre depèn de la composició en àcids grassos del pinso (Ajuyah et al., 1991; Cherian et al., 1996). Això concorda amb el fet que quan afegim oli de peix al 2,5% en el pinso s'obtinguin carns més riques en EPA i DHA que no pas aquelles carns que provenen de pollastres alimentats amb pinsos en què s'havia afegit oli de peix al 1,25%. El contingut en EPA i DHA en la carn de pollastres alimentats amb un 2,5% d'oli de peix és aproximadament el doble que el de la carn de pollastres alimentat amb un 1,25%.

Així doncs, l'addició d'oli de peix al 1,25% dóna lloc a carns que, amb 100 g de porció comestible, cobreixen el 20% de la ingesta recomanada pels àcids grassos de la sèrie n-3 (Food and Nutrition Board, 1989) i també per la suma d'EPA i DHA (Simopoulos et al., 2000) (Figura 6).

Els suplements d'acetat d'α-tocoferol (70 i 140 mg/kg de pinso) no influeixen en la composició lipídica de la carn de pollastre. Aquest fet està d'acord amb els resultats trobats en altres treballs (Lin et al., 1989; O'Neill et al., 1998a). A més, la suplementació amb acetat d'α-tocoferol provoca un augment en el contingut d'α-tocoferol de la carn de pollastre crua, la qual cosa també està d'acord amb altres treballs anteriors (Cherian et al., 1996; O'Neill et al., 1998a; Grau et al., 2001a).

L'augment en el contingut d'α-tocoferol provoca un major estabilitat oxidativa d'aquestes mostres quan són congelades durant 15 dies o 5 mesos, doncs els valors de l'índex de l'ATB no presenten diferències significatives entre els diferents tractaments (Figura 7) ni tampoc amb el control (una mostra comercial recent cuita i emmagatzemada a temperatures de congelació durant un dia). Així doncs, les dosis d'acetat d'α-tocoferol afegides a la dieta (70 i 140 mg/kg) són, primer, de similar eficàcia enfront l'oxidació i, segon, suficients per protegir les carns durant un emmagatzematge llarg a temperatures de congelació.

Per altra banda es coneix que l'addició d'olis de peix en els pinsos dels pollastres per sobre del 2% pot causar un detriment de la qualitat sensorial de la carn de pollastre (Hargis i van Elswyk, 1993). Aquest fet està relacionat amb la major susceptibilitat cap a l'oxidació de les carns riques en AGPI. Tanmateix, en el nostre estudi, l'addició d'oli de peix en el pinso (1,25% o 2,5%) no provoca diferències significatives entre els diferents tractaments sobre l'acceptabilitat dels consumidors després de 15 dies o 5 mesos de conservació a temperatures de congelació (Figura 7). Fins i tot, després de 5 mesos de congelació, tampoc s'observen diferencies entre les diferents mostres provinents de cada tractament i la mostra control, que és una mostra comercial fresca recent cuita i emmagatzemada durant un dia a congelació. Per tant, es pot dir que els consumidors no aprecien de manera diferent les mostres enriquides en AGPI de la sèrie n-3 de les comercials.

El fet que no trobem diferències sensorials entre les diferents mostres de carn i tampoc a nivell del seu estat oxidatiu concorda amb la bibliografia anterior que indica que la suplementació amb α-tocoferol és molt efectiva per protegir enfront de l'oxidació i que existeix una alta correlació entre l'índex de l'ATB i la pèrdua de la qualitat sensorial de les carns (Bou et al., 2001; Ang i Lyon et al., 1990). Aquests resultats s'expliquen per l'efecte protector que té l'α-tocoferol en aquestes mostres riques en AGPI. De fet, quan les dietes no han estat suplementades amb α-tocoferol, només afegint un 0,75% d'oli de peix a la dieta dels pollastres ja s'observen problemes sensorials (Gonzalez-Esquerra i Leeson, 2000).

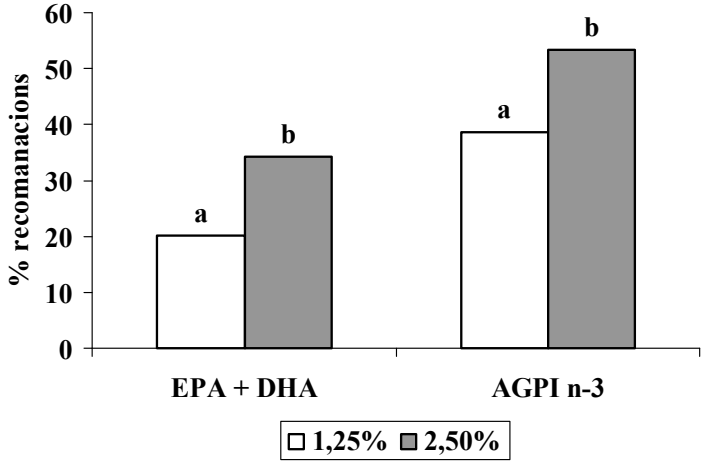

Figura 6. Aportació de 100 g de porció comestible de carn de pollastres alimentats amb un 1,25% o bé un 2,5% d'oli de peix sobre les recomanacions de ingesta (%) d'AGPI n-3 (Food and Nutrition Board, 1989) i d'EPA + DHA (Simopolos et al., 2000).

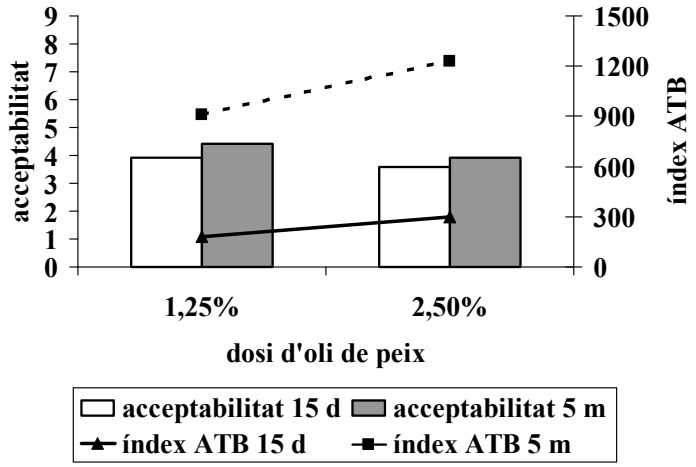

Figura 7. Acceptabilitat dels consumidors (0 = poc acceptable, 9 = molt acceptable) i valors de l'índex de l'ATB (μg de malondialdehid/kg) de la carn de pollastre en funció de la dosi d'oli de peix (1,25% i 2,5%) afegida al pinso i dels temps d'emmagatzematge a -20 ºC.

- **Efecte de la substitució de l'oli de peix durant els darrers dies del cicle productiu (objectiu 2)**

En pinsos de pollastres suplementats amb 100 mg/kg d'acetat d'α-tocoferol, la substitució o no en el pinso del 1,25% de l'oli de peix per la mateixa dosi d'oli de llinosa o de greix animal durant els darrers 5 dies de vida de l'animal (pinso de retirada) no provoca canvis en el contingut de greix total de la carn de pollastre crua (barreja de pit i cuixa amb pell). Tanmateix, la substitució del 1,25% d'oli de peix en el pinso per la mateixa dosi d'oli de llinosa durant el darrers cinc dies de vida provoca una disminució significativa en els nivells de pràcticament tots els àcids grassos de la sèrie n-3, excepte per l'àcid linolènic que augmenta (Figura 8). Així doncs, la substitució de l'oli de peix en el pinso per oli de llinosa dóna lloc a carns (calculat a partir de 100 g de porció comestible) que aporten el 55,6% de la ingesta dietètica recomanada per l'àcid linolènic (Institute of Medicine, 2002) i el 16,6% de la recomenada per la suma d'EPA i DHA (Simopoulos et al., 2000), mentre que les carns procedents d'animals alimentats amb oli de peix durant tota la vida aporten el 11,6% de la ingesta recomanada per l'àcid linolènic i el 21,1% de la ingesta recomanada per la suma d'EPA i DHA.

La substitució del 1,25% d'oli de peix en el pinso per la mateixa dosi de greix animal provoca una disminució dels àcids grassos de la sèrie n-3 similar a la produïda per la substitució amb oli de llinosa, exceptuant el fet que l'àcid linolènic no augmenta significativament (Figura 8). Així doncs, la substitució de l'oli de peix per greix animal dóna lloc a carns que aporten un 16,9% de les ingestes dietètiques recomanades per la suma d'EPA i DHA (Simopoulos et al., 2000). A més, la substitució amb greix animal provoca un increment significatiu dels àcids grassos monoinsaturats.

Per altra banda, les diferents substitucions dietètiques no provoquen canvis en l'estabilitat oxidativa de la cuixa de pollastre cuita als 74 dies ni als 18 mesos d'emmagatzematge a congelació (Figura 9). Aquest fet explicaria que tampoc es trobin diferències en l'acceptabilitat sensorial per part dels consumidors en ambdós períodes (Figura 9). A més, l'índex de l'ATB, que està correlacionat amb la pèrdua de la qualitat sensorial i la generació d'olors desagradables en les carns, no presenta diferències significatives entre tractaments (O'Neill et al., 1998a; Lyon et al., 1988;

Bou et al., 2001). A la vegada, les mostres provinents dels diferents tractaments tampoc són diferents d'una mostra control. Aquesta mostra control és una mostra comercial fresca recent cuita i emmagatzemada durant un dia en les mateixes condicions que les mostres estudiades.

Així doncs, la substitució de l'oli de peix, per altres fonts lipídiques durant el darrers dies de vida, és una estratègia útil per modificar la composició en àcids grassos de la carn de pollastre i així buscar un equilibri entre la seva estabilitat oxidativa i valor nutritiu. A més, gràcies a una suplementació suficient amb tocoferol aquests canvis en la composició d'àcids grassos no repercuteixen en un canvi de l'acceptabilitat per part dels consumidors.

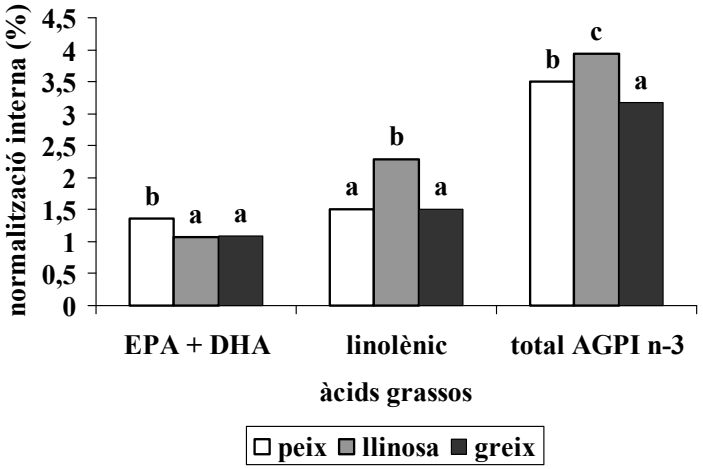

Figura 8. Composició en AGPI de la sèrie n-3 de la carn de pollastres alimentats amb un 1,25% d'oli de peix, llinosa o greix animal els cinc darrers dies de vida expressada en normalització interna (%).

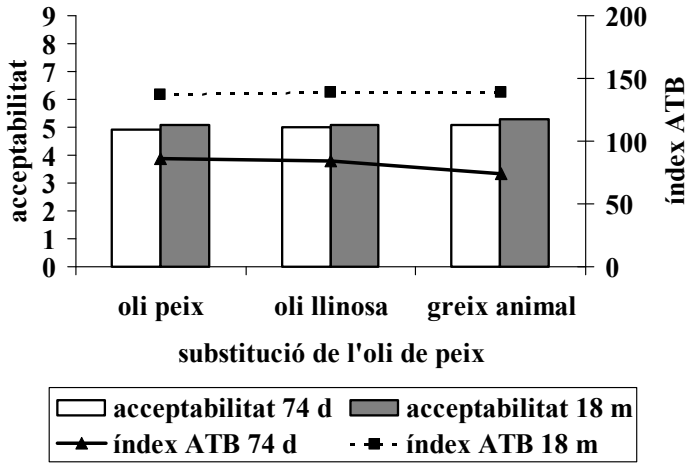

Figura 9. Acceptabilitat dels consumidors (0 = poc acceptable, 9 = molt acceptable) i valors de l'índex de l'ATB (μg malondialdehid/kg) de la carn de pollastre en funció de la substitució o no de l'oli de peix per altres font lipídiques (oli de llinosa o greix animal) així com del temps d'emmagatzematge a -20 ºC.

- **Influència de la suplementació amb Zn i Se (objectiu 3)**

La suplementació de 200, 300 o 600 mg de Zn/kg de pinso no té cap efecte sobre el contingut de Zn en la carn de pollastre. Si bé la suplementació de Zn no té efectes sobre els paràmetres productius, la dosis de 600 mg/kg mostra una lleugera tendència a disminuir el pes de l'animal. Aquests efectes negatius sobre els paràmetres productius de l'animal, en especial sobre el pes de l'animal, havien estat descrits anteriorment a dosis superios a les assajades en aquest estudi (Sandoval et al., 1998).

Els suplements de Zn assajats tampoc tenen cap influència sobre la composició en àcids grassos, el contingut en α-tocoferol, l'estabilitat oxidativa, l'acceptabilitat i la ranciesa de la carn de pollastre.

Tanmateix, l'efecte més remarcable de la suplementació amb Zn és l'efecte sobre el contingut en Se de la carn. En augmentar el suplement de Zn (0, 300 o 600 mg/kg) és produeix un increment en el contingut de Se tant en la barreja de pit i cuixa de pollastre (Figura 10) com en la cuixa de pollastre. Aquest fet no s'havia descrit anteriorment i no es coneix exactament quins factors hi poden estar involucrats.

Tanmateix, es coneix que el Se s'excreta per biometilació i que diferents formes de Se es poden donar dins la carn de pollastre (Foster i Sumar, 1997; Schrauzer, 2000; Daun et al., 2004). Algunes d'aquestes formes de Se poden interactuar amb diferents compostos com la metal·lotioneina (Jacob et al., 1999; Chen i Maret, 2001). Aquesta proteïna intracel·lular, rica en cisteïna, controla, mitjançant els grups tiol, els nivells de Zn dins de l'organisme i està involucrada en altres funcions com per exemple la detoxificació de metalls i la reducció de diversos oxidants cel·lulars (Schwarz et al., 1994; Maret, 2000, 2003; Coyle et al., 2002; Klotz et al., 2003). Per aquesta raó, hom pensa que diferents formes de Se poden unir-se a la metal·lotionina o altres selenoproteïnes que, a altes dosis de Zn en la dieta, poden presentar un metabolisme alterat i, com a conseqüència, augmentar els nivells de Se en la carn de pollastre.

La suplementació de Se en forma de selenit sòdic (0 vers 1,2 mg Se/kg) no provoca un augment significatiu en el contingut de Se en la carn de pollastre (Figura 11). El seleni inorgànic és absorbit de forma passiva i pot ser excretat o entrar a formar part específicament de selenoproteïnes funcionals en forma de selenocisteïna (Thomson, 1998; British Nutrition Foundation, 2001). Així doncs, si es detecta un increment en els nivells de selenoproteïnes funcionals a partir de la suplementació de Se amb formes inorgàniques possiblement indica que els requeriments en aquest element no estaven totalment coberts (Thomson, 1998; Mahan i Parret, 1996).

Per contra, l'addició d'un extracte de llevats ric en Se en el pinso (0 vers 0,2 mg Se/kg) provoca un increment significatiu en el contingut de Se tant en la barreja de pit i cuixa de pollastre (Figura 11). Aquest extracte majoritàriament conté Se en forma de selenometionina. Aquest fet explicaria que la carn de pollastre acumuli més Se, doncs la selenometionina pot acumular-se de forma inespecífica en diverses proteïnes com per exemple les dels teixits musculars (Thomson, 1998; Surai, 2002).

La suplementació de les dues formes de Se no provoca cap canvi en la composició d'àcids grassos, el contingut en α-tocoferol, l'estabilitat oxidativa, i l'acceptabilitat de la carn de pollastre.

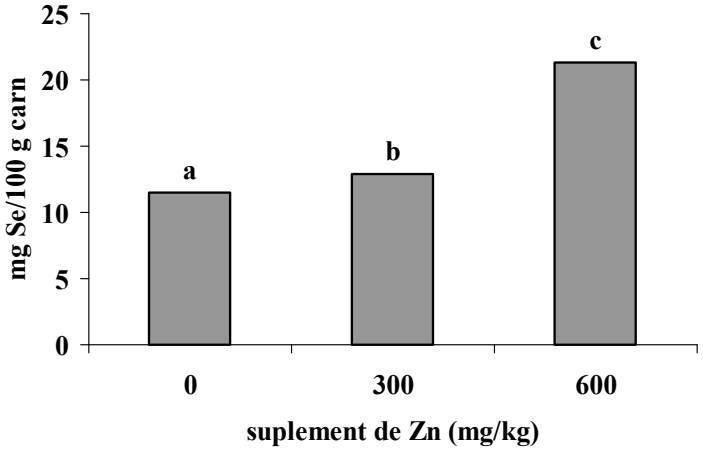

Figura 10. Efecte del suplement de Zn sobre el contingut de Se en la barreja de pit i cuixa de pollastre crua amb pell.

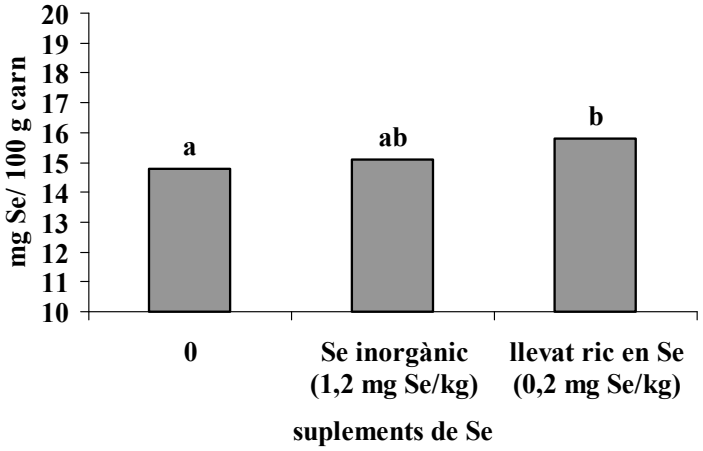

Figura 11. Efecte de la forma de Se sobre el contingut de Se en la barreja de pit i cuixa crua amb pell.

- **Influència de l'addició d'olis oxidats (objectiu 4)**

Es preparen diferents olis escalfats a partir d'un oli de gira-sol refinat (anomenat oli fresc). Després d'escalfar-lo 12 dies a una temperatura de 55-60 ºC

s'obté un oli de gira-sol amb un alt índex de peròxids, raó per la qual se l'anomena oli peroxidat. Quan el mateix oli fresc s'escalfa a una temperatura de 190-195 ºC durant 28 h s'obté un oli de gira-sol amb un alt índex de *p*-anisidina i un elevat contingut en polímers, raó per la qual se l'anomena oli molt oxidat. De la barreja al 50% d'oli de gira-sol fresc i oli de gira-sol molt oxidat s'obté un oli amb valors de variables d'oxidació intermedis, raó per la qual se l'anomena oli oxidat.

La composició en àcids grassos d'aquests olis varia en funció del tractament tèrmic que han rebut. L'oli peròxidat té un contingut menor en àcid linoleic i àcid linolènic degut a l'oxidació que aquests han sofert. L'escalfament a altes temperatures també provoca una disminució en àcid linoleic i linolènic, però a més, i degut a les elevades temperatures, es formen diferents isòmers dels àcids grassos (Figura 12). Aquest darrer fet també ha estat descrit també per altres autors (Juanéda et al., 2003; Sébédio et al., 1988).

Concretament, en escalfar a temperatures de 190 ºC té lloc la formació d'isòmers de configuració de l'àcid linoleic amb un doble enllaç *trans* (*c*,*t*/*t*,*c*-LA) o amb els dos dobles enllaços en posició *trans* (*t*,*t*-LA) i també de l'àcid oleic, éssent majors els continguts en aquests isòmers en l'oli molt oxidat. A més, també es formen isòmers de posició com els isòmers de l'àcid linoleic conjugat (CLA): el 9*c*,11*t*-CLA i el 10*t*-12*c*-CLA, i un conjunt d'àcids grassos amb 18 àtoms de carboni amb dues insaturacions en configuració *trans* no interrompudes per un grup metil (*ditrans*-NMIOD) (Figura 12).

Degut als tractaments tèrmics aquest olis van patir un pèrdua significativa en el contingut en α-tocoferol. Tenint en compte això, quan es van formular els diferents pinsos amb els diferents olis, es van rectificar les pèrdues d'α-tocoferol afegint en els pinsos acetat d'α-tocoferol per tal de què tots aportessin el mateix contingut en α-tocoferol a la dieta dels animals.

L'addició d'olis de gira-sol amb diferent grau d'oxidació no va provocar canvis en el contingut de greix de la cuixa de pollastre crua amb pell. En relació a l'addició d'oli peroxidat, aquest no provoca canvis en la composició d'àcids grassos de la carn de pollastre tot i que la seva composició reflexa les tendències observades en l'oli peroxidat així com en el pinso formulat a partir d'aquest. Contràriament, l'addició en el pinso d'olis que han estat escalfats a temperatures superiors a 190 ºC provoquen

canvis en la composició d'àcids grassos de la carn de la cuixa de pollastre crua (Figura 13). Els canvis observats en la carn reflexen en certa mesura el diferent contingut en isòmers dels àcids grassos dels olis utilitzats. De fet, és coneix que les carns poden tenir diferents contingut en àcids grassos *trans* i que aquestes diferències venen sobretot donades pel contingut d'aquests en la dieta (Aro et al., 1998; Szymczyk et al., 2001; Badinga et al., 2003; Simon et al., 2000). Així doncs, en les carns que provenen de pollastres que han rebut el pinso amb oli de gira-sol molt oxidat s'observa un major contingut en àcid linoleic amb un o dos dobles enllaços *trans* i també un major contingut en *ditrans*-NMIOD (Figura 13). Les carns provinents de pollastres que han menjat pinsos amb oli de gira-sol oxidat també tenen un major contingut en àcid linoleic amb un doble enllaç *trans* i *ditrans*-NMIOD respecte a les carns que provenen d'animals que han rebut pinsos formulats amb oli fresc (Figura 13).

L'anàlisi discriminant de les mostres de cuixa de pollastre indica que el contingut per a diferents isòmers d'àcids grassos i en especial el contingut en *ditrans*-NMIOD permet distingir aquelles carns que provenen de pollastres alimentats amb pinsos en què s'han afegit olis oxidats a altes temperatures (Figura 14). A més, es va poder establir una recta amb un alt coeficient de detreminació (r^2 = 0,876) entre el contingut de *ditrans*-NMIOD en el pinso i el seu contingut en la cuixa de pollastre.

El subministrament de pinsos en què s'han afegit olis oxidats, però en els quals s'han corregit les pèrdues en α-tocoferol, no provoca canvis en el contingut d'α-tocoferol, en el contingut de minerals (Fe, Zn, Cu i Se), ni en les variables d'oxidació (índex de l'ATB i contingut en hidroperòxids) de la carn de la cuixa de pollastre crua.

En relació a la carn de la cuixa de pollastre cuita, el subministrament d'olis oxidats tampoc provoca canvis en l'acceptabilitat per part dels consumidors després de 4 mesos d'emmagatzematge a temperatures de congelació. Tampoc en l'índex de l'ATB després de 30 mesos de conservació a -20 ºC.

A partir de l'observació d'aquests darrers valors de l'índex de l'ATB es va creure convenient accelerar l'oxidació de les mostres per tal de poder avaluar la ranciesa d'aquestes. Per aquesta raó, després de 30 mesos d'emmagatzematge a -20 ºC, les mostres es van deixar durant 9 dies a temperatures d'entre 4-5 ºC i tot seguit es va fer l'avaluació sensorial de l'aroma i flavor a ranci (amb un panel de tastadors

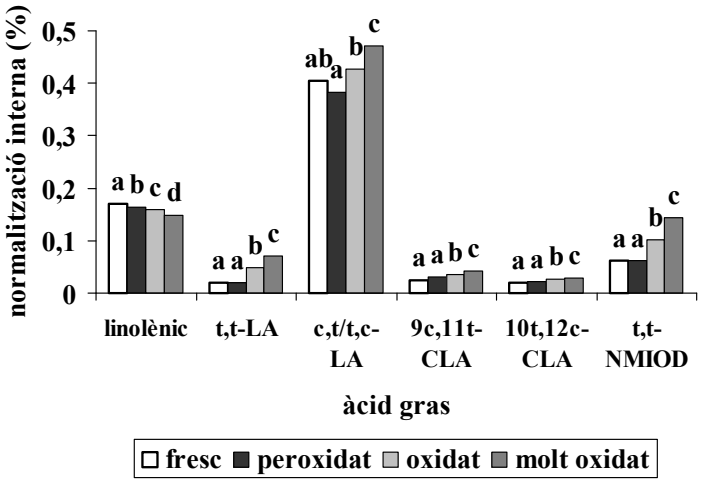

Figura 12. Composició en àcid linolènic i en isòmers de l'àcid linoleic dels diferents olis de gira-sol emprats, expressats en normalització interna (%). LA = àcid linoleic; CLA = àcid linoleic conjugat; NMIOD = àcid gras amb 18 àtoms de carboni i 2 dobles enllaços no interromputs per un grup metil.

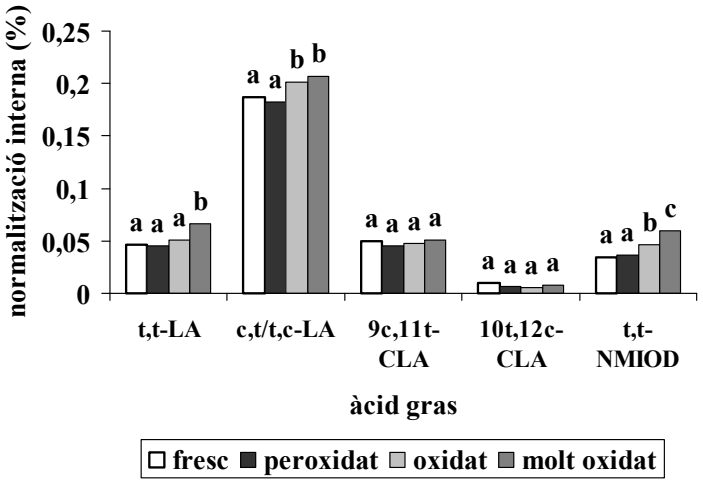

Figura 13. Composició en isòmers de l'àcid linoleic en les cuixes de pollastres alimentats amb els diferents olis, expressats en normalització interna (%). LA = àcid linoleic; CLA = àcid linoleic conjugat; NMIOD = àcid gras amb 18 àtoms de carboni i 2 dobles enllaços no interromputs per un grup metil.

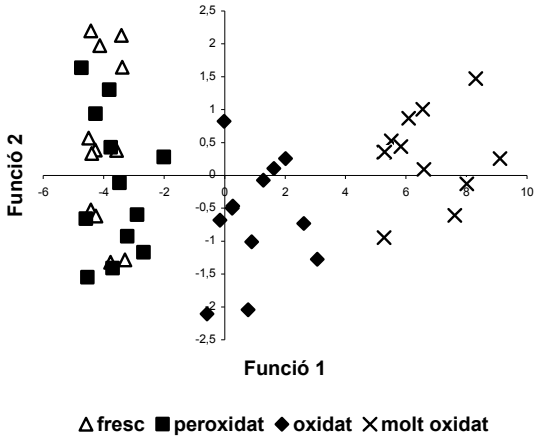

Figura 14. Quadre de distribució de les puntuacions respecte les dues funcions principals de l'anàlisi discriminant fet en les cuixes de pollastres alimentats amb diferents olis (fresc, peròxidat, oxidat i molt oxidat).

entrenats). Aquesta avaluació sensorial no va resultar afectada per l'addició d'olis oxidats al pinso. El mateix va passar per la determinació de l'índex de l'ATB mesurat després d'emmagatzemar les mostres durant 9 dies a temperatura de refrigeració (després d'haver estat congelades durant 30 mesos).

La suplementació amb acetat d'α-tocoferol no provoca canvis en la composició de la carn de la cuixa de pollastre, exceptuant l'increment en el contingut d'α-tocoferol. Està àmpliament descrit que la suplementació amb acetat d'α-tocoferol provoca l'augment d'α-tocoferol en la carn de pollastre (Cherian et al., 1996; de Winne i Dirinck, 1996). Al mateix temps, també s'observen valors d'oxidació menors quan s'ha afegit aquest suplement a la dieta, tant pel valor de l'índex de l'ATB com pel contingut en hidroperòxids lipídics (HPL) (Figura 15), posant de manifest les propietats antioxidants que té el tocoferol en el control d'aquestes i d'altres variables d'oxidació (Grau et al., 2001a, b).

De manera similar, en carn de la cuixa cuita, els valors de l'índex de l'ATB als 4 mesos d'emmagatzematge a congelació són menors quan les carns provenen de pollastres que han rebut el suplement d'acetat d'α-tocoferol. Tot i això, l'acceptabilitat de les mostres no presenta diferències entre la suplementació o no amb acetat d'α-

tocoferol (Figura 16). Tanmateix, quan s'avalua la ranciesa després de 30 mesos a congelació seguits de 9 dies a refrigeració, les mostres no suplementades amb acetat d'α-tocoferol presenten unes puntuacions en aroma i flavor a ranci més elevades i el mateix passa amb els índexs de l'ATB després de 30 mesos a -20 ºC mesurats abans i després d'emmagatzemar 9 dies més a temperatures de refrigeració (Figura 17).

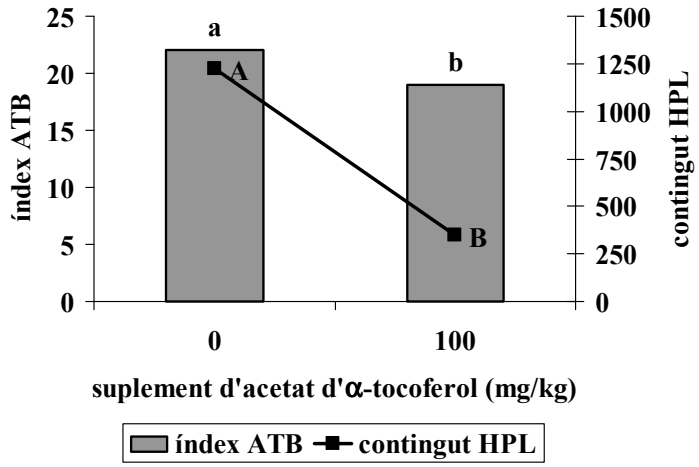

Figura 15. Efecte de la suplementació amb acetat d'α-tocoferol sobre l'índex de l'ATB (μg malondialdehid/kg) i el contingut en HPL (μmol d'hidroperòxid de cumè/kg) en carn de cuixa pollastre crua.

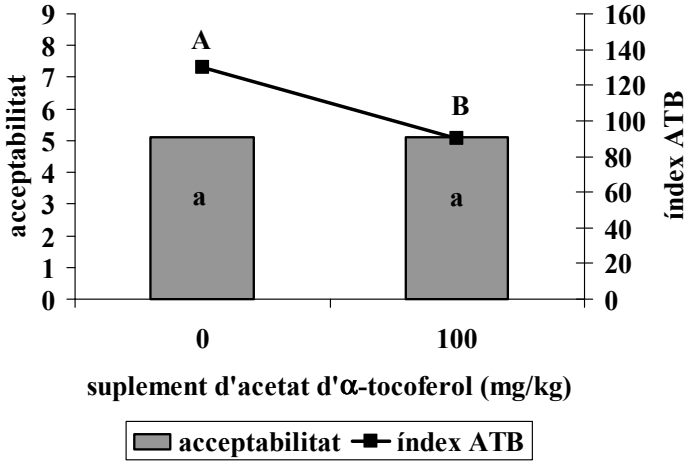

Figura 16. Efecte de la suplementació amb acetat d'α-tocoferol sobre l'acceptabilitat i l'índex de l'ATB (μg malondialdehid/kg) en carn de cuixa de pollastre cuita després de 4 mesos d'emmagatzematge a -20 ºC.

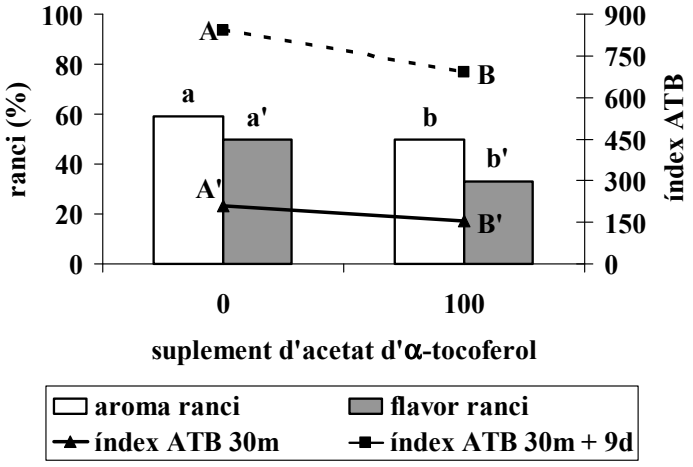

Figura 17. Efecte de la suplementació amb acetat d'α-tocoferol sobre l'aroma i el flavor a ranci de la carn de cuixa de pollastre cuita després d'emmagatzemar les mostres 30 mesos a -20 ºC més 9 dies a 4-5 ºC així com sobre l'índex de l'ATB (μg malondialdehid/kg) mesurat després de 30 mesos a -20 ºC (30 m) i després de 30 mesos a -20 ºC més 9 dies a 4-5 ºC (30 m + 9 d).

3. CONCLUSIONS

Sobre la determinació de minerals en pinsos i carn de pollastre:

- La mineralització de mostres de carn de pollastre en tub obert provoca pèrdues per volatilització del Se, sobretot si les mostres es duen a sequedat, raó per la qual aquest procediment només es recomana per a la determinació de Fe i Zn en carn de pollastre.

- La mineralització en tub tancat i per l'acció combinada de l'addició d'una barreja d'àcid nítric i peròxid d'hidrogen juntament amb l'ús de microones permet la correcta determinació i quantificació del Fe, Zn, Se i Cu en carn de pollastre. L'addició d'àcid fluorhídric en l'etapa final de la mineralització permet la dissolució del precipitat de material silícic i la correcta quantificació de Fe, Zn, Se i Cu en pinsos.

Sobre els efectes de la modificació de la dieta en el valor nutritiu, l'estabilitat oxidativa i la qualitat sensorial de la carn de pollastre:

- L'addició en el pinso d'oli de peix al 1,25% durant les tres darreres setmanes de vida provoca un enriquiment substancial en EPA i DHA en la carn de pollastre (aporta aproximadament el 20% de la ingesta dietètica recomanada per la suma d'aquests àcids grassos). La transferència d'aquests àcids grassos del pinso a la carn és molt alta, doncs quan s'afegeix un 2,5% d'oli de peix al pinso, la carn conté pràcticament el doble d'EPA i DHA que quan la dosi era del 1,25%.

- La suplementació amb acetat d'α-tocoferol dels pinsos enriquits en oli de peix provoca un augment en el contingut d'α-tocoferol en la carn de pollastre. A

més, en les condicions assajades les dues dosis d'acetat d'α-tocoferol (70 i 140 mg/kg) confereixen una bona estabilitat oxidativa a les carns, mesurada a través de l'índex de l'ATB. Aquest fet provoca que no hi hagin diferències en l'acceptabilitat sensorial després d'emmagatzemar les mostres congelades durant 5 mesos.

- La substitució de l'oli de peix durant els últims 5 dies de vida dels pollastres per oli de llinosa dóna lloc a carns més riques en àcid linolènic (aproximadament aporten el 55% de les recomanacions per aquest àcid gras) i amb un menor contingut en EPA i DHA (16% de les recomanacions per la suma d'aquests àcids grassos). Per contra la substitució d'oli de peix per greix animal dóna lloc a carns menys riques en AGPI de la sèrie n-3, si bé encara tenen un contingut apreciable en EPA i DHA comparable al de la carn obtinguda quan es substitueix l'oli de peix per oli de llinosa.

- La suplementació dels pinsos amb Zn fins a dosis de 600 mg/kg no té cap efecte en el contingut d'aquest element en la carn de pollastre. Tampoc té cap efecte en l'estabilitat oxidativa, mesurada mitjançant l'índex de l'ATB, ni en l'acceptabilitat sensorial de la carn de pollastre cuita.

- La suplementació de Se en formes orgàniques provoca un increment en el contingut d'aquest element en la carn de pollastre, mentre que la suplementació amb selenit sòdic no el provoca. En cap cas, l'addició de Se en el pinso provoca canvis en l'estabilitat oxidativa, mesurada mitjançant l'índex de l'ATB, ni en l'acceptabilitat de la carn de pollastre cuita.

- El contingut de Se en la carn de pollastre augmenta significativament quan augmenta el suplement de Zn en el pinso.

- L'addició al 6% d'olis oxidats a temperatures de 190-195 ºC als pinsos provoca canvis en la composició en àcids grassos de la carn de pollastre. L'escalfament dels olis a aquestes temperatures comporta la formació diferents isòmers de

posició i configuració dels àcids grassos que quan son subminitrats en la dieta dels animals poden fer augmentar el seu contingut en la carn de pollastre. De fet, l'augment amb *ditrans*-NMIOD permet discriminar entre les carns procedents d'animals alimentats amb pinsos que contenien olis escalfats a altes temperatures (190-195 ºC) i les carns procedents d'animals que han rebut pinsos amb oli fresc o oli oxidat a 55-60 ºC.

- L'addició d'olis amb diferent grau d'oxidació als pinsos (6%), però igual contingut en α-tocoferol, no provoca canvis en el contingut d'α-tocoferol ni en les variables d'oxidació (l'índex d'ATB i contingut d'hidroperòxids) de la cuixa de pollastre crua.

- L'addició d'aquest percentatge d'olis oxidats tampoc provoca canvis en l'avaluació de l'acceptabilitat de les cuixes de pollastre cuites ni en l'índex de l'ATB després de 4 mesos d'emmagatzematge a congelació. Tampoc s'observen canvis en l'aparició de la ranciesa ni en l'índex de l'ATB després de 30 mesos d'emmagatzematge a – 20 ºC seguits de 9 dies de conservació a 4-5 ºC.

- L'estabilitat oxidativa de les cuixes de pollastres que han estat alimentats amb pinsos que contenien olis oxidats, únicament es veu influenciada per l'addició en el pinso d'acetat d'α-tocoferol. Aquesta suplementació del pinso amb acetat d'α-tocoferol (100 mg/kg) provoca un disminució de l'índex de l'ATB i del contingut d'hidroperòxids en la carn crua i de l'índex de l'ATB i la ranciesa en la carn cuita.

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VI. ANNEXES

ABREVIACIONS EMPRADES

Taula 11. Llistat de les abreviatures emprades en el texte escrit en Català.

ABREVIATURA	NOM COMPLET
ADP	Adenosin difosfat
AGI	àcid gras insaturat
AGPI	acid gras poliinsaturat
AOCS	American Oil Chemists' Society
ASTMD	American Society for Testing Materials
ATB	Àcid tiobarbitúric
BHA	Butil hidroxianisol
BHT	Butil hidroxitoluè
c	Configuració *cis*
COMA	Committe on the Medical Aspects
CV	Coeficient de variació
DHA	Àcid docosahexaenoic
EPA	Àcid eicosapentaenoic
FAO	Food and Agriculture Organization
GPx	Enzim glutatió-peroxidasa
GSH	Glutatió reduït
GSSG	Glutatió oxidat
HPL	Hidroperòxid lipídic
·OH	Radical hidroxil
P-	complex protoporfirina
R·	Radical d'un àcid gras
RO·	Radical alcoxil
ROO·	Radical peroxil
ROOH	Hidroperòxid
SOD	Superoxid-dismutasa
TBHQ	Ter-butil hidroquinona
t	Configuració *trans*
USDA	United States Department of Agriculture
WHO	World Health Organization

Taula 12. Llistat de les abreviatures emprades en el texte escrit en Anglès.

ABREVIATURA	NOM COMPLET
α-TA	α-tocopheryl acetate
AF	Animal fat
ANOVA	Analysis of variance
BHT	Butylated hydroxytoluene
BW	Body weight
CLA	Conjugated linoleic acid
d	Day
DHA	Docosahexaenoic acid
DMOX	Dimethyloxazoline
EDTA	Ethylendiaminetetraacetate
EPA	Eicosapentaenoic acid
FA	Fatty acid
FAME	Fatty acid methyl ester
FO	Fish oil
FOX	Ferrous oxidation-xylenol orange
FSO	Fresh sunflower oil
GPx	Glutathione peroxidase
h	Hour
LA	Linoleic acid
LO	Linseed oil
min	Minute
mo	Month
MT	Metallothionein
MUFA	Monounsaturated fatty acid
NMIOD	Non-methylene interrupted octadecadienoic
OSO	Oxidised sunflower oil
PSO	Peroxidised sunflower oil
PUFA	Polyunsaturated fatty acid
SEM	Standard error of the mean
SeMet	Selenomethionine
SFA	Saturated fatty acid
TBA	Thiobarbituric acid
vol	Volume
VOSO	Very oxidised sunflower oil
wt	Weight

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COMUNICACIONS A CONGRESSOS

Autors:	R. Bou, F. Guardiola, A. Tres, M. Baucells, R. Codony
Títol:	Effect of Dietary Fish Oil, and α-Tocopheryl Acetate and Zn Supplementation on the Composition and Acceptability of Chicken Meat.
Forma de presentació:	Pòster
Congrés:	2nd Euro Fed Lipid Congress. Managing Oils & Fats Supplies for Human Needs
Lloc:	Strasbourg (França)
Data:	6-8 novembre 2002
Entitat organitzadora:	Euro Fed Lipid
Caràcter:	Internacional

Autors:	R. Bou, R. Codony, J. Boatella, J. Galobart, F. Guardiola.
Títol:	Improvement of Chicken Meat Quality, with Special Emphasis on Nutritional Value, Through Dietary Treatments.
Forma de presentació:	Presentació oral
Congrés:	25th World Congress and Exhibition of the ISF
Lloc:	Burdeaux (França)
Data:	12-15 octubre 2003
Entitat organitzadora:	ISF
Caràcter:	Internacional

Autors:	R. Bou, F. Guardiola, A. C. Barroeta, R. Codony
Títol:	Increase of geometrical and positional fatty acid isomers in dark meat from broilers fed heated oils.
Forma de presentació:	Pòster
Congrés:	3rd Euro Fed Lipid Congress. Oils, Fats and Lipids in a Changing World
Lloc:	Endinburgh (Escòcia)
Data:	5-8 setembre 2004
Entitat organitzadora:	Euro Fed Lipid
Caràcter:	Internacional