Universitat de Girona

FAT AND SALT REDUCTION AND SUBSTITUTION IN SMALL-CALIBER NON-ACID FERMENTED SAUSAGES

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Dipòsit legal: Gi. 1806-2014
http://hdl.handle.net/10803/283747

PhD THESIS

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PhD Programme in Technology

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Thesis presented at the University of Girona to apply for the PhD degree

Dr. Maria Dolors Guàrdia Gasull and Dr. Xavier Serra Dalmau, researchers of the Food Technology Programme of IRTA in Monells (Girona),

WE DECLARE:

That the thesis entitled **"Fat and salt reduction and substitution in small-caliber non-acid fermented sausages"** presented by **Héctor Mora Gallego** to obtain the doctoral degree has been completed under our supervision and meets the requirements to apply for an International Doctorate.

Dr. Maria Dolors Guàrdia Gasull   Dr. Xavier Serra Dalmau

Girona, May, 5th 2014

The author gratefully acknowledges the European Community financial participation under the Sixth Framework Programme for Research, Technological Development and Demonstration Activities, for the Integrated Project Q-PORKCHAINS FOOD-CT-2007-036245.

Agradecimientos

Cuando hace ya cinco años comencé el proyecto de realizar una tesis doctoral, no tenía ni idea de todo lo que iba a suponer en mi vida, no sólo por el trabajo realizado sino por el hecho de haber vivido y compartido tantos momentos y experiencias, tanto profesionales como personales. Mi etapa trabajando en IRTA y viviendo en Girona es algo que siempre llevaré dentro y por ello quiero expresar mi agradecimiento a las siguientes personas, sin las cuales este trabajo no hubiera sido posible:

A IRTA, por concederme la beca de doctorado y darme así la oportunidad de formarme en el campo de la investigación, de trabajar en el proyecto Q-Porkchains y de llevar a cabo esta tesis.

A mis directores de tesis, Xavier Serra y Maria Dolors Guàrdia, por ser grandes profesionales y personas, por todo lo que he aprendido de ellos, por su dedicación y la calidad aportada a este trabajo y a todos los que he tenido el gusto de compartir con ellos, por ayudarme siempre que lo he necesitado y por ser comprensivos en los momentos difíciles. Ha sido un placer trabajar con vosotros.

A Cinto Arnau, jefe del programa de Tecnología Alimentaria de IRTA, por su orientación y buenos consejos en la realización de este trabajo.

A Pere Gou, por su contribución en los artículos que se incluyen en esta tesis y el gran trabajo estadístico realizado.

A Luis Guerrero y Anna Claret por su ayuda y consejos en los estudios de análisis sensorial y de consumidores, y por todo lo que he aprendido con ellos sobre este tema. Luis, gracias por tu ayuda con la estadística, tu buen humor y tus consejos.

A todos los compañeros y compañeras de IRTA y CENTA con quien he convivido estos años. A todos los técnicos, investigadores y becarios de IRTA que con su trabajo han contribuido a que esta tesis sea posible: Cristina, Jordi, Quim, Bernardo, Marta, Grau, Dani, Raquel, Bekele, Gustavo, Tòfol, Jeroni, Dioni, Ramix, Xevi, Josep, Núria… por su ayuda con las fabricaciones y análisis de muestras y datos. A Nico, Elvira y Anna por facilitarme el trabajo en el laboratorio de del CENTA. Y por supuesto a Eva, por darme la oportunidad de ponerme en contacto con IRTA y realizar allí mi tesis.

A Dolors Parès, por su ayuda durante el Máster de Biotecnología Alimentaria y a Mònica Toldrà, mi tutora de la tesis en la UdG.

A los compañeros y compañeras del Máster, por los buenos momentos dentro y fuera de la UdG, y por la amistad que aún conservo con algunos y algunas.

A René Lametsch y Rikke Miklos por su contribución en los artículos que se incluyen en esta tesis y por hacer que mi estancia en la Universidad de Copenhague fuera muy agradable.

A los compañeros y amigos que he conocido a raíz de estos cuatro años de trabajo, y que al final han acabado siendo parte de mi vida en Girona: Israel y Luis (muy buenas las excursiones, caminatas por la montaña y viajes), Zhen y Jie, Pedro, Elisa, Duarte, Madda, Emine, Burak, Arianna y Vincent, Viki, Diego, Mauro y María, y más gente que seguro se me olvida.

En especial, querría agradecer enormemente a las personas que considero que han sido mi familia en Girona, con quienes he compartido esta etapa tan importante de mi vida, y que siempre han estado conmigo en los mejores y en los peores momentos. Muchas gracias, Marc y Cris, Amós, Nico, Helena, Rodri, Vane y Amanda. Sin vosotros y vosotras hubiera sido mucho más difícil todo. Sois grandes amigos y amigas.

A mis amigos y amigas de Alcázar de San Juan, por estar siempre ahí, por interesarse en el trabajo que he estado haciendo estos años y por hacerme sentir siempre cerca de casa a pesar de haber estado lejos.

Y por último, agradezco a mi familia, y muy especialmente a mis padres Pedro y Merce y a mi hermana Ade, todo su apoyo, su comprensión y su cariño. Porque siempre habéis creído en todo lo que he hecho y siempre me habéis animado a seguir adelante. Porque sois un ejemplo de cómo afrontar la vida por difícil que venga, unos luchadores de los que he aprendido mucho, y eso se refleja en lo que yo he llegado a ser y a conseguir. Por ello, este trabajo es vuestro también y os lo dedico especialmente a vosotros. Os quiero.

Contents

PhD thesis presentation

This PhD thesis was carried out under the Integrated Project Q-PORKCHAINS FOOD-CT-2007- 036245 under the Sixth Framework Programme for Research, Technological Development and Demonstration Activities of the European Community. The project started in January 2007 with 44 partners from 15 EU countries, 1 partner from Norway, 1 partner from USA, and 4 partners from INCO target countries (China, Brazil, and South Africa). An open call in the 3rd year of the project supplemented the consortium with additional 13 partners, 1 research organisation and 12 Small and Medium Enterprises (SMEs) which further developed and applied Q-PorkChains' results in pilot projects.
The underlying rationale for the project is to be found in the dramatic changes in the international pork market over the past decade. Pork production is an important socioeconomic factor in the European Union (EU) as more than 20% of the world pork production is produced here. Pork and pork products represent an important part of the diet in the EU. In several of the member states the proportion of pork exceeds 50 % of all meat consumed. But Europe faces increasing competition with regard to pork production and the subsequent processing and retailing of pork products. China, Brazil, USA and Canada are challenging the European Union's self-sufficiency and leading position in the pork sector. Additionally, European consumers are becoming increasingly sophisticated, demanding and powerful. They require foods that are of high quality, safe, diverse and healthy. Preferably they should be produced environmentally friendly and respect ethical aspects such as animal welfare issues.

In order to defend the position of the EU and to explore new production and market opportunities, Q-PorkChains addressed the need for developing innovative, integrated, and sustainable production chains, which produce high quality pork products, matching consumer demands.

The Q-PorkChains project was structured in six research modules (MD) and two horizontal modules bridging the research community, the pork industry and the society at large with the following general objectives:

- **MD I. Consumer/market analysis**: To develop new tools for marketing and development of pork-based products based on mapping and assessment of behaviour towards the pig production chain as citizens and as consumers.
- **MD II. Diversity, Flexibility and Sustainability of Farm-level Production Systems**: To develop tools and systems for improved responsiveness of sustainable production systems at farm-level towards society demands.
- **MD III. Product Development - Quality, Nutrition and Convenience**: To develop innovative technologies for improved pork products to match consumer demands in relation to quality, nutrition, and convenience.
- **MD IV. Integration and Sustainable Management of the Production Chain**: To identify and develop tools for integration and efficient sustainable management of a diversified European production and distribution system.
- **MD V. New Biology as a Tool for Control of Pork Quality**: To develop and apply new and appropriate molecular control tools in the production of pork.
- **MD VI. Synthesis of Existing Knowledge on Pork Quality, Safety, and Welfare**: To develop prediction models for pork quality, safety, and welfare as a consequence of the production systems.
- **MD A. Pilot and Demonstration Chains**: To facilitate cooperation with SMEs on pilot research and demonstration activities and develop inter-organisational collaboration along pork chains and networks.
- **MD B. Education, Training, and Dissemination**: To transfer knowledge from the project to users at all levels.

This PhD thesis was carried out within Module III (Work Package III.1) with the general objective to develop innovative technologies for improved pork products to match consumer demands making products that are nutritious, convenient, of high quality and safety, and produced from the best quality pork. Module III was divided in three work packages: nutritional enhancement (WP III.1), quality optimisation (WP III.2) and development of convenience pork products (WP III.3).

In WPIII.1, the activities carried out in Task III.1.a were focused on reducing sodium content in both fermented sausages and cooked ham and fat reduction in fermented sausages.

As a result of the activity of WP III.1 Task III.1a, three scientific publications that comprise this doctoral thesis were obtained.

Published works

Mora-Gallego, H., Serra, X., Guàrdia, M. D., Miklos, R., Lametsch, R., & Arnau, J. (2013). Effect of the type of fat on the physicochemical, instrumental and sensory characteristics of reduced fat non-acid fermented sausages. *Meat Science, 93*, 668–674.

Quality index of the journal according to JCR Science Edition 2012:
Impact factor: 2.754
Position in Food Science & Technology Category: 17/124 (1^{st} quartile)

Mora-Gallego, H., Serra, X., Guàrdia, M. D., & Arnau, J. (2014). Effect of reducing and replacing pork fat on the physicochemical, instrumental and sensory characteristics throughout storage time of small caliber non-acid fermented sausages with reduced sodium content. *Meat Science, 97*, 62–68.

Quality index of the journal according to JCR Science Edition 2012:
Impact factor: 2.754
Position in Food Science & Technology Category: 17/124 (1^{st} quartile)

Mora-Gallego, H., Guàrdia, M. D., Serra, X., Gou, P., & Arnau, J. Sensory characterization and consumer acceptability of KCl and sunflower oil addition in small-caliber non-acid fermented sausages with a reduced content of NaCl and fat. *Meat Science*, *Submitted*. (MEATSCI-D-14-00237).

Quality index of the journal according to JCR Science Edition 2012:
Impact factor: 2.754
Position in Food Science & Technology Category: 17/124 (1^{st} quartile)

Abbreviations

β	Regression coefficient ($P < 0.05$) of the covariable
%TEF	Total expressible fluid
a*	Instrumental redness Lab-CIE (1976)
a_w	Water activity
AESAN	Agencia Española de Seguridad Alimentaria
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists' Society
ASTM	American Society for Testing and Materials
b*	Instrumental yellowness Lab-CIE (1976)
BEDCA	Spanish Food Composition Database
BF	Batch of non-acid fermented sausages with 5% backfat (Paper I)
BF3	Batch of small-caliber non-acid fermented sausages with 3% backfat (Paper II)
BF20	Batch of small-caliber non-acid fermented sausages with 20% backfat (Paper II)
CIE	Commission Internationale de l'Éclariage
CNC	Coagulase negative cocci
CT	Batch of small-caliber non-acid fermented sausages made of lean trimmings with 20% fat and 2.5% NaCl (Paper III)
DAGs	Diacylglycerols
DAG	Batch of non-acid fermented sausages with 5% diacylglycerols (Paper I)
DASH	Dietary Approaches to Stop Hypertension
DFDM	Defatted dry matter
EFSA	European Food Safety Authority
EVOO	Extra-virgin olive oil
F_0	Initial force in Stress Relaxation Test
F_2	Force at 2 seconds in Stress Relaxation Test
F_{90}	Force at 90 seconds in Stress Relaxation Test
FDA	United States Food and Drug Administration
FICT	Fédération Française des Industriels Charcutiers Traiteurs
FOS	Fructooligosacharides
FOSHU	Food for Special Health Use
FSAI	Food Safety Authority of Ireland

GLM	General Linear Model
GRAS	Generally Recognized as Safe
HDL	High-density lipoprotein
HUT	Home-use test
K-lactate	Potassium lactate
L	Batch of small-caliber non-acid fermented sausages made of lean trimmings with 10% fat and 1.5% NaCl (Paper III)
L*	Instrumental lightness Lab-CIE (1976)
LAB	Lactic acid bacteria
LDL	Low-density lipoprotein
L-KCl	Batch of small-caliber non-acid fermented sausages made of lean trimmings with 10% fat, 1.5% NaCl, and 0.64% KCl (Paper III)
Lean	Batch of non-acid fermented sausages with no added fat (Paper I)
MAG	Monoacylglycerols
MAP	Modified Atmosphere Packaging
M:P	Moisture:protein ratio
MUFA	Monounsaturadted fatty acid
n–3	Omega-3 poliunsaturated fatty acid
n–6	Omega-6 poliunsaturated fatty acid
pH_{24h}	pH at 24 h of sausages manufacture
pH_{final}	pH in the final product (end of drying process)
PUFA	Polyunsaturated fatty acid
RH	Relative humidity
RMSE	Root mean square error of the linear model in the statistical analysis
S	Batch of small-caliber non-acid fermented sausages made of shoulder 3D with 10% fat and 1.5% NaCl (Paper III)
SAS	Statistical Analysis System
SC	Sodium caseinate
SFA	Saturated fatty acid
S-KCl	Batch of small-caliber non-acid fermented sausages made of shoulder 3D with 10% fat, 1.5% NaCl and 0.64% KCl (Paper III)
S-KCl-1.5SO	Batch of small-caliber non-acid fermented sausages made of shoulder 3D with 10% fat, 1.5% NaCl, 0.64% KCl and 1.5% sunflower oil (Paper III)
S-KCl-3SO	Batch of small-caliber non-acid fermented sausages made of shoulder 3D with 10% fat, 1.5% NaCl, 0.64% KCl and 3% sunflower oil (Paper III)

SO	Batch of non-acid fermented sausages with 5% sunflower oil (Paper I)
SO3	Batch of small-caliber non-acid fermented sausages with 3% sunflower oil (Paper II)
SPI	Soy protein isolate
SR	Stress Relaxation
TAG	Triacylglycerols
TBA	Thiobarbituric acid
TFA	Trans fatty acid
TPA	Texture Profile Analysis
UFA	Unsaturated fatty acid
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
X_{DFDM}	Water content on a defatted-dry-matter basis
X	kg H_2O/kg dry matter
WHO	World Health Organization
Y_2	Force decay at 2 seconds in Stress Relaxation Test
Y_{90}	Force decay at 90 seconds in Stress Relaxation Test
$Y(t)$	Force decay in Stress Relaxation test

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Summary

The evidence for adverse cardiovascular effects of sodium and fat is supported by a number of observational studies during the last three decades. As a result of the campaigns of organizations like the World Health Organization (WHO), nowadays, many consumers are conscious about the need to reduce the fat and sodium daily intake, and they have started to demand fat and sodium-reduced meat products in order to improve the healthiness of their diet. Nevertheless, fermented sausages are among the meat products where fat and sodium reduction is more complicated, because fat and sodium chloride contribute together in many of their characteristic technological and sensory properties, i.e., water-holding capacity, texture, color, flavor and aroma. For this reason, many problems in the manufacture, processing, and in the final product are associated to low fat and salt contents.

The main objective of this Thesis is to develop fermented sausages with lower fat and sodium content by means of innovative processing and the use of fat and sodium chloride (NaCl) substitutes. A total of three studies with specific objectives were carried out. In the first study (Paper I), the effect of sunflower oil and pork lard-based diacylglycerols on the sensory attributes, physicochemical parameters and instrumental texture of reduced fat non-acid fermented sausages was assessed. The second study (Paper II) evaluated the effect of pork fat reduction and its substitution by sunflower oil on the instrumental and sensory properties throughout storage time of small caliber non-acid fermented sausages with reduced sodium content (with partial substitution of NaCl by potassium chloride (KCl) and potassium lactate). Finally, in the third study (Paper III), the effect of the simultaneous reduction of pork fat and NaCl and the addition of KCl and sunflower oil on the quality and the consumer acceptability of small caliber non-acid fermented sausages was evaluated.

Results for the first study showed that reduced fat non-acid fermented sausages containing less than 12.5% of final fat content (added fat: 5% backfat, 5% sunflower oil and 5% diacylglycerols) had a good overall sensory quality. Sausages with sunflower oil showed higher sensory ratings in desirable ripened odor and flavor attributes, and improved texture, i.e., lower hardness and chewiness (both sensory and instrumental) and higher crumbliness. Sausages with diacylglycerols showed a similar behavior to that of backfat, so they could be a good alternative to produce healthier reduced fat non-acid fermented sausages. Results for the second study showed that reduced fat sausages (20% final fat content vs. 44% of a standard sausage) with acceptable

sensory characteristics can be obtained by adding to the shoulder lean (8% fat content) during the grinding, either 3.3% backfat (3% fat content) or 3% sunflower oil, both previously finely comminuted with lean. Furthermore, sunflower oil showed to be suitable for partial pork backfat substitution in very lean fermented sausages, conferring desirable sensory properties similar to those of sausages with standard fat content. The sensory quality of the sausages was maintained after three-month cold storage in modified atmosphere. Results for the third study showed that the simultaneous reduction of fat and NaCl increased weight loss, moisture content, water activity, instrumental redness, instrumental texture parameters (hardness, chewiness and cohesiveness) and sensory attributes (darkness, hardness and elasticity) but did not significantly affect the consumer acceptability. The addition of 0.64% KCl to the leanest batches decreased water activity and barely affected instrumental texture parameters and consumer acceptability. Sunflower oil addition decreased hardness, chewiness and cohesiveness and increased crumbliness, but it may negatively affect consumer acceptability. The simultaneous reduction of fat and NaCl with the addition of 0.64% KCl was the preferred option by the consumers.

These results confirm that it is possible to obtain fermented sausages with reduced fat and sodium content with similar physicochemical, instrumental and sensory characteristics than those of a standard product, and with good consumer acceptability. In addition, sunflower oil and KCl are confirmed to be suitable pork fat and NaCl substitutes in fermented sausages.

Resumen

A lo largo de las últimas tres décadas, diferentes estudios han evidenciado los efectos adversos de la ingesta de grasa y sodio en la salud, sobre todo aquellos relacionados con enfermedades cardiovasculares. Actualmente y como resultado de las campañas realizadas por organismos como la Organización Mundial de la Salud (OMS), muchos consumidores son conscientes de la necesidad de reducir la ingesta diaria de grasa y sodio y demandan a la industria alimentaria productos con un contenido reducido de estos ingredientes para conseguir una dieta más saludable. Sin embargo, los embutidos crudos-curados están entre los productos cárnicos en los cuales la reducción de grasa y sodio es más complicada debido a la contribución conjunta de la grasa y la sal en muchas de sus propiedades tecnológicas y sensoriales, tales como la capacidad de retención de agua, el color, el sabor, el aroma y la textura. Por ello, la reducción de grasa y sal en la elaboración de embutidos crudos-curados no es evidente y puede conllevar defectos en el producto final.

El objetivo principal de esta tesis es desarrollar embutidos crudos-curados con un contenido reducido en grasa y sodio mediante un procesado innovador y el uso de sustitutos de grasa y cloruro sódico (NaCl). Se realizaron 3 estudios cada uno de ellos con unos objetivos específicos. En el primer estudio (Artículo I) se evaluó el efecto de la utilización de aceite de girasol y de diacilgliceroles obtenidos de grasa de cerdo sobre los atributos sensoriales, los parámetros fisicoquímicos y la textura instrumental de embutidos crudos-curados no ácidos reducidos en grasa. En el segundo estudio (Artículo II) se evaluó el efecto de la reducción de la grasa de cerdo y su sustitución por aceite de girasol sobre las propiedades instrumentales y sensoriales de embutidos crudos-curados no ácidos tipo *fuet* con contenido reducido en sodio (con sustitución parcial de NaCl por cloruro potásico (KCl) y lactato potásico) a lo largo de su almacenamiento. Finalmente, en el tercer estudio (Artículo III) se evaluó el efecto de la reducción simultánea de grasa de cerdo y NaCl, así como la adición de KCl y aceite de girasol sobre la calidad y la aceptabilidad por parte de los consumidores de embutidos crudos-curados no ácidos tipo *fuet*.

Los resultados del primer estudio mostraron que los embutidos crudos-curados no ácidos con un contenido final en grasa inferior al 12,5% (grasa añadida: 5% de grasa canal, 5% aceite de girasol y 5% diacilgliceroles) presentaron una buena calidad sensorial. Los embutidos elaborados con aceite de girasol se puntuaron con intensidades superiores de olor y flavor a curado y con menor intensidad de dureza y

masticabilidad (sensorial e instrumental) y con mayor desmenuzabilidad. Los embutidos con diacilgliceroles mostraron un comportamiento similar a los embutidos con la misma cantidad de grasa canal, por lo que podrían ser una buena alternativa para obtener embutidos reducidos en grasa más saludables. Los resultados del segundo estudio mostraron que es posible obtener *fuets* reducidos en grasa (20% de contenido final vs 44% de un producto estándar) sensorialmente aceptables, mediante la adición, durante el picado del magro de paleta de cerdo (8% en contenido de grasa), de un 3,3% de grasa canal (contenido en grasa del 3%) o un 3% de aceite de girasol, ambos previamente picados muy finamente con una parte de magro. Además, se demostró que el aceite de girasol es adecuado para la sustitución parcial de grasa de cerdo en embutidos muy magros, confiriendo propiedades sensoriales deseables similares a las de los *fuets* con un contenido de grasa estándar. La calidad sensorial de los *fuets* envasados en atmósfera modificada se mantuvo después de tres meses de almacenamiento en refrigeración. Los resultados del tercer estudio mostraron que la reducción simultánea de grasa y NaCl aumentó la pérdida de peso de las piezas, el contenido de humedad, la actividad de agua, el color rojo medido instrumentalmente, los parámetros de textura instrumental (dureza, masticabilidad y cohesividad) y los atributos sensoriales (intensidad de color, dureza, elasticidad); sin embargo, no afectó significativamente a la aceptabilidad de los consumidores. La adición de 0,64% de KCl a los lotes más magros disminuyó la actividad de agua pero apenas afectó a los parámetros de textura instrumental y a la aceptabilidad de los consumidores. La adición de aceite de girasol disminuyó la dureza, la masticabilidad y la cohesividad y aumentó la desmenuzabilidad, pero puede afectar negativamente a la aceptabilidad de los consumidores. La reducción simultánea de grasa y NaCl con la adición de 0,64% de KCl fue la opción preferida por los consumidores.

Estos resultados confirman que es posible obtener embutidos crudos-curados con contenido reducido en grasa y sodio con similares características fisicoquímicas, instrumentales y sensoriales a un producto estándar, y con una buena aceptabilidad de los consumidores. Además, el aceite de girasol y el KCl quedan confirmados como sustitutos adecuados de la grasa y del NaCl para este tipo de embutidos.

Resum

Diversos estudis realitzats en les darreres tres dècades han evidenciat els efectes adversos de la ingesta de greix i sodi sobre la salut, especialment relacionats amb les malalties cardiovasculars. Actualment, fruit de les campanyes informatives realitzades per diversos organismes com l'Organització Mundial de la Salut (OMS), molts consumidors són conscients de la necessitat de reduir la ingesta diària de greix i de sodi i, demanden productes carnis amb un contingut reduït d'aquests ingredients per a una alimentació més saludable. Quan es tracta de reduir el contingut de greix i de sodi en productes carnis, especialment en embotits crus curats, és complicat a causa de la contribució d'ambdós (greix i clorur sòdic) en moltes de les propietats tecnològiques i sensorials que els són pròpies, com la capacitat de retenció d'aigua, el color, el sabor, l'aroma i la textura. Per aquest motiu, molts problemes durant l'elaboració, processat i en el producte final estan relacionats amb la reducció de greix i sal en embotits.

L'objectiu principal d'aquesta tesi és desenvolupar embotits crus curats amb un contingut reduït de greix i sodi mitjançant un processat innovador i la utilització de substituts de greix i de clorur sòdic (NaCl). Es van realitzar 3 estudis, cadascun amb uns objectius específics. En el primer estudi (Article I) es va avaluar l'efecte de la utilització d'oli de gira-sol i de diacilglicerols obtinguts de greix de porc en les característiques sensorials, els paràmetres fisicoquímics i la textura instrumental d'embotits crus curats no àcids reduïts en greix. En el segon estudi (Article II) es va avaluar l'efecte de la reducció de greix de porc i la seva substitució per oli de gira-sol en les propietats instrumentals i sensorials durant la conservació d'embotits crus curats no àcids tipus fuet amb un contingut reduït de sodi (amb substitució parcial del NaCl per clorur potàssic (KCl) i lactat potàssic). Finalment, en el tercer estudi (Article III), es va avaluar l'efecte de la reducció simultània de greix de porc i NaCl, així com l'addició de KCl i d'oli de gira-sol en la qualitat i l'acceptabilitat per part dels consumidors d'embotits crus curats no àcids tipus fuet.

Els resultats del primer estudi van evidenciar com els embotits amb un contingut final de greix inferior al 12,5% (greix afegit: 5% de greix canal, 5% d'oli de gira-sol i 5% de diacilglicerols) van presentar una qualitat sensorial satisfactòria. Els embotits amb oli de gira-sol es van caracteritzar per presentar una intensitat superior d'olor i de flavor de curat, menor intensitat de duresa i masticabilitat (sensorial i instrumental) i, una elevada esmicolabilitat. Els embotits elaborats amb diacilglicerols van presentar un comportament similar a aquells elaborats amb la mateixa quantitat de greix canal de

manera que la utilització de diacilglicerols podria ser una bona opció per obtenir embotits més saludables ja que el seu ús permet reduir la quantitat de greix. Els resultats del segon estudi van demostrar que és possible obtenir fuets reduïts en greix (20% de contingut de greix final vs 44% d'un embotit estàndard) sensorialment acceptables, mitjançant l'addició durant el picat del magre d'espatlla de porc (amb un 8% de contingut de greix) d'un 3,3% de greix canal (amb un contingut de greix del 3%) o un 3% d'oli de gira-sol, prèviament ambdós picats amb magre molt finament. També, es va demostrar que l'oli de gira-sol és adequat per a la substitució parcial del greix de porc en embotits molt magres ja que confereix al producte obtingut unes propietats sensorials similars a les que caracteritzen els fuets estàndards. La qualitat sensorial dels fuets després de 3 mesos d'emmagatzemament en condicions de refrigeració i envasats en atmosfera modificada es va mantenir. Els resultats del tercer estudi van demostrar que la reducció simultània de greix i de NaCl va causar un increment en la pèrdua de pes dels fuets, en el contingut d'humitat, en l'activitat d'aigua, en el color vermell (mesurat de forma instrumental), en els paràmetres de textura instrumental (duresa, masticabilitat i cohesivitat) i en alguns atributs sensorials (intensitat de color, duresa i elasticitat); contràriament, no va afectar significativament l'acceptabilitat dels consumidors. L'addició d'un 0,64% de KCl als lots més magres va disminuir l'activitat d'aigua i, gairebé, no va afectar els paràmetres de textura instrumental i l'acceptabilitat dels consumidors. L'addició d'oli de gira-sol va disminuir la duresa, la masticabilitat i la cohesivitat però pot afectar negativament l'acceptabilitat dels consumidors. La reducció simultània de greix i NaCl amb l'addició d'un 0,64% de KCl va ser l'opció més acceptada per part dels consumidors.

Aquests resultats confirmen que és possible elaborar embotits crus curats amb un contingut reduït en greix i sodi amb característiques fisicoquímiques, instrumentals i sensorials similars a les del producte estàndard i amb una bona acceptabilitat per part dels consumidors. També, es confirma que l'oli de gira-sol i el KCl són uns substituts adequats del greix i del NaCl per aquest tipus d'embotits.

1 INTRODUCTION

Fermented sausages are meat products of great importance to the meat industry in Europe, especially in countries like Germany and those of the Mediterranean area (Spain, Italy, France…). However, these meat products contain high fat and sodium contents and public health organizations recommend limiting their consumption.

The evidence for adverse cardiovascular effects of sodium and fat is supported by a number of observational studies (Grundy, 1984; Rose & Connolly, 1999; Slattery, Edwards, Boucher, Anderson, & Caan, 1999) during the last three decades. These studies indicate an association of increasing risk of mortality from cardiovascular diseases with high sodium and fat intake. An excessive consumption of fat and sodium is also linked to other diseases like hypertension, which is one of the major causes of development of cardiovascular diseases, and to obesity and certain types of cancer.
As a result of the campaigns of organizations like the World Health Organization (WHO), nowadays, many consumers are conscious about the need to reduce the fat and sodium daily intake, and they have started to demand fat and sodium-reduced meat products in order to improve the quality of their diet. Nevertheless, fermented sausages are among the meat products where fat and sodium reduction is more complicated, because fat and sodium chloride (salt) contribute together in many of their characteristic technological and sensory properties, i.e., water-holding, texture, color, flavor and aroma. For this reason, many problems in the manufacture, processing, and in the final product are associated to low fat and salt contents. Therefore, meat companies need to develop innovative formulations that are able to maintain the desirable characteristics of these products, not simply by reducing the fat and salt content in order to obtain healthier fermented sausages with good consumer acceptability.

Although fat and salt reduction in fermented sausages is difficult, many studies have shown that it is possible to obtain acceptable reduced fat and reduced sodium products. In these studies, substitutes of different nature, i.e., vegetable fats, proteins, and carbohydrates, have been used to replace animal fat with positive results from the technological and sensory point of view. Among these substitutes, vegetable and fish oils alone or in combination with proteins, hydrocolloids like konjac, glucomannan and cellulose derivatives, and vegetable fibers like inulin and cereal and fruit dietary fiber, contribute to obtain acceptable reduced fat fermented sausages. Likewise, acceptable reduced sodium sausages have been obtained by replacing sodium chloride by other chloride salts such as potassium, magnesium and calcium chloride, alone or in mixtures. In order to minimize the negative effect of these substitutes on the flavor of the sausages, flavor enhancers like potassium lactate, amino acids and yeast extracts have been used as salt substitutes with positive results.

1.1. Effect of fat and sodium on human health

Animal fat, besides supplying much of the energy that the human body requires (9 kcal/g), is a source of liposoluble vitamins (A, D, E and K), essential fatty acids and precursors of compounds (e.g., prostaglandins) that regulate a number of physiological functions. In the right proportions, fat is therefore an essential component of any balanced diet (Jiménez-Colmenero, 2000; Vural, Javidipour, & Ozbas, 2004). On the other hand, sodium is also an essential nutrient required for normal cellular function. It is involved in the maintenance of the extracellular fluid, and together with potassium in maintaining total body water homeostasis. Sodium contributes to establish the membrane potential of most cells and plays a direct role in the action potential required for the transmission of nerve impulses and muscle contraction. The active absorption of sodium from the lumen of the gastrointestinal tract is important in the absorption of nutrients (European Food Safety Agency [EFSA], 2005). Dietary deficiency of sodium is very uncommon due to the widespread occurrence of sodium in foods.

Rapid changes in diets and lifestyles that have occurred with industrialization, urbanization, economic development and market globalization have accelerated over the past two decades. In both developed and newly developed countries, one of the main effects of these changes has been the increased consumption of energy-dense diets high in fat, particularly saturated fat (WHO, 2003). In Europe, the average total energy intakes coming from fat in adults range from less than 30% to 47% (EFSA, 2010). The lipid content in edible lean meat today is less than 5% (Chizzolini, Zanardi, Dorigoni, & Ghidini, 1999), so it can no longer be considered an energy-rich food. However, in some commercial meat products, the percentages can be as high as 40–50% (Jiménez-Colmenero, 2001). This is the case of *salami* or some types of Spanish dry-fermented sausages, e.g., *fuet*.

Animal fat is rich in saturated fatty acids (SFAs) and cholesterol, which have been linked to obesity, hypertension, cardiovascular disease and several types of cancer such as colon, breast and prostate cancer (Grundy, 1994; National Cancer Institute [NCI], 1984; Rose & Connolly, 1999; Slattery, Edwards, Boucher, Anderson, & Caan, 1999). Meat fat is structured in triacylglycerols (TAGs) containing mainly monounsaturated fatty acids (MUFAs) and SFAs. Meat lipids usually contain less than 50% SFAs, and up to 70% MUFAs and polyunsaturated fatty acids (PUFAs) (Romans, Costello, Carlson, Greaster, & Jones, 1994). The percentage of unsaturated fatty acids changes depending on the type of meat, e.g., poultry and pork contains more unsaturated fatty acids (approx 10–15% of total fatty acids) than beef and lamb, and also a notable amount of PUFAs. *Trans* fatty acids (TFAs) comprise about 1–2% of total fatty acids across all types of meat (Valsta, Tapanainen, & Männistö, 2005). TFAs have been reported to be detrimental for health. When compared with the intake of equal amounts of calories from saturated or *cis* unsaturated fats, the consumption of TFA raises levels of low-density lipoprotein (LDL) cholesterol, reduces levels of high-density lipoprotein (HDL) cholesterol, and increases the total cholesterol/HDL cholesterol ratio, which is a powerful cardiovascular disease risk predictor (Stampfer, Sacks, Salvini, Willett, & Hennekens, 1991; Ascherio, Katan, Zock, Stampfer, & Willet, 1999; Jahreis, 2005). MUFAs and PUFAs reduce the LDL cholesterol, although PUFAs also depress the HDL cholesterol (Mattson & Grundy, 1985). Therefore, a high presence of unsaturated fatty acids instead of SFAs and TFAs in the meat products would result in a healthier diet. Thus, a number of scientific authorities and nutritional organizations including the WHO (2003), the Food and Agriculture Organization of the United Nations (FAO, 2010) and the EFSA (2010) have reported guidelines for achieving an optimal dietary fat intake, which should ideally account for between 20 and 35% of total diet energy. According to dietary recommendations for the intake of specific fatty acids, no more than 10% of calorie intake should be from SFAs, 6-11% should be from PUFAs (n–6, 2.5–9%; n–3, 0.5–2%), around 10-15% should be from MUFAs, and less than 1% should be from TFAs. It is also recommended to limit cholesterol intake to 300 mg/day (WHO, 2003).

Meat as such is relatively poor in sodium, containing only 50–90 mg of sodium per 100 g (Romans et al., 1994). However, the sodium in meat products is much higher because of the salt content, which can be as much as 2% in heat-treated products (e.g., sausages) and as much as 6% in uncooked cured products, in which drying (loss of moisture) increases the proportion even further. Estimations taking eating habits into account suggest that approximately 20–30% of common salt intake comes from meat and meat products (Wirth, 1991). Further studies performed by several authors and health and food authorities evaluated the contribution of different food groups to the total sodium chloride (NaCl) intake of different countries of Europe and USA showing differences related to different food traditions. In Ireland, cured and processed meat products contribute 20.5 % to the sodium intake (Food Safety Authority of Ireland

[FSAI], 2005). Similarly, meat and meat products contribute 20.8% in UK and 21.0% in USA to the sodium intake (Engstrom, Tobelmann, & Albertson, 1997; Scientific Advisory Committee of nutrition [SACN] (2003). In Spain, processed meat products (dry-fermented sausages, dry-cured ham, cooked ham, cooked turkey, sausages…) and fresh meat contribute 30.2% to the sodium intake (Agencia Española de Seguridad Alimentaria y Nutrición [AESAN], 2009). In Finland, meat dishes contribute 27% to the NaCl intake (KTL-National Public Health Institute, 2007).

The association between excessive sodium intake and the development of hypertension (Dahl, 1972; Fries, 1976; MacGregor & Sever, 1996; MacGregor & de Wardener, 2002) has prompted public health and regulatory authorities to recommend reducing dietary intake of NaCl. Hypertension is a major risk factor in the development of cardiovascular disease. The results of the DASH sodium study (Dietary Approaches to Stop Hypertension) showed a graded linear relation between salt intake and blood pressure (Appel et al., 2011). Furthermore, a high salt intake has been linked to increased risk of stomach cancer, increased risk of renal stones, decreased bone mineral density (He & MacGregor, 2007) and left ventricular hypertrophy (Messerli & Ketelhut, 1993). The mean daily sodium intakes of populations in Europe range from about 3–5 g (about 8–11 g salt) (Brandsma, 2006; EFSA, 2005; Kilcast & Angus, 2007). These values are well in excess of the maximum intake value recommended by the WHO (WHO, 2012) (2 g sodium/day in adults, corresponding to 5 g NaCl). Other reports from the Food Safety Authority of Ireland [FSAI] (2005) and the SACN (2003) in the UK have shown that the average daily sodium intake from foods in Irish and UK adults has been estimated as 3.3–3.9 g (8.3–10 g NaCl).

1.2. Effect of fat and sodium on the properties of meat products

Fat and NaCl contribute together to important technological, texture and sensory properties of meat and meat products, being basic for consumer acceptability. As the salt level rises, the increase in saltiness is more noticeable in meat products with higher fat levels (Matulis, McKeith, Sutherland, & Brewer, 1995). In cooked sausages, the fat content affects the perception of saltiness, depending on the formulation (Ruusunen, Simolin, & Puolanne, 2001, Ruusunen, Tirkonnen, & Puolanne, 2001). These authors showed that an increase in meat protein reduced perceived saltiness. Ruusunen et al. (2005) reported that meat content had a stronger effect than fat content on perceived saltiness. This study showed that more salt was needed in ground beef patties of high meat content to achieve the same perceived saltiness as in products of low meat content.

Fat is an essential component for meat products as it provides several features positively related to their sensory and technological quality, contributing to the appearance, flavor, texture, mouthfeel, juiciness and overall sensation of lubricity of meat products. In the case of the texture, changes in the fat type and content also determine differences in instrumental texture tests, such as the Texture Profile Analysis (TPA). From a technological point of view, fat slows down the dehydration process in dried meat products (Wirth, 1988). Moreover, fat increases the feeling of satiety during meals (Akoh, 1998). From a sensory standpoint, the main functionalities of meat products affected by fat are flavor, texture and appearance, as explained next.

Flavor**:** Fat modifies the perception of flavor compounds influencing the balance, intensity and release of flavor and affecting their distribution and migration (Hughes, Cofrades, & Troy, 1997; Lucca & Tepper, 1994).

Texture**:** Fat exerts considerable influence on the binding, rheological and structural properties of meat products and it plays an important role in the formation of meat emulsions in comminuted products (García, Dominguez, Galvez, Casas, & Selgas, 2002). In products like dry-fermented sausages, fat has important technological functions. It helps to loosen the sausage mixture and this aids the continuous release of moisture from the inner layers of the product; a process necessary for undisturbed fermentation and aromatization (Bloukas, Paneras, & Fournitzis, 1997; Muguerza, Fista, Ansorena, Astiasaran, & Bloukas, 2002).

Appearance**:** The main parameters to assess meat products in sensory tests are related to color. It depends, among other factors, on the fat content and type (Ahmed, Miller, Lyon, Vaughters, & Reagan, 1990; Moller & Skibsted, 2007) and on myoglobin and its state within the meat used to manufacture the product (Hand, Hollingswort, Calkins, & Mandigo, 1987). Fat plays an important role in the color of meat products and lipid oxidation affects negatively the color and flavor of meat and meat products. Regarding the color, lipid oxidation affect the oxidation of haem pigments (myoglobin and haemoglobin) whose red color turns into brown (Rohlík, Pipek, & Pánek, 2010). Some studies carried out with different meat products, i.e., frankfurters, hamburgers and dry-fermented sausages, have shown changes in both instrumental and sensory color depending on the fat content and type (Choi et al., 2009; Jiménez-Colmenero et al., 2003; Muguerza et al., 2002; Park et al., 2005).

Sodium chloride also provides a number of important functionalities to meat products:

Flavor: The perceived saltiness of NaCl in meat products is mainly due to the Na^+ cation with Cl^- anion modifying the perception (Miller & Barthoshuk, 1991). Sodium chloride also confers a characteristic flavor to meat products, enhancing flavor intensity in processed meat (Gillette, 1985; Matulis et al., 1995). Thus, salt reduction does not only decrease the perceived saltiness but it also weakens the overall flavor in meat products.

Texture: Salt plays an important role in the final texture of meat products, because the level of sodium chloride directly influences the swelling of the myofibrilar meat proteins myosin and actin causing an increase in water-holding capacity. Sodium chloride increases viscosity of meat batters increasing the interactions between proteins that are able to form networks and stabilize the interface of meat fats forming stable emulsions (Desmond, 2006, 2007). Improvement of water-holding capacity also reduces cooking loss thereby increasing tenderness and juiciness of meat products (Desmond, 2006).

Color: The salt content affects instrumental color measurements and sensory color perception. Several studies on different meat products, i.e., frankfurters, breakfast sausages and dry-fermented sausages, showed changes for instrumental color parameters lightness (L*), redness (a*) and yellowness (b*) with the salt reduction and substitution by different sodium chloride replacers (Gimeno, Astiasarán, & Bello, 1999, 2001; Jiménez-Colmenero, Ayo, & Carballo, 2005).

Preservative: Salt prevents spoilage through the reduction of water activity due to the presence of ions exerting osmotic pressure effects on the microorganisms and, thus, increasing the shelf life of the meat products. Therefore, when the salt content in meat products is reduced below typically used levels, the product has a shorter shelf life or may no longer be safe without addition of other preservatives (Madril & Sofos, 1985).

1.3. Fat and salt reduction and substitution in fermented sausages

1.3.1. General introduction to fermented sausages

Fermentation and drying can be considered the oldest way to preserve raw meat. The historical origin of fermented meat products is still unknown, although bibliographical research has traced it back more than 2500 years in China. Proof of sausage production was first documented in ancient Greece, where it may have been encouraged by the existing climate conditions (Liepe, 1983). The Romans inherited this tradition from the Greeks, and from then on, fermented sausages spread to central, eastern, and northern Europe, as well as to America and Australia where fermented sausages were recognized as the heritage of European immigrants (Demeyer, 2004; Vignolo, Fontana, & Fadda, 2010). Despite the widespread production of fermented sausages, Europe is still the major producer and consumer of these meat products, production and per capita consumption figures being highest in Germany, Italy, Spain, and France (Lücke, 1998; Fédération Française des Industriels Charcutiers Traiteurs [FICT], 2002; Di Cagno et al., 2008).

Fermented sausages can be defined as a meat product made of a mixture of mainly beef and pork meat, and less often of poultry, mutton, lamb, goat, horse, ostrich, and game meat (Vural & Özvural, 2007); pork fat, salt, curing agents, sugar, spices, and in many cases starter cultures are added. According to Demeyer (2004) and Lebert, Leroy, & Talon (2007), fat must be firm, white, and fresh, with a high melting point and a low content of polyunsaturated fatty acids to avoid rancidity and fat exudation and for a clear-cut surface of the sausages.

Fig. 1 shows data about the most common parameters used in fermented sausages manufacture in Europe. However, the values concerning temperature, relative humidity and time are not exhaustive as there are many different products in other countries in which these parameters can change, e.g. Chinese *lup cheong* with no pH drop due to a very fast drying, or semi-dry summer sausages with low pH that may be fermented at temperatures as high as 40 °C (Ferrini, Arnau, Guàrdia, & Comaposada, 2014).

Processing of fermented sausages includes meat and fat selection, grinding, mixing, stuffing, fermentation, drying and/or smoking and packaging for retail distribution. Bones and intermuscular fatty tissue must be removed from the meat, and connective tissue and membranes trimmed off. Meat and fat are chilled or frozen and comminuted to the desired particle size. A grinder machine or a bowl-cutter is used. The grinder machine consists of a rotating set of knives and a chopping plate that confer the desired meat and fat particle size, whereas the cutter consists of a rotating set of knives in a rotating bowl. Grinding is usually carried out under vacuum to avoid drying and changes in lean and fat color, that are request to be red and white, respectively (Vignolo, Fontana, & Fadda, 2010). Once meat and fat have been ground, they are mixed with additives: curing salts (NaCl, nitrates and nitrites), ascorbic acid, colorants, sugars (lactose, dextrose), spices, aromatic herbs, and starter cultures: lactic acid bacteria (LAB) and/or coagulase negative cocci (CNC). The process can be carried out in the bowl cutter during the chopping or in a vacuum mixer machine after the meat and fat have been ground in the grinder machine, depending on the product.

After mixing, the meat batter is immediately stuffed firmly into natural (pork, beef/ox) or synthetic (collagen, cellulose, plastic) water-vapour-permeable casings. This process is carried out in a stuffer machine under vacuum in order to prevent abnormal colors or flavors, and to avoid the formation of air pockets (Vignolo, Fontana, & Fadda, 2010).

After stuffing, sausages are fermented at 12–24 ºC during 1–7 days for dry sausages, and at 25–34 ºC during 1–2 days for semi-dry sausages. The fermentation step includes a pH decrease from approximately 5.7 to its lower value, which could vary from 5.5 (hard salamis) to 4.6 (or even 4.2 in high-temperature fermented sausages) depending on the sausage style (Petäja-Kanninen & Puolanne, 2007). Meat fermentation is a low-energy, biological acidulation, preservation method, which results in unique and distinctive meat properties, such as flavor and palatability, color, microbiological safety, tenderness, and a host of other desirable attributes of fermented sausages. The process is caused by "cultured" or "wild" microorganisms, which decrease the pH. The process depends on several parameters such as the proportions and quality of the raw material, use of starters, temperature and relative humidity, loss of water and microbial flora (Rosselló, Barbas, Berna, & López, 1995). Lactic acid originates from the natural conversion of glycogen reserves in the carcass tissues and from the added sugar during product fermentation. A desirable fermentation product is the outcome of acidulation caused by lactic acid production and lowering the water activity (a_w) caused by the addition of salt (curing) and drying. Both natural and controlled fermentations involve lactic-acid bacteria (LAB) (Ockerman, & Basu, 2007). Another technologically important bacterial group in sausages fermentation is coagulase negative cocci (CNC). Actually, most starter cultures, today, consist of LAB and/or CNC, selected for their metabolic activity. The most common LAB species identified are *Lactobacillus sakei*, *Lactobacillus curvatus*, and *Lactobacillus plantarum*, with *L. sakei* prevailing. Among CNC, *Staphylococcus xylosus* and *Staphylococcus carnosum* are the most common species identified from traditional products. Pediococci and enterococci have also been often identified from fermented sausages (Vignolo, Fontana, & Fadda, 2010). The reduction of pH and the lowering of water activity are both microbial hurdles that produce a safe product (Ockerman, & Basu, 2007).

After fermentation sausages are air dried or smoked depending on the sausage type. Air drying is generally used for Spanish, French, Italian and Greek fermented sausages. The drying for these products is highly dependent on temperature, air velocity, and caliber of sausages, which are responsible of the duration of the process. Normally, it varies from 4 to 12 weeks. Temperature usually ranges between 10 and 15 ºC. The relative humidity (RH) during fermentation varies from a minimum of 63% to 75%, to a maximum of 86% to 95% in dry-fermented sausages, leading to variable a_w (0.83 to 0.95) in traditional sausages at the end of drying (Lebert et al., 2007). Lipolysis and proteolysis are key processes during ripening of fermented sausages. These biochemical events may originate from endogenous muscle enzymes or from bacterial enzymes (Molly et al., 1997). Lipid degrading phenomena, implying the release of free fatty acids and carbonyl compounds, and denaturation and partial fragmentation of proteins, are important in the development of the characteristic taste and flavor of the final product (Eim, Simal, Rosselló, & Femenia, 2008).

Once the ripening process is finished, appropriate packaging systems should be applied in order to delay or prevent unfavorable quality changes, i.e., discoloration, lipid oxidation, rehydration, dehydration, and microbial spoilage in the products during storage and distribution. Among available packaging systems, vacuum packaging and modified atmosphere packaging (MAP) have been widely used for fermented sausages.

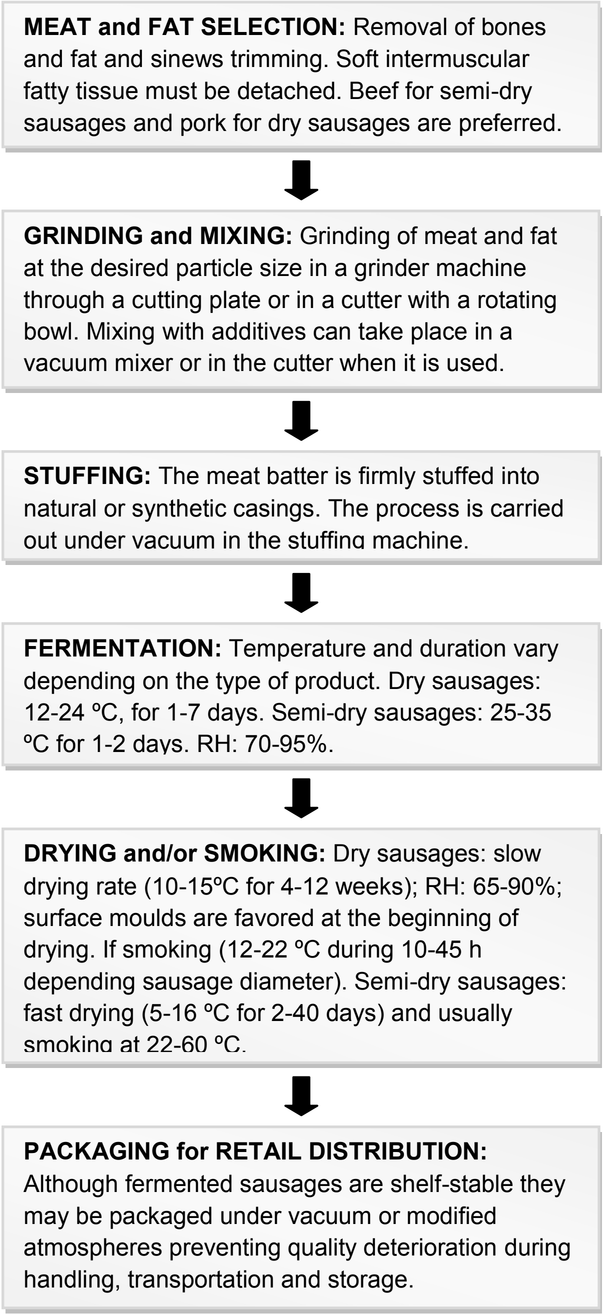

Fig.1. Processing of fermented sausages (modified from Vignolo & Fadda, 2010).

In general, dry-fermented sausages have a final pH ranging between 5.2 and 5.8, a moisture lower than 30%, and a moisture: protein ratio (M:P) lower than 2.3:1, and a_w ranging between 0.85 and 0.91. Semi-dry fermented sausages have a more acid final pH (4.7 to 5.2–5.4), a moisture of 35% or higher, higher a_w values (>0.90–0.91), and a M:P ratio ranging from 2.3:1 to 3.7:1.

1.3.2. Limiting factors of fat and salt reduction and substitution in fermented sausages

Fermented sausages are among the meat products where fat reduction is more complicated because fat confers important sensory properties, i.e., flavor, texture and mouthfeel (Mendoza, García, Casas, & Selgas, 2001). When fat content is reduced and protein content from meat increases, salty flavor is less noticeable (Matulis et al., 1995; Ruusunen et al., 2005). Thus, NaCl reduction is also complicated in reduced fat fermented sausages as levels of both NaCl and NaCl substitutes must be modified to obtain a product with similar sensory attributes to a traditional fermented sausage. Fat also affects important technological functions such as water release during drying (Wirth, 1988). One of the most detrimental effects of fat reduction in fermented sausages is a harder instrumental and sensory texture (Bloukas et al., 1997; Muguerza et al., 2002). Another main potential problem posed by these modifications is how they may influence the rate and extent of lipid oxidation, which in turn affects quality characteristics and has health implications (Jiménez-Colmenero, 2007). Therefore, the modification of the composition of fermented sausages by reducing its fat and NaCl content can lead to negative changes in the sensory characteristics of these products that could affect the consumer acceptability. Some important physicochemical, instrumental and sensory characteristics of fermented sausages that could be affected by fat and NaCl reduction and substitution are explained next.

1.3.2.1. Effect on physicochemical characteristics

Using a low amount of animal fat during sausage processing can affect some important product characteristics, especially during the drying process. Such parameters, i.e., water content, water activity, and pH determine the characteristics and attributes that define the quality of the final product (flavor, odor, appearance, texture, microbiological safety, etc.).

The weight losses depend on the temperature and relative humidity of the ripening room, the air velocity inside the room and the ripening time, the degree of comminution of the meat mixture (Klettner, Roedel, Ott, & Ponert, 1985), the width and the material of the casings and the amount of fat in the sausage (Del Nobile et al., 2009). The higher the fat content, the lower the weight losses over the same period (Klettner & Roedel, 1980; Bloukas et al., 1997).

Fat is reduced in favor of lean meat; therefore, reduced fat fermented sausages have higher water content which means faster water losses. In finely-granulated sausages like *salami* and *saveloy*, fat reduction presents problems due to the loss of moisture during the drying process. For example, 32% fat is added to conventional salami during manufacture, rising to 40% after one week and 45–50% after four weeks of ripening (Wirth, 1988). Even if the fat used in its manufacture was reduced from 30% to 5%, the fat level would still be 15% after one week. The lowest acceptable final fat content for this kind of product has been reported to be around 20–30% depending on the extent of drying (Jiménez-Colmenero, 2000). Nevertheless, acceptable sensory quality attributes of *Milano salami* with 13% fat were reported (Chizzolini, 1997) replacing fat with pre-gelatinized rind, surimi paste and tapioca starch and modifying the processing technology to slow down dehydration.

At the same weight loss, sausages with lower fat level present higher water content. This fact leads to higher water activity (a_w) in reduced fat fermented sausages at early stages of the drying process (Olivares, Navarro, Salvador, & Flores, 2010), which has been related to a higher pH decline.

The pH decline has also been associated to fat content and ripening time (Ordóñez, Hierro, Bruna, & de la Hoz, 1999; Marco, Navarro, & Flores, 2008; Olivares, Navarro, & Flores, 2009). Faster pH decline with the fat reduction was reported in fermented sausages by Olivares et al. (2010) and Soyer, Ertas, & Üzümcüoglu (2005).

Likewise, salt reduction also determines changes in the physicochemical parameters of fermented sausages. In dry-fermented products a simple salt reduction cannot be made, because a low water activity has to be reached in order to control the microbial flora (Ruusunen & Puolanne, 2005). Stahnke (1995) found that a low NaCl content favors pH decrease. Gimeno, Astiasarán, & Bello (1998, 1999, 2001) replaced NaCl with potassium, magnesium and calcium ascorbates and were able to reduce the NaCl content by about 50%. The only relevant difference was the lower consistency which is to be expected when the chloride ions are replaced with ascorbate ions that do not effectively react with myofilaments. Regarding the combined effect of pH and water-holding capacity, when the drying starts the water-holding is high (pH 5.6 and ionic strength ca. 0.8–1.0 in the water phase) but starts to decrease with the decrease of pH and increase of ionic strength (Ruusunen & Puolanne, 2005). On the other hand, the addition of solutes decreases a_w (Tapia, Alzamora, & Chirife, 2007). Thus, the reduction of NaCl content in fermented sausages causes a_w increase, which favors the lactic acid bacteria growth, resulting in a pH decrease.

To sum up, special attention needs to be paid to adjust the processing conditions of reduced fat and salt fermented sausages in order to obtain similar physicochemical characteristics than conventional products, i.e., a_w and pH values, and suitable water content, which will condition suitable sensory attributes and, therefore, consumer acceptability.

1.3.2.2. Effect on instrumental color and visual appearance

Fermented sausages coarsely granulated can present an uneven appearance because large fat particles can be revealed in the cut surface, suggesting a high fat content (Wirth, 1988). For this reason, finely granulated reduced fat sausages would be more suitable from the visual point of view. Del Nobile et al. (2009) reported unacceptable appearance and changes in sensory color ratings in *salami* manufactured with 100% substitution of pork backfat with extra-virgin olive oil, as soaked with either whey-protein-based crumb or white pan bread. This *salami* presented bad sliceability, casings separated from the fermented meat, and the oil was not completely enclosed inside the matrix but collected under the casing.

Changes in instrumental color parameters L* (lightness), a* (redness) and b* (yellowness) have been reported in fermented sausages with fat reduction and substitution. Del Nobile et al. (2009) obtained the lowest lightness (L*) for the instrumental color analysis in salami with 100% substitution of pork backfat with extra virgin olive oil, which agrees with the decrease of this parameter with the fat reduction reported by Bloukas et al. (1997) and Muguerza et al. (2002) in Greek dry-fermented sausages and *chorizo de Pamplona*, respectively. Additionally, several studies have reported a lightness decrease with decreasing moisture content: in dried pork *M. Longissimus* (Serra, Arnau, & Colleo, 2009) and in dry-cured minced pork (Ferrini, Comaposada, Arnau, & Gou, 2012; Holmgaard Bak et al., 2012). Thus, in reduced fat fermented sausages, both the less numerous white fat particles and the higher moisture content (as a result of a higher lean content) could result in a darker visual color and in lower instrumental L* values. Muguerza et al. (2002) reported no changes in a* values with fat reduction in dry-fermented sausages, whereas sensory evaluation scored low fat sausages (10% fat) as being redder than medium-fat and high-fat sausages (20 and 30% fat, respectively). Muguerza, Gimeno, Ansorena, & Astiasarán (2004) and Salazar, García, & Selgas (2009) reported higher a* values with the fat reduction in dry- fermented sausages. The red color of fermented sausages develops as the result of the reaction of nitric oxide, produced from nitrites, with myoglobin producing nitric oxide myoglobin. As a result, variations in the myoglobin content (among other factors such as pH, weight loss during processing, etc.), linked with changes in composition (related to fat reduction strategy), affect the product color (Liaros, Katsanidis, & Bloukas, 2009). Regarding instrumental yellowness, Muguerza et al. (2002) reported higher b* values in sausages in which 20% of pork fat was replaced by olive oil.

Another problem derived from the animal fat substitution by vegetable oils is the lipid oxidation due to the higher amount of unsaturated fatty acids, particularly polyunsaturated (Jiménez-Colmenero, 2007; Lee et al., 2006), which could result in an undesired color instead of the typical color of fermented products. Replacing beef fat with olive oil has been reported to favor lipid oxidation in traditional Turkish dry-fermented sausage (Kayaardi & Gök, 2003). Likewise, oxidation has been also reported in dry-fermented sausages containing fish oil extract during curing (Muguerza, Ansorena, & Astiasarán, 2004).

NaCl content also affects the final color in dry-fermented meat products (Moller & Skibsted, 2007). Salt modifications can produce changes in the microorganism growth, which would affect color development (Quintanilla et al., 1994). Gimeno et al. (1999) reported higher instrumental lightness and yellowness, and lower sensory color intensity in *chorizo de Pamplona* with a mixture (2.29%) of different salts (NaCl, KCl and $CaCl_2$) with an equivalent ionic strength to that of the control manufactured with 2.6% NaCl. Gou, Guerrero, Gelabert, & Arnau (1996) observed a decrease of color uniformity in dry-fermented sausages with 60% substitution of NaCl by KCl, and a decrease of color intensity with 70% substitution. Calcium ascorbate used as salt substitute has been reported to increase instrumental redness and yellowness and lower lightness in dry-fermented sausages with 45% NaCl substitution (Gimeno et al., 2001).

1.3.2.3. Effect on odor and flavor

The amount and type of fat used in the formulation of fermented sausages will determine their final sensory attributes. Flavor formation in dry-fermented sausages is mainly related to lipolysis (Gandemer, 2002) through the generation of free fatty acids that are further subjected to lipid oxidation reactions producing a large variety of volatile compounds (Zanardi, Ghidini, Battaglia & Chizzolini, 2004). Moreover, fat acts as a precursor and a solvent for aroma compounds (Leland, 1997).

Chevance et al. (2000) indicated that fat reduction in salami increased the release of odor compounds. Nevertheless, Olivares, Navarro, & Flores (2011) studied the effect of fat content on lipid changes and the generation of volatile compounds during the processing of dry-fermented sausages with different fat levels (10, 20, and 30%), and also the sensory attributes and consumer acceptability. These authors reported that fat reduction decreased the generation of lipid derived volatile compounds during processing. They also reported a consumer preference for sausages with medium and high fat levels (20 and 30%) related to some aroma compounds which contribute with pleasant aroma nuances. Muguerza et al. (2002) studied the effect of total fat level (30%, 20%, and 10%) and partial replacement of pork backfat with olive oil (0% and 20%) on the processing and quality characteristics of fermented sausages. These authors found that both fat level and olive oil replacement affected the sensory odor and taste of sausages. In a further study, Muguerza, Ansorena, Bloukas, & Astiasarán (2003) found that the reduction of fat in fermented sausages increased thiobarbituric acid (TBA) values, total aldehydes, and typical lipid oxidation compounds such as hexanal, 2-hexenal, and 2-octenal, and related those increases to the changes in odor and flavor previously reported. In addition, when pork fat is replaced by vegetable oils the fatty acid profile of the product changes increasing the percentage of unsaturated fatty acids, which could increase lipid oxidation (Muguerza, Ansorena, & Astiasarán (2004). Severini, De Pilli, & Baiano (2003) reported differences in *salami* flavor between the basic formulation and formulations with extra-virgin olive oil used as fat substitute (33.5, 50 and 66.5% substitution). These differences were more evident after 30 days of storage because of a piquant note developed in the oil containing products presumably as a result of lipid oxidation.

Regarding the effect of NaCl reduction and its substitution in dry-fermented sausages, substitution levels of between 30 and 40% could be achieved with only slight sensory changes in the product (Gou et al., 1996). KCl has similar functional properties to NaCl, but its addition to meat products is mainly limited by its bitter taste (Askar, El-Samahy, & Tawfik, 1994). When KCl is used in a high percentage (> 40%) a decrease in aroma and taste has been reported in fermented sausages (Campagnol, dos Santos, Wagner, Terra, & Pollonio, 2011; Guàrdia, Guerrero, Gelabert, Gou, & Arnau, 2008). Gelabert, Gou, Guerrero, & Arnau (2003) reported an increase in bitter taste at the 40% level of substitution. Likewise, these authors concluded that the partial substitution (above 40%) of NaCl with different mixtures of KCl/glycine and K-lactate/glycine showed negative flavor effects which did not permit an increase in the level of substitution compared to those obtained with the individual components. Gimeno et al. (1998) used a mixture of 1.00% NaCl, 0.55% KCl, 0.23% $MgCl_2$, and 0.46% $CaCl_2$ to replace NaCl in dry-fermented sausages. These authors reported lower sensory acceptability of sausages including this mixture due to their lower salty taste.

Nevertheless, there are only few studies about the effect of salt substitution on aroma. Campagnol et al. (2011) studied the volatile compounds generated in fermented sausages when NaCl was replaced by KCl and yeast extracts. These authors reported an evident decrease in aroma and taste after 50% substitution. Corral, Salvador, & Flores (2013) studied the effect of a 16% salt reduction and its substitution by KCl in the production of aroma active compounds in slow fermented sausages. These authors reported that salt reduction affected the sausage quality producing a reduction in the sensory scores for aroma and taste. The aroma was affected due to the reduction in sulfur and acids and the increase of aldehyde compounds. Furthermore, the substitution by KCl did not improve the aroma of the sausages.

1.3.2.4. Effect on instrumental and sensory texture

The consistency of fermented sausages increases due to acidification and drying. After acidification, which causes aggregation of myofibrillar proteins to form a gel, drying is a major factor affecting binding and rheological properties (Arnau, Serra, Comaposada, Gou, & Garriga, 2007). Several studies have reported a relationship between hardness and water content in dry-cured ham and loin (Serra, Ruiz-Ramírez, Arnau, & Gou, 2005; Ruiz-Ramírez, Arnau, Serra, & Gou, 2005a, 2006; Ruiz-Ramírez, Serra, Gou, & Arnau, 2005b). According to these authors, when the fat content is reduced, the water content increases. This higher water content leads to faster water losses (Klettner & Roedel, 1979).

Increases in hardness of dry-fermented sausages with fat content reduction (10, 20, and 30%) were reported by Muguerza et al. (2002). Increases in hardness and chewiness with fat content reduction have also been reported in fermented sausages with 6 and 10% fat and dietary fruit fibers used as fat substitutes (apple, orange, and peach) (García et al., 2002), in slow fermented sausages with 10% fat (Olivares et al., 2010), and in dry-fermented sausages with 6 and 15% fat with short-chain fructooligosaccharides (FOS) used as fat substitutes (Salazar et al., 2009).

Changes in the texture of fermented sausages with vegetable oils used as fat replacers have also been reported. The way to incorporate the oil can affect the texture of the sausages. In this sense, Bloukas et al. (1997) found that fermented sausages with direct incorporation of olive oil as liquid (10 and 20% of pork fat substitution) were softer than control sausages from the instrumental and sensory point of view, whereas those in which the olive oil was incorporated as pre-emulsified fat with soy protein isolate were harder than the control. Severini et al. (2003) reported a decrease of *salami* firmness (maximum recorded force on the output of the texture analyzer expressed as Newtons) with the increase of the amount of liquid extra-virgin olive oil used as pork fat substitute. Muguerza, Gimeno, Ansorena, Bloukas, & Astiasarán (2001) reported that the incorporation of pre-emulsified olive oil in the Spanish fermented sausage *chorizo de Pamplona* (20, 25 and 30% of pork fat substitution) also implied a modification of instrumental Texture Profile Analysis (TPA) parameters giving lower values for hardness in modified products. In the same way, Del Nobile et al. (2009) observed decreases in the instrumental Warner-Bratzler shear force test parameters hardness, cohesiveness, gumminess, and chewiness in Italian *salami* with 100% replacement of pork fat by extra-virgin olive oil adsorbed on whey-protein-based crumb or on white pan bread.

Several studies have reported texture changes in fermented sausages with the substitution of NaCl by different replacers. Gou et al. (1996) reported a loss of sensory cohesiveness in fermented sausages at substitution levels higher than 30% with K-lactate and higher than 50% with glycine. Likewise, Gelabert et al. (2003) stated that NaCl substitution by K-lactate at levels of 30 and 40% presented problems in texture. Lactate inhibits the growth of lactic acid bacteria during fermentation (Gou et al., 1997), which could be related to a lower pH drop. Lower chloride content and the delay in pH decrease in K-lactate substitutions could explain the modifications detected in texture at the 30% and higher levels. The drop in pH, the chloride content and the drying process promote cohesiveness and hardness, which are important characteristics for ease of slicing (Vignolo, Pesce de Ruiz, & Oliver, 1988). Guàrdia et al. (2008) reported increases in crumbliness and pastiness and decreases in hardness and cohesiveness at 50% substitution of NaCl by K-lactate in dry-fermented sausages. These products were rejected by consumers in a consumer acceptability study.

1.3.3. Fat substitutes in fermented sausages

Even though fat substitution supposes some limitations to the quality of fermented sausages, many studies have demonstrated the success of several types of fat substitution strategies in such meat products. In these studies, new formulations or modifications of the traditional ones were carried out in order to decrease the amount of animal fat, reducing calories and, in some cases, changing the fatty acid profile of the new products. In these new formulations, the strategy of replacing fat by lean meat have been widely used (Liaros et al., 2009; Mendoza et al., 2001; Muguerza et al., 2002; Olivares et al., 2010). In addition, different ingredients have been used to replace animal fat in order to obtain fermented sausages with healthier profiles.

Fat substitutes can be divided into three groups in relation to their different nature: derived from fat, derived from protein, and derived from carbohydrate (American Dietetic Association, 2005; Wylie-Rosett, 2002). The origin of the substitute is determinant to confer the technological, physicochemical and sensory characteristics to the final product. Furthermore, the way to incorporate the fat substitute and the type of manufactured product is determinant to decide a suitable strategy in the manufacture, processing and conservation of the reduced-fat product.

Concerning the way of incorporation of the fat substitutes to the meat product, Jiménez-Colmenero (2007) described some different technological options for substitutes derived from fat:

***Incorporation as liquid oil*:** Vegetable and marine origin lipids incorporated directly, as they are liquid at room temperature. The control of some factors such as lipid oxidation, and negative changes in the texture and sensory properties is required.

***Incorporation as pre-emulsified oil*:** Oil-in-water emulsions with one or various emulsifiers, typically proteins, carbohydrate derivatives, or mixes of both. The pre-emulsion is usually made before the product manufacture to stabilize the lipid ingredient improving some properties that could be affected in the case of the direct incorporation as liquid.

Incorporation as encapsulated oil: Microencapsulation has been used with the purpose of stabilising active substances, controlling the release of active substances, masking unpleasant tastes or smells (Kolanowski & Laufenberg, 2006; Pelser, Linssen, Legger, & Houben, 2007), or avoiding lipid oxidation. It is based on the formation of oil emulsions using proteins, polysaccharides, lecithin and other low molecular weight emulsifiers. The emulsions are then spray-dried (the low-cost microencapsulation technology commonly used in the food industry) to form microcapsules.

***Incorporation as solid fats*:** Some vegetable fats like palm oil are solid at room temperature because of their high solid glyceride content. It is common to incorporate these fats melted to facilitate the proper mixture with the rest of ingredients during the manufacture of meat products. Melting point of fats is a factor to be taken into account as it can affect sensory attributes, e.g., conferring a waxy texture and an aftertaste if fat is solid at 35–40 ºC which can affect consumer acceptance (Babji et al., 1998, Babji, Alina, Yussof, & Wan Sulaiman, 2001). Techniques like interesterification and partial hydrogenation have been used to increase the consistency of fats with high melting points.

The most common fat substitutes in fermented sausages are vegetable oils alone or mixed with proteins through different technological options. Other products such as fish oil, cereal and fruit fibers, inulin, or short chain fructooligosacharides have also been successfully used to obtain reduced-fat fermented sausages.

Reformulation by replacing the animal fat by vegetable oils has been recognized as an interesting way to improve the fatty acid profile of dry-fermented sausages. Olive oil was the first vegetable fat used for that purpose because of its high proportion of the monounsaturated oleic acid. Olive oil is a vegetable oil with a very high MUFA content. It contains 56.3–86.5% MUFA, particularly oleic acid. This oil is also rich in tocopherols and phenolic substances which act as antioxidants. Vitamin E to PUFA content ratio is high in olive oil, therefore it is considered to have a high biological value (Viola, 1970). Bloukas et al. (1997) found that 20% of pork backfat could be substituted with commercial olive oil in fermented sausages with acceptable appearance, firmness, odor and taste. Muguerza et al. (2001) replaced 25% of pork backfat with olive oil in *chorizo de Pamplona* obtaining similar color and texture attributes than commercial sausages. Regarding changes in the lipid profile of fermented sausages, Muguerza et al. (2003) reported an increase of MUFA content and a decrease in oxidation after two months of storage in Greek dry-fermented sausages with 20% of the total pork replaced by olive oil. Del Nobile et al. (2009) also obtained a better fatty acid profile in *salami* with 60 and 100% of pork backfat substituted by whey-protein-based crumb and white pan bread soaked in extra-virgin olive oil (EVOO), showing lower saturated fatty acid and higher MUFA than control. Moreover, these authors also reported sensory attributes similar to control in sausages with 60% whey-protein-based crumb with extra-virgin olive oil. Likewise, Severini et al. (2003) reported that it is possible to produce salami with 5% EVOO, corresponding to a 33.3% substitution of pork backfat regarding the traditional product, with good taste but healthier fatty acid profile.

Although olive oil has been the most commonly used, other vegetable oils such as cottonseed, palm, soy, linseed, canola, flaxseed, hazelnut and sunflower oil have been successfully included in different types of fermented sausages. Interesterified palm and cottonseed oils have been used to substitute 20, 60 and 100% beef fat in Turkish semi-dry-fermented sausages obtaining acceptable sensory scores, lower saturated to unsaturated (SFA/UFA) and higher polyunsaturated to saturated (PUFA/SFA) fatty acid ratios (Vural, 2003). Soy oil has been used as a successful pork fat replacer in *chorizo de Pamplona* (15, 20 and 25% substitution) obtaining texture, color, and sensory properties comparable to those of the commercial product (Muguerza et al., 2003). Ansorena & Astiasarán (2004) and Valencia, Ansorena, & Astiasarán (2006) reported improved nutritional quality and no oxidation problems in dry-fermented sausages with partial substitution of pork backfat by linseed oil and antioxidants. In the study of Valencia et al. (2006), the nutritional benefits of linseed oil and antioxidants containing products were maintained after 5 months of storage in refrigeration, especially under vacuum or MAP. Pelser et al. (2007) replaced 10, 15 and 20% pork backfat with canola oil and flaxseed oil pre-emulsified with soy protein isolate, and 15 and 20% pork backfat with flaxseed oil encapsulated or pre-emulsified with sodium caseinate in Dutch-style fermented sausages (*cervelat*). The addition of canola oil and encapsulated flaxseed oil resulted in a comparable shelf life as the control in terms of lipid oxidation. In addition, physical and sensory analysis showed that the sausages with encapsulated fish oil and flaxseed oil resembled the control most. Yildiz-Turp and Serdaroglu (2008) reported higher overall acceptability in Turkish fermented sausages (*sucuk*) with hazelnut oil replacing 15% of beef fat.

Sunflower oil has a number of characteristics which make this oil a suitable alternative to replace animal fat in meat products. First of all, it is a cheap ingredient in comparison with other oils such as the most commonly used olive oil. Technologically, sunflower oil offers advantages like a solidification point of –16 ºC to –17 ºC (Bockisch,1998) that allows the storage at low temperature and the immediate incorporation to meat products in liquid form. Moreover, according to the composition indicated by the Codex Alimentarius (Codex-Stan 210-1999), the SFA content of regular sunflower oil is lower than that in corn (maximum 22%), cottonseed (maximum 32%), peanut (maximum 28%), and soybean (maximum 20%) oils, and higher than the saturated content of safflower (maximum 12%) and rapeseed (maximum 12%) oils. The linolenic acid content (18:3) of regular sunflower oil is fairly low (always lower than 0.3%), giving the oil a good oxidative stability (Grompone, 2005). Finally, sunflower oil has higher tocopherol level than other vegetable oils like corn, soybean, peanut and cottonseed oils (Codex-Stan 210-1999). Tocopherols have an important vitamin E activity and also function as free radical scavengers conferring antioxidant activity (Grompone, 2005).

Sunflower oil has been incorporated to different meat products with positive results. Paneras & Bloukas (1994) reported 67% lower total fat, 40–45% lower saturated fatty acids, 50–53% lower calories, reduced cholesterol and 20% higher meat protein in low-fat frankfurters (10% fat, 12.5% protein) with sunflower oil, compared to control (29.1% animal fat, 10.4% protein). These products showed similar sensory quality and shelf-life than the control without fat reduction. Choi et al. (2013) replaced the pork backfat level in frankfurters from 30% to 20% with a mix of sunflower oil (0, 5, 10, 15, and 20%) and *makgeolli* lees fiber (2%) and studied their physicochemical, textural and sensory properties. The results of this study show that incorporating sunflower seed oil and *makgeolli* lees fiber into the formulation successfully reduced animal fat in frankfurters, while improving quality characteristics. Ambrosiadis, Vareltzis, & Georgakis (1996) compared beef frankfurters and cooked *salamis* (containing 19.5% pork back fat) with samples with pork backfat substitution by different vegetable oils: soya-seed oil (19.5%), sunflower oil (19.5%, 24%, 27.5%), cotton-seed oil (19.5%), corn-seed oil (19.5%) or palmine (19.5%). The full-fat control obtained the highest acceptability scores followed by sunflower oil samples. Papavergou, Ambrosiadis, & Psomas (1995) used sunflower oil to replace animal fat in comminuted cooked sausages. When processed, products were assessed for their stability with respect to autoxidation and change in sensory properties during vacuum-packed storage in a domestic refrigerator at 4 °C. Data obtained indicated that changes in TBA values and sensory properties of products produced using corn oil, sunflower oil and hydrogenated vegetable fat were similar to those observed for reference material produced using lard.

Although sunflower oil has been successfully incorporated in some meat products as discussed above, no studies about the use of this vegetable oil as animal fat substitute in fermented sausages had been published so far. Thus, the present thesis includes three papers concerning the use of sunflower oil as fat substitute in fermented sausages.

Fish oils are sources of very-long chain polyunsaturated fatty acids (PUFA) of the n–3 family such as eicosapentaenoic acid and docosahexaenoic acid. Their positive health effects are well recognized: antithrombogenic, antiinflamatory and hypotriglyceridemic properties, inhibit the formation of atherosclerotic plaques and prevent arrhythmias, prevention of autoimmune disorders, Crohn’s disease, breast, colon and prostate cancers, rheumatoid arthritis and particularly cardiovascular diseases (Alexander, 1998; Connor, 2000; Rose & Connolly, 1999). For that reason, several authors have included fish oils in the formulation of fermented sausages in order to reduce animal fat and to obtain healthier lipid profiles. Valencia et al. (2006) manufactured fermented sausages (*chorizo de Pamplona*) with 25% of pork backfat replaced by pre-emulsified deodorized fish oil from a mix of different fishes. These authors obtained higher PUFA + MUFA/SFA and lower n–6/n–3 ratios, without affecting sensory properties and oxidation status with regard to conventional sausages. Likewise, Pelser et al. (2007) used encapsulated fish oil to replace 15 and 20% of pork backfat in fermented Dutch style sausages, obtaining a lower n–6/n–3 ratio and similar sensory attributes than the conventional product without fat reduction.

Fat substitutes obtained by interesterification of animal fat with different vegetable oils have received particular attention in several studies. Different oils such as omega-3 rich oils have been enzimatically interesterified to produce a new range of triacylglycerols (TAGs) with healthier nutritional profiles, due to the reduction of saturated fatty acids and the increase of MUFAs and PUFAs. On the other hand, pork lard diacylglycerols (DAGs) produced from enzymatic glycerolysis of TAGs have been also found to be healthier as they result in lower fat accumulation in the human body (Flickinger & Matsuo, 2003; Maki et al., 2002; Meng, Zou, Shi, Duan, & Mao, 2004; Murase, Aoki, Wakisaka, Hase, & Tokimitsu, 2002). In this case, the beneficial health effects arise from its glyceride structure and not the fatty acid composition (Cheong & Xu, 2011).

DAG-based lard presents a number of properties which make this fat a good alternative to substitute conventional pork lard in meat products. DAGs have the ability to form emulsions and retain water due to the surface activity caused by a polar group in the molecular structure (Nakajima, 2004). Thus, stronger water retention could reduce the need for salt and phosphates as water-holding properties can be enhanced by the own characteristics of DAGs. In this aspect, this type of substitute could be a good alternative to combine with salt reduction in meat products. On the other hand, DAGs have higher melting points compared to TAGs, which could suppose a possibility to improve the texture of some products (Miklos, Xu, & Lametsch, 2011). In fact, meat emulsions containing 10, 50 and 100% DAG as fat substitute have been reported to increase the water-holding and fat binding and to be harder and more elastic than those prepared with pork lard (Miklos et al., 2011). Likewise, Cheong, & Xu (2011) found that lard DAG emulsions incorporated at different proportions as a substitute to pure lard fat were found to be more stable with lower total expressible fluid and almost no oiling out. Therefore, DAG is a promising ingredient which application to meat products is to be taken into account as it can help to improve some problems associated to fat reduction. Nevertheless, no studies concerning the use of DAGs in fermented sausages have been published before the first of the papers included in this thesis.

Table 1
Oil-based fat substitutes in fermented sausages regarding the way of incorporation, the type of product and the level of fat substitution (%).

Substitute	Incorporation	Product	Substitution (%)	Source
Fish oil	Encapsulated and deodorized	Dutch style fermented sausage	15, 20%	Pelser et al. (2007)
	Pre-emulsified/SPI[1]	Chorizo de Pamplona	25%	Valencia et al. (2006a)
Olive oil	Pre-emulsified/SPI	Dry-fermented sausage	10, 20%	Bloukas et al. (1997)
	Pre-emulsified/SPI	Dry-fermented sausage	66%	Kotsopoulos et al. (2008)
	Pre-emulsified/SPI	Chorizo de Pamplona	10, 15, 20, 25, 30%	Muguerza et al. (2001)
	Liquid: pre-mixed with SC[2]	Salami	33.5, 50, 66.5%	Severini et al. (2003)
	Pre-emulsified/SPI	Turkish sucuk	20, 40, 60%	Kayaardi & Gök (2003)
	Pre-mixed with whey protein-based crumb and white pan bread	Salami	60, 100%	Del Nobile et al. (2009)
Linseed oil	Pre-emulsified/SPI	Chorizo de Pamplona	25%	Ansorena & Astiasarán (2004)
	Pre-emulsified/SPI	Chorizo de Pamplona	25%	Valencia et al. (2006b)
	Pre-emulsified/SC	Dutch style fermented sausage	10, 15, 20%	Pelser et al. (2007)
	Encapsulated	Dutch style fermented sausage	15, 20%	Pelser et al. (2007)
Canola oil	Pre-emulsified/SPI	Dutch style fermented sausage	10, 15, 20%	Pelser et al. (2007)
Soy oil	Pre-emulsified/SPI	Chorizo de Pamplona	15, 20, 25%	Muguerza et al. (2003)
Palm oil	Interesterified	Semi-dry fermented sausage	20, 60, 100%	Vural et al. (2003)
Cottonseed oil	Interesterified	Semi-dry fermented sausage	20, 60, 100%	Vural et al. (2003)
Hazelnut oil	Pre-emulsified with whey protein powder	Turkish sucuck	15, 30, 50%	Yildiz-Turp & Serdaroglu (2008)

[1]Soy protein isolated.
[2]Sodium caseinate.

Many vegetable fibers have been successfully used as alternatives to animal fat in fermented sausages. Inulin is a soluble dietary fiber composed of a blend of fructose polymers extracted from plants. It is used as fat substitute because of its contribution to better mouthfeel, enhanced flavor and low caloric value (1.0 kcal/g) (Izzo & Franck, 1998). Moreover, it is fermented in the colon resulting in increased bifidobacteria and production of short chain fatty acids favoring calcium absorption and retention. The combination of these favorable effects could reduce the risk of colon cancer (Izzo & Franck, 1998). Mendoza et al. (2001) manufactured low-fat content dry-fermented sausages with different percentages of inulin. Results showed that the addition of powdered inulin at a concentration of 11.5% gave better sensory results than control low-fat products without addition of inulin, but still statistically lower than control high-fat products.

Amorphous cellulose obtained from cereals is a promising fat substitute. It is an insoluble fiber with no caloric value and free of any flavor. Due to its high water-holding capacity, amorphous cellulose increases viscosity, conferring a succulence and texture similar to that of fat (Torres, 2002). For these reasons, amorphous cellulose is a good alternative to obtain energy-reduced fermented sausages with healthier fatty acid profiles. Moreover, the sensory attributes of the product can be improved not only in terms of aroma and flavor but also in terms of texture. Campagnol, dos Santos, Wagner, Terra, & Pollonio (2012b) reported that the substitution of up to 50% of the pork backfat content by amorphous cellulose gel can be accomplished without a loss of product quality, enabling the production of fermented sausages with the levels of fat and cholesterol decreased by approximately 45% and 15%, respectively.

The effect of the addition of cereal and fruit dietary fiber in reduced fat fermented sausages has been also studied. García et al. (2002) manufactured dry-fermented sausages with 6 and 10% pork backfat, with addition of cereal (wheat and oat) and fruit (peach, apple and orange) dietary fibers, at 1.5 and 3% concentrations. They obtained an energy reduction close to 35%. Sausages containing 10% pork backfat and 1.5% fruit fiber, especially those with orange fiber, showed sensory characteristics similar to conventional high fat products.

Fructooligosaccharides (FOS) are recognized as a natural food ingredient and classified as dietary fiber in almost all European countries (Flamm et al., 2001). They are considered as a prototype prebiotic which stimulates the growth of colonic microbiota (bifidobacteria and lactobacilli) which ferment it and produce short-chain carboxylic acids that enhance mineral absorption (Bounik, Flourie, & Riottot, 1996; Coudray et al., 1997; Coussement & Franck, 2001; Flamm, Glinsmann, Kritchevsky, Prosky, & Roberfroid, 2001; Harland & Narula, 2001). Salazar et al. (2009) reported that reduced fat (15%) dry-fermented sausages (*salchichón* type) with short-chain FOS reduced hardness making the sausage easier to chew, and obtained higher acceptability than sausages with 30% fat.

Konjac glucomannan is a polysaccharide produced by the plant *Amorphophallus konjac*. It is a non-digestible fiber of high interest in food technology including meat products because of their low caloric value, the physiological benefits, and their capacity to form gels when combined with other ingredients (starch, carrageenans, gellan gum). Ruiz-Capillas, Triki, Herrero, Rodriguez-Salas, & Jiménez-Colmenero (2012) used konjac gels as fat analogs to substitute up to 80% of pork backfat in dry-fermented sausages, obtaining acceptable sensory characteristics and achieving an energy reduction of 24.5%.

Table 2

Carbohydrate-based fat substitutes in fermented sausages regarding the way of incorporation, the type of product and the level of fat substitution (%).

Substitute	Incorporation	Product	Substitution (%)	Source
Inulin	Powder	Dry-fermented sausage	75% reduction + 7.5 or 12.5% inulin	Mendoza et al. (2001)
	Aqueous solution	Dry-fermented sausage	75% reduction + 7.5, 12.5% inulin	Mendoza et al., 2001
Fruit fiber: orange, apple, peach	Direct incorporation	Dry-fermented sausage	60, 75% reduction + 1.5, 3% fiber	García et al. (2002)
Cereal fiber: wheat, oat bran	Direct incorporation	Dry-fermented sausage	60, 75% reduction + 1.5% fib re	García et al. (2002)
Konjac flour	Direct incorporation as a solid gel (aqueous solution with cornstarch, i-carrageenan and $Ca(OH)_2$)	Dry-fermented sausage	50, 80%	Ruiz-Capillas et al. (2012)
FOS[1]	Aqueous solution	Dry-fermented sausage (salchichón type)	70, 85, 94% reduction + 2, 4, 6% FOS	Salazar et al. (2009)
Amorphous cellulose	Direct incorporation as a gel (aqueous solution)	Fermented sausage	25, 50, 75, 100%	Campagnol et al. (2012b)

[1]Fructooligosaccharides.

1.3.4. Salt substitutes in fermented sausages

Salt reduction in fermented meat products has been successfully achieved through different strategies such as the use of KCl alone (Gou et al., 1996; Ibáñez, Quintanilla, Astiasarán, & Bello, 1997; Ibáñez, Quintanilla, Cid, Astiasarán, & Bello, 1996; Ibáñez et al., 1995) or together with other chloride salts ($CaCl_2$, $MgCl_2$) (Gimeno et al., 1998; Zanardi, Ghidini, Conter & Ianieri, 2010). Moreover, different flavor enhancers have been used in several studies (lactate, amino acids, yeast extracts) (Gou et al., 1996; Gelabert et al., 2003; Guàrdia et al., 2008; Campagnol et al., 2011).

Gou et al. (1996) manufactured dry-fermented sausages with different levels of NaCl substitution by KCl, K-lactate and glycine, and studied the effect of this substitution on the texture, flavor and color characteristics of these products. These authors reported acceptable flavor up to 40% substitution for the three salt replacers. The most important flavor defects were the bitter taste of KCl, the lactate flavor of K-lactate, and reductions in acid taste, salty taste and piquantness and increases in sweetness caused by the addition of glycine. Acceptable sensory texture was reported up to 30% substitution with K-lactate, 40% with KCl, and 50% with glycine. After these levels, a loss of cohesiveness was detected.

Ibañez et al. (1997) did not find significant differences between products manufactured with 3% of NaCl (control) and modified products manufactured with 1.5% NaCl and 1% KCl. The sodium reduction in these products was about 25%, in comparison with the traditional formulation (Ibañez et al., 1996). In another work, the same authors found that the nitrosylation process and carbohydrate heterofermentative activity of the starter cultures was favored in dry-fermented sausages manufactured with a mixture containing 1.37% NaCl and 0.92% KCl in comparison with sausages manufactured with 2.73% NaCl (Ibañez et al., 1995).

Gimeno et al. (1998 and 1999) used mixtures KCl, $CaCl_2$, and $MgCl_2$ to replace 61.5% of NaCl in *chorizo de Pamplona*, avoiding the negative effects observed with an excessive concentration of each one. They obtained acceptable products in terms of texture and color. Later, these authors (Gimeno et al., 2001) used calcium ascorbate to replace 46% of NaCl in *chorizo* and reported that the product had acceptable values of instrumental color and texture and no problems from a microbiological point of view.

Gelabert et al. (2003) used KCl, K-lactate and glycine, alone or mixtures of these substitutes, to replace NaCl in fermented sausages. These authors concluded that critical levels of NaCl substitution by KCl, K-lactate or glycine, regarding flavor and texture, were: 40% for KCl, 30% for K-lactate and 20% for glycine. A negative bitter taste was detected in sausages with KCl at a level of 40% substitution. From a safety point of view, the three substitutes for NaCl were acceptable at 40% substitution. The substitution of NaCl by mixtures of KCl/glycine and K-lactate/glycine, at levels of 40–70% gave important flavor (less saltiness, bitterness and sweetness) and textural differences. Therefore, the individual substitutes showed more advantages than the mixtures.

Guàrdia, Guerrero, Gelabert, Gou, & Arnau (2006) evaluated the consumer acceptability towards small-caliber fermented sausages with 50% molar substitution of NaCl by six mixtures of KCl (0–50%) and K-lactate (0–50%). The preference study showed no differences between the control batch without salt substitution and batches with 50% KCl and 40% KCl + 10% of K-lactate substitution levels. According to these results and from a sensory point of view, the authors concluded that it is possible to reduce NaCl content in small-caliber fermented sausages by 50% and to obtain a product acceptable for consumers. In a later consumer study, the same authors (Guàrdia, Guerrero, Gelabert, Gou, & Arnau, 2008) reported that small-caliber fermented sausages with 50% substitution of NaCl by KCl showed similar sensory attributes scores than those of the control without salt substitution. In addition, these sausages were acceptable for most consumers.

Apart from K-lactate and glycine, other flavor enhancers have been used in fermented sausages in order to solve the lower sensory acceptability derived of the decrease in aroma and taste. Campagnol et al. (2011) improved the sensory quality of reduced-fat fermented sausages with 50% KCl by the addition of 2% yeast extract. This product increased volatile compounds from amino acid and carbohydrate catabolism that contributed to the suppression of taste defects caused by KCl. In a later study, the same authors (Campagnol, dos Santos, Terra, & Pollonio, 2012a) used lysine, disodium guanylate, and disodium inosinate in fermented sausages with 50% replacement of NaCl by KCl. They reported that lysine, at a concentration of 1% with disodium inosinate (300 mg/kg) and disodium guanylate (300 mg/kg), reduced the sensory defects caused by the replacement of 50% NaCl with KCl, allowing the preparation of sensory acceptable fermented sausages with a 50% decrease in sodium.

Table 3

NaCl substitutes and flavor enhancers in fermented sausages regarding the type of product and the level of NaCl substitution.

Substitute / Flavor enhancer	Product	Substitution (%)	Source
KCl	Dry-fermented sausage	0–60% (progressive substitutions of 10%)	Gou et al. (1996)
	Dry-fermented sausage	50% reduction (1.5%NaCl + 1% KCl)	Ibáñez et al. (1995, 1996, 1997)
	Dry-fermented sausage	0–40% (progressive substitutions of 10%)	Gelabert et al. (2003)
	Small caliber fermented sausages (*fuet* type)	0–50% (progressive substitutions of 10%)	Guàrdia et al. (2006, 2008)
K-lactate	Dry-fermented sausage	0–100% (progressive substitutions of 10%)	Gou et al. (1996)
	Dry-fermented sausage	0–40% (progressive substitutions of 10%)	Gelabert et al. (2003)
	Small caliber fermented sausages (*fuet* type)	0–50% (progressive substitutions of 10%)	Guàrdia et al. (2006, 2008)
Glycine	Dry-fermented sausage	0–100% (progressive substitutions of 10%)	Gou et al. (1996)
	Dry-fermented sausage	0–40% (progressive substitutions of 10%)	Gelabert et al. (2003)
KCl + glycine	Dry-fermented sausage	40–70%	Gelabert et al. (2003)
KCl + glycine + disodium guanylate + disodium inositate	Fermented sausage	50%	Campagnol et al. (2012a)
K-lactate + glycine	Dry-fermented sausage	40–60%	Gelabert et al. (2003)
KCl + MgCl2 + CaCl2	Dry-fermented sausage	61.5%	Gimeno et al. (1998, 1999)
Calcium ascorbate	Chorizo	46%	Gimeno et al. (2001)
KCl + yeast extract	Fermented sausage	50% (2% yeast extract)	Campagnol et al. (2012)

2 OBJECTIVES

A main objective and three specific objectives were considered in this thesis, according with the objectives proposed in the Q-Porkchains project that aims to improve the nutritional quality of pork meat products.

2.1. MAIN OBJECTIVE

Nutritional enhancement of pork products through the development of fermented sausages with lower fat and sodium content by means of innovative processing and the use of fat and sodium chloride substitutes.

2.2. SPECIFIC OBJECTIVES

1. Study of the effect of sunflower oil and pork lard-based diacylglycerols as pork-fat substitutes on the sensory attributes, physicochemical parameters and instrumental texture of reduced fat non-acid fermented sausages.

2. Study of the effect of pork fat reduction and its substitution by sunflower oil on the physicochemical, instrumental and sensory properties throughout storage time of small caliber non-acid fermented sausages (*fuet* type) with reduced sodium content (with partial substitution of NaCl by KCl and K-lactate) and without direct addition of nitrate and nitrite.

3. Study of the effect of the simultaneous reduction of fat and NaCl and the addition of KCl and sunflower oil on the quality and the consumer acceptability of small-caliber non-acid fermented sausages.

3 EXPERIMENTAL DESIGN

In this chapter, the formulation, the main differences in the manufacture and processing, and the analysis methods used to evaluate the quality of the non-acid fermented sausages are described briefly.

The formulation of the batches manufactured in the three studies is shown in Table 4. Regarding the fat addition, in Papers I and II, fat was added by mincing it together with lean in a bowl chopper until forming a finely comminuted paste that was later added to the rest of the lean of each batch during grinding (before mixing). In the case of paper III, the sunflower oil was directly added into the mixer. With respect to the drying level, in Paper I all the batches were dried until reaching the same water content on a defatted dry-matter basis (X_{DFDM}). In Paper II all the batches were dried until reaching the same weight loss (49%). In Paper III, all the batches were dried until reaching the same water content on a defatted desalted dry-matter basis (X_{DFDSDM}).

The following analyses were performed to evaluate the physicochemical and sensory quality of the non-acid fermented sausages:

Instrumental color: color measurement in the CIE-Lab space (Commission Internationale de l'Éclairage [CIE], 1976).

Instrumental texture: Texture Profile Analysis (TPA) (Bourne, 1978); Stress Relaxation test (SR test). The SR test was performed only in papers I and II.

Physicochemical parameters: water activity (a_w) measurement; moisture content determination (Association of Official Analytical Chemists [AOAC], 1990); total fat determination by Soxhlet extraction (International Organization for Standardization [ISO] 1443, 1973); pH measurement in a homogenized sample solution (method described by Choi et al., 2009); water content on a defatted dry-matter basis (X_{DFDM}) in Paper I and water content in a defatted desalted dry-matter basis in Paper III, calculated from the chemical composition of each batch.

Sensory quality: analysis performed by a trained panel (American Society for Testing and Materials [ASTM], 1981; ISO 8586-1, 1993; ISO 8586-2, 1994). Quantitative Descriptive analysis using a non-structured scoring scale where 0 means absence of the descriptor and 10 means high intensity of the descriptor (Amerine, Pangborn, & Roessler, 1965).

Consumer acceptability: (only in Paper III) carried out with eighty-four consumers. Evaluation of the acceptability using a non-structured scoring scale (0 = extremely disliked and 10 = extremely liked).

Table 4

Formulation of the different batches of non-acid fermented sausages manufactured in the three studies (Papers I; II and III) according to the type of meat used, the type and amount of added fat, the amount of NaCl and the type and amount of NaCl substitute.

Paper	Batch code	Meat type	Added fat	NaCl	NaCl Substitute
I	Lean	Ham lean	-	2.0%	-
	BF	Ham lean	5% pork backfat	2.0%	-
	SO	Ham lean	5% sunflower oil	2.0%	-
	DAG	Ham lean	5% DAGs	2.0%	-
II	BF20	Shoulder lean 3D	20% pork backfat	1.4%	0.422% KCl + 1.325% K-lactate (78% purity)
	BF3	Shoulder lean 3D	3% pork backfat	1.4%	0.422% KCl + 1.325% K-lactate (78% purity)
	SO3	Shoulder lean 3D	3% sunflower oil	1.4%	0.422% KCl + 1.325% K-lactate (78% purity)
III	CT	Lean trimmings 80:20	-	2.5%	-
	L	Lean trimmings 90:10	-	1.5%	-
	L-KCl	Lean trimmings 90:10	-	1.5%	0.64% KCl
	S	Shoulder lean 3D	-	1.5%	-
	S-KCl	Shoulder lean 3D	-	1.5%	0.64% KCl
	S-KCl-1.5SO	Shoulder lean 3D	1.5% sunflower oil	1.5%	0.64% KCl
	S-KCl-3SO	Shoulder lean 3D	3% sunflower oil	1.5%	0.64% KCl

4 RESULTS

4.1. PAPER I

Mora-Gallego, H., Serra, X., Guàrdia, M. D., Miklos, R., Lametsch, R., & Arnau, J. (2013). Effect of the type of fat on the physicochemical, instrumental and sensory characteristics of reduced fat non-acid fermented sausages. *Meat Science, 93*, 668–674.

Mora-Gallego, H., Serra, X., Guàrdia, M. D., Miklos, R., Lametsch, R., & Arnau, J. (2013). "Effect of the type of fat on the physicochemical, instrumental and sensory characteristics of reduced fat non-acid fermented sausages". *Meat Science*, 93, 668-674

http://dx.doi.org/10.1016/j.meatsci.2012.11.042

http://www.sciencedirect.com/science/article/pii/S0309174012004032

Received 18 July 2012

Received in revised form 2 November 2012

Accepted 10 November 2012

Abstract

Four batches of reduced fat non-acid fermented sausages were manufactured with pork-ham lean, and the addition of no fat (Lean), 5% pork backfat (BF), 5% sunflower oil (SO) and 5% diacylglycerols (DAGs). The effect of the type of fat as pork-fat substitute on some physicochemical parameters, instrumental color and texture and sensory attributes of the sausages was studied. Results showed that reduced fat non-acid fermented sausages containing less than 12.5% of fat (BF, SO and DAGs) had a good overall sensory quality. This means a fat reduction of more than 70% compared with the average fat content of standard fermented sausages of similar characteristics. Sausages with SO showed higher sensory ratings in desirable ripened odor and flavor attributes and improved texture defined by lower hardness and chewiness (both sensory and instrumental) and higher crumbliness. Sausages with DAGs showed a similar behavior to that of BF, so they could be a good alternative to produce healthier reduced fat non-acid fermented sausages.

Keywords

Fat reduction; Fat substitute; Diacylglycerol; Fermented sausage; Sensory quality; Instrumental texture

3.1. PAPER II

Mora-Gallego, H., Serra, X., Guàrdia, M. D., & Arnau, J. (2014). Effect of reducing and replacing pork fat on the physicochemical, instrumental and sensory characteristics throughout storage time of small caliber non-acid fermented sausages with reduced sodium content. *Meat Science, 97*, 62–68.

Mora-Gallego, H., Serra, X., Guàrdia, M. D., & Arnau, J. (2014). “Effect of reducing and replacing pork fat on the physicochemical, instrumental and sensory characteristics throughout storage time of small caliber non-acid fermented sausages with reduced sodium content”. *Meat Science*, 97, 62-68

http://dx.doi.org/10.1016/j.meatsci.2014.01.003

http://www.sciencedirect.com/science/article/pii/S0309174014000047

Received 20 June 2013

Received in revised form 10 December 2013

Accepted 6 January 2014

Abstract

The effect of pork fat reduction (from 44% to 20% final fat content) and its partial substitution by sunflower oil (3% addition) on the physicochemical, instrumental and sensory properties throughout storage time of small caliber non-acid fermented sausages (fuet type) with reduced sodium content (with partial substitution of NaCl by KCl and K-lactate) and without direct addition of nitrate and nitrite (natural nitrate source used instead) was studied. Results showed that sausages with reduced fat (10% initial fat content) and with acceptable sensory characteristics can be obtained by adding to the shoulder lean (8% fat content) during the grinding, either 3.3% backfat (3% fat content) or 3% sunflower oil, both previously finely comminuted with lean. Furthermore, sunflower oil showed to be suitable for partial pork backfat substitution in very lean fermented sausages, conferring desirable sensory properties similar to those of sausages with standard fat content. The sensory quality of the sausages was maintained after three-month cold storage in modified atmosphere.

Keywords

Fat reduction; Fat substitute; Sodium reduction; Fermented sausage; Sensory quality; Instrumental texture

3.1. PAPER III

Mora-Gallego, H., Guàrdia, M. D., Serra, X., Gou, P., & Arnau, J. Sensory characterization and consumer acceptability of small-caliber non-acid fermented sausages with sunflower oil and KCl as fat and NaCl substitutes. *Meat Science, Submitted*. (MEATSCI-D-14-00237).

Embargoed until publication

Mora-Gallego, H., Guàrdia, M. D., Serra, X., Gou, P., & Arnau, J. "Sensory characterization and consumer acceptability of KCl and sunflower oil addition in small-caliber non-acid fermented sausages with a reduced content of NaCl and fat". *Meat Science*, Submitted. (MEATSCI-D-14-00237).

http://www.journals.elsevier.com/meat-science/

Abstract

The effect of the simultaneous reduction of fat (from 20% to 10% and 7%) and salt (from2.5% to 1.5%) and the addition of 0.64% KCl and sunflower oil (1.5% and 3.0%) on the physicochemical, instrumental colour and texture, sensory properties and consumer acceptability of small calibre non-acid fermented sausages (*fuet* type) was studied. This simultaneous reduction increased weight loss, % of moisture, a_w, redness (a*),instrumental texture parameters (hardness, chewiness and cohesiveness) and sensory attributes (darkness, hardness, elasticity) but did not significantly affect the consumer acceptability. The addition of 0.64% KCl to the leanest batches decreased the a_w and barely affected instrumental texture parameters and consumer acceptability. Sunflower oil addition decreased hardness, chewiness and cohesiveness and increased crumbliness, but it may have negatively affected the consumer acceptability. The simultaneous reduction of fat and NaCl with the addition of 0.64% KCl was the preferred option by the consumers.

Keywords

Fat reduction; Salt reduction; Fermented sausages, Sensory attributes, Acceptability

5 GENERAL DISCUSSION

In this chapter, the effect of pork fat reduction and salt reduction and substitution on the physicochemical characteristics, instrumental color and texture, sensory quality and consumer acceptability of non-acid fermented sausages will be reviewed and discussed, including the results obtained in Papers I, II and III.

5.1. Effect of the type of fat on the physicochemical, instrumental and sensory characteristics of reduced fat non-acid fermented sausages

In Paper I, small caliber non-acid fermented sausages made with pork-ham lean (Lean batch) were used as control batch in order to assess the technological and sensory changes and/or improvements of non-acid fermented sausages. Pork-ham lean was used instead of shoulder lean because of its lower fat content. The batches with added fat were manufactured with pork-ham lean and the addition of 5% of different types of fat, i.e., pork backfat (BF batch), sunflower oil (SO batch) and pork lard DAGs produced from enzymatic glycerolysis of TAGs (DAG batch). In addition, no microbial starter was added; therefore, the fermentation was carried out by the natural microbial flora of the meat. All batches were dried to reach the same water content on a defatted-dry-matter basis (X_{DFDM}). Thus, in Paper I, the effect of the type of fat on the quality of the sausages was studied.

In Paper II, small caliber non-acid fermented sausages made with pork shoulder and 20% pork backfat (BF20 batch) were used as control, resembling the typical formulation of a commercial product. Two reduced fat batches were manufactured including 3.3 % pork backfat (which corresponds to 3% added fat) and 3% sunflower oil (BF3 and SO3 batches, respectively). Regarding the previous experiment, the level of added backfat was reduced from 5% in the sausages of Paper I to 3% in this experiment due to the use of pork shoulder which has a higher amount of fat than pork ham lean. Therefore, even though less amount of backfat was added, the final fat content of the sausages was higher in Paper II than in Paper I. Finally, sunflower oil was the only fat substitute used due to the positive results obtained in Paper I, and because this oil is cheaper and easier to obtain than DAGs. In this study, the characteristics of the fermented sausages were analyzed at 1 and 3 months of storage in modified atmosphere (MAP; 80% N_2:20% CO_2) at 3 °C. Thus, the effect of the storage time on the physicochemical, instrumental and sensory characteristics and its interaction with the fat type/level will be discussed.

In Paper III, small caliber non-acid fermented sausages were made using pork meat from different cuts and different fat contents (no backfat was added): lean trimmings 80:20 (lean:fat proportion) used as control (CT batches), lean trimmings 90:10 (L batches), and shoulder 3D (approx 7% fat; S batches). In this study, sunflower oil was included in two S batches at levels of 1.5% and 3%. Similarly to Paper I, the sausages were dried to reach the same water content in a defatted-dry-matter basis, and in this case also the NaCl and KCl contents, which were different between batches, were taken into account. Thus, the parameter used to establish the end of the drying process was the water content in a defatted-desalted-dry-matter-basis (X_{DFDSDM}).

5.1.1. Effect on physicochemical characteristics

Differences in the initial moisture content and in the level and type of fat among batches determined the differences in the weight loss during the drying process in the three studies, as expected. According to Klettner & Roedel (1980), the higher the fat content, the lower the weight losses over the same time. Thus, batches with higher fat content needed more time to reach the X_{DFDM} (Paper I), weight loss (%) (Paper II), or X_{DFDSDM} (Paper III) established to stop the drying process.

In Paper I, the SO and DAG batches showed lower weight loss than the BF batch because the sunflower oil and DAGs contained no water in comparison with the backfat (water~8 %; USDA, 2012a). Moreover, the three types of fat were added to the meat as a mixture of the fat with a part of the ham-lean (emulsion). This emulsion could cover more easily the particles of ground meat, especially in the case of sunflower oil and DAGs without cellular structure and connective tissue, which facilitates fat to cover the ground meat particles delaying the release of water. Bloukas, Paneras, & Fournitzis (1997) reported similar results for fermented sausages with 10% and 20% of the total pork backfat replaced by olive oil incorporated as a liquid, resulting in lower weight losses than the control (33% pork backfat). Del Nobile et al. (2009) also found lower weight losses in *salami* with olive oil incorporated in liquid form as pork backfat substitute.

In Papers I and III, despite having established a common target drying level for all batches (X_{DFDM} and X_{DFDSDM}, respectively), they showed slightly significant differences among them. In order to correct for these differences, X_{DFDM} and X_{DFDSDM} were included as covariables in the statistical analyses for instrumental color, instrumental texture, and sensory evaluation.

Water activity (a_w) increased with the X_{DFDM}, as expected, in Paper I. In Paper III, a_w decreased with moisture and showed lower values in the CT batch, which had higher fat level and lower moisture content than the reduced fat batches. In this study, the reduced fat sausages with sunflower oil also showed similar a_w values than the CT batch, which could be related to the fact that the oil would cover the meat particles causing a slower release of water during the drying process, as in the case of Paper I. In paper II, all batches were dried to the same weight loss (49%). Similarly to the Papers I and III, due to the higher fat content of BF20 batch, these sausages showed lower moisture and a_w values than those of the reduced fat batches, as expected.

Some traditional Spanish sausages, e.g., *fuet*, and some types of *chorizo* ripened at low temperature do not include starter cultures in their formulation, and fermentation is produced by the natural microbial flora at low constant temperature of approx 12 ºC. The characteristic sensory attributes of these products, i.e., colour, texture, flavour, are mainly determined by the low temperature of the process, and the quick decrease of water activity due to the small calibre (<30 mm in *fuet*) (Ruiz-Ramírez, Gou, & Arnau, 2003). No starter was added to the sausages in Paper I, whereas sausages in Papers II and III included starter cultures. In the three cases, a pH increase occurred during the drying process. This could be due to a combination of several factors such as the effect of K-lactate addition (Guàrdia, Guerrero, Gelabert, Gou, & Arnau, 2008) (papers I and II) and the absence of starter cultures in the mixture (Paper I), the low drying temperature used during the first steps of drying, and the effect of the mould which usually increases pH at the end of the ripening and the production of ammonia from amino acids (Grazia, Romano, Bagni, Roggiani, & Guglielmi, 1986; Lücke, 1986; Roncalés, Aguilera, Beltrán, Jaime, & Peiro, 1991).
In the three studies, the batches with lower fat level showed higher pH. Beriain, Iriarte, Gorraiz, Chasco, & Lizaso (1997) and Roncalés et al. (1989) related pH increases after fermentation to the proteolysis phenomena which would involve a rise in the content of basic nitrogen compounds and, thus, the partial neutralization of the acidity. Therefore, in our reduced fat sausages, with more protein content than the control ones, a higher degree of proteolysis would occur, causing a pH increase.

The feasibility of reduced fat products depends, among other factors, on the degree of fat reduction from a technological point of view, the effects on the sensory characteristics and, therefore, the acceptability of the product (Jiménez-Colmenero, 1996). In fermented sausages, this limit is established at about 20–30% (of fat content) in the final product depending on the degree of drying (Wirth, 1988). However, some authors have obtained positive results including less amount of fat in different types of fermented sausages. Acceptable sensory quality of *Milano salami* with 13% fat were reported (Chizzolini, 1997) replacing fat with pre-gelatinised rind, surimi paste and tapioca starch and modifying the processing technology to slow down dehydration. García, Domínguez, Gálvez, Casas, & Selgas (2002) reported sensory characteristics similar to conventional high fat products in dry-fermented sausages containing 10% pork backfat and 1.5% fruit fibre.

In Paper I, the final fat content of the sausages (Lean: 4.8%, Backfat: 12.3%, Sunflower oil: 11.2% and DAG: 12.2%) represent fat reductions of more than 70% regarding a standard small calibre non-acid fermented sausage (*fuet* type) with a final fat content of around 42%. Thus, these sausages can be considered as reduced fat products according to Regulation (EC) 1924/2006 of the European Parliament and of the Council on nutrition and health claims made on foods, which considers a product as reduced fat when the reduction in fat content is at least 30% compared to a similar product. The fat content of the pork shoulder used in Paper II was higher than the pork ham-lean used in Paper I. Therefore, reduced fat sausages made of pork shoulder (BF3 and SO3) showed higher final fat contents after drying (approx 20%, data not shown) than sausages made of pork ham-lean (approx 5% for Lean and approx 12% for batches with 5% added fat), even though the sausages made of pork shoulder included less added fat (3%). Reduced fat sausages of Paper II also showed lower fat reduction (43 to 45%, in comparison with the BF20 batch with a final fat content of 44.0% or a standard *fuet* type sausage with a final fat content of around 42%. This reduction higher than 30% was enough to consider the sausages of Paper II as reduced fat. As in the case of paper I, the sausages of Paper III had lower final fat contents (33.2% in the CT batch and 13.0 to 18.6% in the reduced fat batches, data not shown) than the sausages of Paper II. Likewise, the fat reduction was higher than that the achieved in Paper II (39 to 56% with respect to the CT batch, and 44 to 61% with respect to a standard *fuet* type sausage with 42% fat).

5.1.2. Effect on instrumental color and visual appearance

Regarding instrumental color, several studies have reported significantly higher instrumental lightness (L*) with the increasing fat level in Greek traditional sausages (Papadima & Bloukas, 1999) and *sucuk*, a Turkish traditional fermented sausage (Soyer, Ertas, & Üzümcüoglu, 2005). Similarly, Bloukas et al. (1997) and Muguerza, Fista, Ansorena, Astiasarán, & Bloukas (2002) also found that L* values increased with the fat level in dry-fermented sausages. In these studies, Bloukas et al. (1997) and Muguerza et al. (2002) also reported that the partial substitution of pork backfat by olive oil produced lighter sausages, showing the influence of the type of fat on the instrumental lightness of these fermented meat products. On the contrary, Del Nobile et al. (2009) obtained the lowest lightness in salami with 100% substitution of pork backfat with extra virgin olive oil.

In our research work, the increase of instrumental lightness with the fat level observed in the three papers was mainly attributed to the higher content of white backfat particles in the batches with higher backfat content. Thus, in Paper I, BF and DAG sausages were lighter than the SO sausages, as pork backfat and DAGs are solid at room temperature showing a visible white color, whereas sunflower oil is liquid and translucent. In Papers II and III, control batches with the highest backfat content, i. e., BF20 and CT respectively, showed the highest L* values, as expected. In Paper II the SO3 batch showed lower L* values than batches with added backfat, similarly than in Paper I. Nevertheless, in Paper III, the addition of sunflower oil to reduced fat sausages, i.e., S-KCl-3SO and S-KCl-1.5SO batches, did not modify L* values with respect to the reduced fat batches containing only pork fat. The explanation for this result could be that the meat used to manufacture all the reduced fat batches of Paper III had a similar fat content and, thus, a similar amount of white fat particles regardless of the sunflower oil addition. On the contrary, in the reduced fat sausages of Papers I and II, the pork backfat responsible of the white color was replaced by sunflower oil (especially in Paper I, as sausages were made of pork-ham lean with a very low fat content: 4.8%). Finally, in Paper II, lightness was affected by the MAP storage time. The fermented sausages showed significantly higher L* values at 3 months than at 1 month, which could be related to product discoloration although it was not detected in the sensory evaluation. These results agree with Rubio et al. (2008) who also reported higher L* values in *salchichón* at 210 days of MAP storage than at 0 days.

Some studies have shown higher instrumental redness (a*) when the fat content is reduced and, therefore, the lean meat content increases in dry-fermented sausages (Muguerza, Gimeno, Ansorena, & Astiasarán, 2004; Salazar, García, & Selgas, 2009). These results agree with the higher redness of the reduced fat batches (BF3 and SO3) with respect to the control (BF20) in Paper II, whereas no significant differences associated to the fat reduction were observed for a* values in papers I and III. Authors like Bloukas et al. (1997) and Del Nobile et al. (2009) did not find differences for redness depending on the type and fat level in Greek fermented sausages and *salami*, respectively. Del Nobile et al. (2009) reported that differences for a* values among *salami* batches with different fat content were not evident, probably due to the typical heterogeneous color of *salami*, which would be similar to the heterogeneous distribution of the meat and fat particles in our sausages. The differences for a* values found in Paper II could be related to higher differences in the fat content between BF20 (20% backfat) and reduced fat batches (3% backfat in BF3 and 3% sunflower oil in SO3), with respect to Paper I (0% added backfat in Lean batch with respect to 5% added fat in the rest of batches) and Paper III (22% fat in CT and 9–11% fat in reduced fat batches). In Paper III, the use of shoulder (S batch) instead of lean trimmings (L batch) as raw material resulted in a significant decrease of the redness (a*). This is probably explained by the anatomical origin of the lean trimmings, i.e., redder muscles.

The reduced fat fermented sausages of Paper II were affected by the interaction between the MAP storage time and the level and type of fat, showing a decrease of a* values at 3 months with respect to the values shown at 1 month. On the contrary, BF20 sausages did not show any change for redness with the storage time. Some studies have shown increases in redness when the fat content is reduced in dry-fermented sausages (Muguerza, Gimeno, Ansorena, & Astiasarán, 2004; Salazar, García, & Selgas, 2009). Likewise, Kayaardi & Gök (2003) also reported changes in redness with the processing time in Turkish dry-fermented beef sausages (*sucuk*) with 20, 40 and 60% of fat substitution by olive oil.

An increase of b* values with the fat level was observed in Papers I and II, probably because of their higher lipid content which could facilitate increases in yellowness (Papadima & Bloukas, 1999; Soyer et al., 2005). In Paper I, batches with 5% added fat showed higher b* values than the Lean batch (no added fat). Similarly, in Paper II, the BF20 batch (20% backfat control) showed higher yellowness than reduced fat batches. In Paper III, the CT batch (highest fat content) showed significantly higher yellowness than the batches in which shoulder was included. A possible explanation could be that these batches presented lower lipid contents than the batches made with lean trimmings, whose fat content was closer to that of the CT batch. Finally, the yellowness of S-KCl batches tended to increase with the addition of sunflower oil, showing b* values similar to the CT batch when 3% sunflower oil was added.

Concerning the effect of the type of fat on yellowness, Muguerza et al. (2002) reported an increase of b* values in fermented sausages with initial fat levels of 10%, 20% and 30% in which 20% of the total backfat was replaced by olive oil. In our case, no significant differences depending on the type of fat were observed in batches with similar fat level. In Paper II, the SO3 sausages could be expected to show an increase of b* values at 3 months due to the higher unsaturated fatty acids content of sunflower oil, which would make them prone to color changes because of oxidation (Jiménez-Colmenero, 2007). Nevertheless, SO3 *fuets* showed similar values than BF20 sausages. Similarly, Muguerza et al. (2003) reported that yellowness was not significantly affected by soy oil addition (15%, 20% and 25% pork fat substitution) in *chorizo de Pamplona*, which could be explained by the antioxidant effect of vitamin E in the oil used.

Regarding the sensory appearance of the sausage slices, the reduction of the fat level led to higher ratings for the darkness appearance in the three studies. These results agree with those obtained for the instrumental lightness, being attributed to the lower presence of white backfat particles in the reduced fat sausages. Muguerza et al. (2003) reported a decrease of color intensity with the increasing fat level in *chorizo* de Pamplona with 15%, 20% and 25% of soy oil used as pork backfat substitute. Darker dry-fermented sausages were also obtained with the fat reduction by Mendoza, García, Casas, & Selgas (2001) and Olivares, Navarro, Salvador, & Flores (2010). Additionally, several studies have reported a lightness decrease with decreasing moisture content: in dried pork *M. Longissimus* (Serra, Arnau, & Colleo, 2009) and in dry-cured minced pork (Ferrini, Comaposada, Arnau, & Gou, 2012; Holmgaard Bak et al., 2012). In Paper II, the lower moisture content, together with the lower amount of white backfat particles, could be the main causes of the higher darkness of reduced fat fermented sausages. Nevertheless, the effect of moisture content on lightness would be negligible in Papers I and III as shown by the fact that the X_{DFDM} and X_{DFDSDM} covariables were not statistically significant for the instrumental lightness. In papers I and II, in agreement with the instrumental color analysis, the batches containing 5% and 3% sunflower oil were rated darker than the batches containing the same level of backfat or DAGs. The lower darkness of sausages with sunflower oil would be due to the characteristic color of the different types of fat used, i.e., backfat and DAGs are white whereas sunflower oil is translucent. Similarly to the results obtained for the instrumental color analysis, the reduced fat batches with sunflower oil showed similar darkness scores than the reduced fat batches containing only pork fat, because of their similar white fat particles amount.

Fermented sausages with sunflower oil were rated brighter than sausages including other types of fat in Papers I and II, whereas in Paper III no differences were observed among batches. The higher brightness of sunflower oil batches in the first two studies could be explained by the lower melting point of the sunflower oil: between −16 °C and −17 °C (Bockisch, 1998), in comparison with backfat that melts between +30 °C and +40 °C (Ospina-E, Cruz-S, Pérez-Álvarez, & Fernández-López, 2010; Suzuki, Shibata, Kadowaki, Abe, & Toyoshima, 2003) and DAGs that melt around +45 °C (Cheong, Zhang, Xu, & Xu, 2009). Oil exudation could result in insufficient binding between the oil incorporated as liquid and the meat. Oil exudation in fermented sausages in which olive oil is directly incorporated as liquid has been reported by Bloukas et al. (1997).The similar brightness among batches observed in Paper III could be related to technological factors such as the higher fat content of the meat used and the different way to incorporate the oil during the sausages manufacture, i.e., added directly during the mixing of the ingredients instead of previously mixed with a part of the ground meat and added to the rest of the meat in a second grinding.

According to Wirth (1988), higher fat and lower water contents delay the moisture release during the drying process leading to longitudinal folds, shrunken diameters and dry edges, and thus, affecting the round shape of the sausages. In all three studies, the batches with higher fat level (batches with added fat in Paper I, BF20 in Paper II, and CT in Paper III) were rated rounder than the rest of batches.

One of the main problems to consider when adding vegetable oils as pork backfat replacement is the high susceptibility to oxidation of the polyunsaturated fatty acids (PUFAs) contained in these oils (Muguerza et al., 2003). These authors reported a positive effect of olive oil to inhibit oxidation. Oxidation color was analyzed in sausage slices in Paper I. The oxidation color of the external part of the slice, i.e., darker and less red appearance, was similar in BF, SO and DAG batches. Thus, no undesired color related to oxidation was observed with the addition of sunflower oil. In Paper II, the attribute discoloration of the slice was analysed. No discoloration was observed among batches. In Paper III, an increase on visual brown color of the slice surface was observed with de addition of 1.5% and 3% sunflower oil with respect to reduced fat batches without this oil.

All instrumental color parameters, i.e., lightness, redness and yellowness were significantly affected by the fat level, whereas only lightness showed changes depending on the type of fat. Nevertheless, the type of fat used as pork fat substitute affected the sensory appearance of reduced fat non-acid fermented sausages.

5.1.3. Effect on odor and flavor

A higher fat level has been reported to give higher aroma scores to fermented sausages (Mendoza et al., 2001; Muguerza et al., 2002; Olivares, et al., 2010). According to this, In Paper I, the sausages with 5% added fat (BF, SO, and DAG) resulted in sausages with higher ripened odor than the lean sausages. However, in Papers II and III, no significant differences for this attribute and for odor intensity were observed between the fat reduced sausages and those without fat reduction. The reason for these results could be the lower fat level of the pork-ham lean used to manufacture the sausages in Paper I in comparison with the pork shoulder and pork lean trimmings used in Papers II and III. Even though sausages of Paper I included more added fat (5%) than reduced fat sausages of Paper II (3%), the latter had a higher total fat content due to the higher amount of fat in the meat. The same occurred with reduced fat sausages of Paper III without added backfat (the fat content of these sausages was the fat of the lean trimmings, instead of added backfat). For this reason, and taking into account that fat has an important role as a solvent for aroma compounds in dry-fermented sausages (Leland, 1997), it would be easier to obtain odor differences in sausages made with a leaner meat, as the contribution of the added fat to the odor would be higher than in sausages made of fattier meats.

In general terms, the flavor of meat products is influenced by both the amount and the type of fat (Baker, Darfler, & Vadhera, 1969; Park, Rhee, & Ziprin, 1990). The SO sausages of Paper I showed higher score for ripened flavor, probably because its lower melting point facilitates the release of volatile compounds in the mouth. In addition, in dry-fermented sausages, lipids are hydrolyzed by lipases with production of free fatty acids (Countron-Gambotti & Gandemer, 1999; Gandemer, 2002), which are susceptible to oxidation reactions that result in the release of volatile compounds (Zanardi, Ghidini, Battaglia, & Chizzolini, 2004). Vegetable oils, such as sunflower oil, contain higher percentage of polyunsaturated fatty acids (PUFAs) than pork backfat. These PUFAs contribute to increase autoxidation in dry-fermented sausages (Ansorena & Astiasarán, 2004) and, therefore, to the release of volatile compounds that could increase the flavor intensity and ripened flavor. In Paper II, the BF20 sausages were rated with higher flavor intensity than the BF3, whereas no differences were found between BF20 and SO3 sausages. In this case, the higher release of volatile compounds caused by the sunflower oil could increase the flavor intensity and ripened flavor in SO3 sausages (3% added sunflower oil) to a similar level than the BF20 sausages (20% added pork backfat). This result shows the importance of the type of fat used on the flavor attributes of fermented sausages. Nevertheless, no differences for ripened flavor and flavor intensity were observed between batches in Paper III, probably due to the higher pork fat content of both CT and reduced fat sausages that would make the differences between both attributes less noticeable. Likewise, the contribution of adding 1.5% and 3% sunflower oil to these two attributes would be less important.

Fat reduction increased the piquantness of the sausages in Papers I and II. In Paper I, the higher piquantness of the Lean batch in comparison with the BF batch could be related to the higher solubility of some pepper components in fat, which are released more slowly during chewing. In this regard, Wirth (1988) reported changes in the behavior of spices with fat reduction in dry-fermented sausages. No significant differences were observed between the Lean batch and the SO and DAG batches. Likewise, in Paper II, the SO batch was rated more piquant than the full fat BF20 batch, whereas BF3 did not show significant differences with BF20. Thus, a reduction of the amount of pepper in the formulation of fermented sausages should be taken into account due to the increase of piquantness when standard pork backfat content is replaced by lower levels of sunflower oil or DAGs.

No significant differences for saltiness were observed in the three Papers. When fat is replaced by lean meat, the salty flavor associated to NaCl is more noticeable because there is higher water to salt ratio (Wirth, 1988). Therefore, it is necessary to reduce also the amount of salt in order to have a similar salty flavor than the standard fat content. In Paper III, reduced fat and salt sausages (16–22% salt reduction or substitution by KCl) obtained similar scores for saltiness than CT sausages with higher salt but also higher fat level. Regarding these results for Paper III, it could be expected that in Papers I and II, with the same salt content in all batches, the salty flavor would be higher in those batches with lower fat content. The explanation for the similar saltiness observed in Paper I could be that the difference in the final fat content (after drying) among batches was lower (11.2% to 12.3% in added fat batches and 4.8% in the Lean batch) than in Paper III (33.2% in the CT batch, and 13.0% to 18.6% in reduced fat batches). In Paper II, the bitter flavor associated to KCl together with the K-lactate flavor could reduce the perception of saltiness, despite the higher differences in the fat content between the full fat BF20 (44.0%) and the reduced fat batches (19.5% and 19.6%).

All batches of Paper II included the same equimolar substitution of NaCl by 15% KCl. Similarly to saltiness, when fat level is reduced, the bitterness associated to KCl was more intensely perceived in reduced fat batches than in BF20. In Paper III, the reduced fat batches with 0.64% KCl were rated bitterer than the batches without KCl, as expected although the maximum score value was 1.4. Likewise, the maximum score value for bitterness in the reduced fat sausages of Paper II was 1.8., which represents a low value in terms of perceived bitterness.

It is shown that it is possible to obtain reduced fat fermented sausages with similar quality than fermented sausages with standard fat content, in terms of odor and flavor. Finally, the addition of 3% to 5% sunflower oil to reduced fat non-acid fermented sausages conferred desirable ripened odor and ripened flavor attributes.

5.1.4. Effect on instrumental and sensory texture

The water loss during the drying process affects the final texture (Klettner & Roedel, 1979), especially in non-acid fermented sausages where no binding due to pH drop occurs, as in the present studies. In this regard, the covariables water content on a defatted-dry-matter basis (X_{DFDM}) and water content on a defatted-desalted-dry-matter basis (X_{DFDSDM}) were significant in Papers I and III, as shown by the regression coefficients for hardness (both TPA and sensory). On the other hand, increases in TPA hardness and chewiness with fat reduction have been previously reported in reduced fat fermented sausages (García et al., 2002; Olivares et al., 2010; Salazar et al., 2009).

In Paper I, the Lean batch, with no added fat and the lowest X_{DFDM}, showed higher TPA and sensory hardness and also higher initial force (F_0; Stress Relaxation test, SR) than the batches with 5% added fat. Decreases of hardness with the addition of olive oil or sunflower oil have been previously reported in fermented sausages (Del Nobile et al., 2009). Both TPA and sensory chewiness decreased with the addition of 5% fat. Among batches with added fat, the sunflower oil batch (SO) showed the lowest TPA and sensory hardness and chewiness. The sunflower oil contains a higher monounsaturated (MUFA)+polyunsaturated (PUFA)/saturated (SFA) fatty acid proportion (ratio≈8.3; USDA, 2012b) than the pork backfat (ratio≈1.6; USDA, 2012a). The fatty acid composition of diacylglycerols (DAGs) has been reported to be similar to backfat (Cheong & Xu, 2011). In this regard, Beriain et al. (1997) reported higher TPA hardness and chewiness in pork fermented sausages in comparison with mutton fermented sausages as a result of the different fatty acid composition of both fats, expressed as MUFA+PUFA/SFA proportion.

Regarding the texture of the DAG batch, it showed lower TPA hardness and chewiness than the BF batch but was rated harder in the sensory evaluation. The explanation for this difference between instrumental and sensory tests may be due to the different melting points of both types of fat: backfat melts between +30 °C and+40 °C (Ospina-E et al., 2010; Suzuki et al., 2003) and DAGs at around +45 °C (Cheong et al., 2009). TPA test was performed at +15 °C, a temperature at which both backfat and DAGs are solid. However, the sensory analysis of texture in mouth was carried out at a temperature around +37 °C. At this temperature, backfat would melt more easily than DAGs, which would be more solid and perceived as harder by the panelists.

As in Paper I, an increase of TPA and sensory hardness and TPA chewiness with the fat reduction was also observed in Paper III. Likewise, the covariable X_{DFDSDM} had a significant effect on hardness. In this case, the fat reduction determined the increase of hardness and chewiness in the reduced fat batches more than the covariable X_{DFDSDM}, as the CT batch with the lowest hardness also showed a lower X_{DFDSDM} value than the reduced fat batches. In fact, the regression coefficients for TPA and sensory hardness in Paper III (ß= –205.6 and ß= –7.30, respectively) were lower than the coefficients for these parameters in Paper I (ß= –690.6 in TPA and ß= –11.26 in sensory analysis). These results could be due to the fact that sausages in Paper I included higher water and lower fat content. As in Paper I, the sunflower oil addition decreased these parameters to the same values than the CT batch without fat reduction. This supposes an improvement in reduced fat fermented sausages, obtaining similar texture than a product with a standard fat content.

The fermented sausages of Paper II were dried to a water loss level of 49%, instead of drying them to an established X_{DFDM} or X_{DFDSDM}. In addition, the fermented sausages of this study were analyzed at 1 and 3 months of storage at 3º C. The storage time and its interaction with the level and type of fat affected some instrumental texture parameters. The batch without fat reduction (BF20) showed higher TPA and sensory hardness, and higher F_0 than the reduced fat batches. This result can be attributed to their lower water content, which conferred them higher hardness. According to several studies (Ruiz-Ramírez, Arnau, Serra, & Gou, 2005a; Serra, Ruiz-Ramírez, Arnau & Gou, 2005) lower water contents increase hardness in dry-cured meat products. In contrast, TPA hardness and SR initial force (F_0) decreased in BF20 at 3 months, whereas no significant differences for these parameters were observed in reduced fat batches. The reduced fat batch with 3% sunflower oil (SO3) showed the lowest sensory hardness, similarly to the batches including 5% sunflower oil in Paper I. Regarding TPA chewiness, BF20 showed higher values than reduced fat sausages. This parameter decreased in the BF20 batch after 3 months of storage, whereas no changes were observed in the reduced fat batches. SO3 obtained the lowest score for sensory chewiness, whereas no significant differences were observed between BF20 and BF3.The sunflower oil added as liquid in SO3 sausages and the exudated fat in BF20 sausages (both melting at mouth temperature) would reduce the binding between fat and meat particles and change the distribution of fat in the product. In this sense, Bloukas et al. (1997) reported oil exudation in reduced fat dry-fermented sausages with olive oil as fat substitute, which could indicate lower binding with meat particles than pork fat.

Regarding TPA cohesiveness, in Papers II and III, this parameter increased with the fat reduction. In both cases, the batches with a standard fat content, i. e., BF20 and CT, showed lower cohesiveness values than the reduced fat batches. In Paper I, the differences in the fat level between batches with 5 % fat and the Lean batch were lower than the differences between the standard fat and the reduced fat batches of the papers II and III. Therefore, no increase of cohesiveness with the decreasing fat level was observed in Paper I. In Papers I and II, the batches with sunflower oil showed higher cohesiveness than the batches with the same content of backfat and DAGs (DAGs only in Paper I). Higher TPA cohesiveness has been reported by Muguerza et al. (2001) in *chorizo de Pamplona* in which 25 % and 30% of the total pork backfat was substituted by olive oil. On the contrary, in Paper III, sausages with sunflower oil did not show higher cohesiveness than the other reduced fat sausages. This result was probably due to the higher pork fat content in the reduced fat sausages of Paper III in comparison with the reduced fat sausages of papers I and II, which reduced the effect of the addition of 1.5% and 3% sunflower oil on cohesiveness.

Several authors have reported different results concerning the effect of the fat level on TPA springiness in fermented sausages. Mendoza et al. (2001) obtained a decrease of this parameter in dry-fermented sausages with 6.3 % fat in comparison with sausages with 12.5% and 25% fat. This result agrees with the lower springiness of the Lean batch (no added fat) in comparison with the batches with 5% fat in Paper I. In Paper II, a decrease of springiness with the fat reduction was observed in batches with backfat (BF20 and BF3), whereas the batch with 3% sunflower oil (SO3) showed the highest value for this parameter, despite of its lower fat content compared to BF20. However, no significant differences depending on the fat level were observed in Paper III, which agrees with the reported by Olivares et al. (2010) in fermented sausages with different pork backfat contents (10%, 20% and 30%). In the three papers, springiness increased with the addition of sunflower oil. Differences for this parameter were not significant in Paper I because differences in the fat content among batches were lower than in the other two studies. In Papers II and III, reduced fat sausages with sunflower oil showed significant higher springiness than reduced fat sausages with pork fat, and obtained similar values than sausages with a standard higher fat content (Paper III), or even higher (Paper II). Therefore in general terms, the differences observed for springiness depended more on the type of fat than on the fat level. In addition, in Paper II, springiness showed a significant increase at 3 months of MAP storage time. Similar results were reported by Rubio et al. (2007), who found that springiness increased during 150 days of MAP storage time (20% CO_2 / 80% N_2) in *salchichón*.

TPA springiness and cohesiveness are instrumental texture parameters which have been related to attributes used in sensory analysis, i.e., elasticity and crumbliness, respectively (Szczesniak & Kleyn, 1963). Therefore, it could be expected that higher TPA springiness and cohesiveness values result in higher sensory elasticity and lower crumbliness, respectively. Nevertheless, the opposite results were observed in Papers I and III, as batches with the highest sensory elasticity showed the lowest TPA springiness and vice versa. In Paper II, the reduced fat BF3 and SO3 sausages obtained significantly higher elasticity scores than the BF20 sausages in the sensory analysis. On the contrary, BF20 showed higher springiness than BF3 in the TPA test. Furthermore, the batches with sunflower oil showed the highest sensory crumbliness but the highest (or not statistically different from other batches) TPA cohesiveness in the three papers. Thus, the results observed for elasticity and crumbliness, suggested that sunflower oil would cover the meat particles reducing the binding between meat proteins, and thus leading to a less elastic and more crumbly texture in mouth, resembling a standard fermented sausage without fat reduction.

According to Szczesniak, (2002), excellent to good correlations between instrumental and sensory ratings have been reported for hardness in many studies using the Instron texture analyzer, whereas correlations for other parameters have been found less good and product-dependent. TPA springiness can be measured in different ways, but most typically, by the distance of the detected height of the product on the second compression divided by the original compression distance (Texture Technologies, n.d.). Sensory elasticity is a characteristic derived from springiness, which was defined as the 'degree to which a product returns to its original shape once it has been compressed between molars' (Civille & Szczesniak, 1973). Cohesiveness is measured as the area of work during the second compression divided by the area of work during the first compression (Bourne, 1978), whereas crumbliness is a sensory attribute derived from cohesiveness. Amerine et al. (1965) defined crumbliness as the 'ease with which a substance can be separated into smaller particles'. Moreover, there are no single and specific receptors for texture in the mouth because of its multiparametric nature, and some textural parameters are perceived when the food is deformed on chewing with the teeth, manipulated with the tongue and mixed with saliva (Szczesniak, 2002). According to these differences between TPA parameters and sensory attributes, the results obtained from both analyses do not necessarily agree in all cases and the same product can be rated differently in the sensory analysis with respect to the instrumental tests.

Regarding the tactile texture in the sensory analysis, the easiness of removal of the casing from the sausage surface (ease to peel) was significantly affected by the fat type and level. In Paper I, the batches with sunflower oil showed the highest score for this attribute. In Paper III, the sunflower oil batches also showed a tendency to increase the easiness of removal of the casing from the sausage surface. This higher ease to peel score could come from the oil exudation (Bloukas et al., 1997) which reduced the protein-casing interaction throughout the drying process. Similarly, in Paper II, SO3 sausages showed higher ease to peel than BF3 sausages, although BF20 batch showed higher ease to peel than the reduced fat batches. This fact could be related to the highest difference of fat content between the control BF20 batch and the reduced fat batches. Even though the SO3 batch included 3% sunflower oil, the oil exudation would be lower than the backfat exudation in BF20 that could cause higher casing separation.

The sensory analysis in Papers I and II included the attribute 'overall sensory quality' in order to evaluate the general acceptability of the panelists towards the different batches of fermented sausages. Nevertheless, it should be taken into account that the hedonic scores given by a trained sensory panel do not necessarily represent the consumers' opinion.

In Paper III, this attribute was not included in the sensory analysis because a consumer acceptability study was carried out. That study will be discussed in a subsequent section. In Paper I, fermented sausages including 5% sunflower oil obtained higher overall sensory quality than fermented sausages with 5% backfat or DAGs, and Lean sausages with no added fat. In Paper II, no significant differences were observed among batches. In this study, sausages including 3% backfat or sunflower oil showed similar scores than sausages with a standard fat content of 20%. It was demonstrated, therefore, that it is possible to obtain reduced fat non-acid fermented sausages with the same sensory quality that a standard fat content product. In addition, sunflower oil can be regarded as a promising pork fat substitute for fermented sausages from sensory, instrumental and technological points of view.

5.2. Effect of the salt reduction and substitution on the physicochemical, instrumental and sensory characteristics of reduced fat non-acid fermented sausages

A reduction of the standard NaCl content of small caliber non-acid fermented sausages was applied to the batches of Papers II and III. KCl and K-lactate were used as NaCl substitutes in Paper II, whereas only KCl was used in Paper III.

In Paper II, all batches included 1.4% NaCl in their formulation, which represents a 30–35% NaCl reduction with respect to a standard fermented sausage with 2.0–2.2% NaCl. The remaining NaCl percentage until a standard content of 2.2% was replaced by a mixture of 0.42% KCl and 1.33% K-lactate, which represents equimolar substitutions of 15% and 21%, respectively. Regarding the reduction of sodium in the final product, the 1.40% NaCl increased to 2.54% at the end of the drying process. This NaCl content represents over 30% reduction with respect to the 3.68% average NaCl content in *fuet* (Zurera-Cosano et al., 2011).

In Paper III, a control batch with 2.5% NaCl (CT) was manufactured. All batches included 0.015% $NaNO_2$ in their formulation. The reduced NaCl batches contained 1.5% NaCl (40% reduction) with or without 0.64% KCl (equivalent to 0.5% NaCl equimolar substitution). After drying, the sodium content increased up to 1.13% - 1.21% sodium in the final product. This sodium content represents a reduction between 16% and 22% with respect to the 1.45% average sodium content in *fuet* (Zurera-Cosano et al., 2011), and therefore the ‘reduced sodium’ claim could not be applicable.

As all batches in Paper II included the same NaCl, KCl and K-lactate level, the effects of the salt reduction and its substitution in comparison with a product with standard NaCl content were not studied. However, the saltiness and the bitterness conferred by KCl were evaluated in the sensory analysis. Thus, the results for these sensory attributes can be compared with those obtained in Paper III and will be discussed in the section 4.2.3. The discussion of the effect of the NaCl reduction and the addition of 0.64% KCl on the physicochemical parameters (section 4.2.1.), instrumental color (section 4.2.2.) and texture (section 4.2.4.) refers to Paper III.

The effect of the NaCl reduction on the sausages of Paper III was analyzed together with the fat reduction with the objective of studying the effect of both reductions in their formulation. Likewise, to discuss the addition of 0.64% KCl as NaCl substitute, the simultaneous effect of the fat reduction must be taken into account.

5.2.1. Effect of salt reduction and KCl addition on physicochemical characteristics

The simultaneous reduction of NaCl and fat resulted in a significant increase of the weight loss, moisture content and a_w in the small caliber non-acid fermented sausages of Paper III (L *vs.* CT batch). Nevertheless, the addition of 0.64% KCl to the reduced fat batches decreased a_w to values closer to those of the CT batch. In this sense, Comaposada, et al. (2007) found that in minced meat products and dry-cured ham, NaCl can be substituted by KCl at levels of 30% and 35% molar respectively, without important effects on isotherms and on drying kinetics of the product. Likewise, a decrease of weight loss with the addition of 0.64% KCl was observed. Terrell, Ming, Jacobs, Smith, & Carpenter (1981) found that the replacement of added NaCl with any chloride salts (with an equivalent ionic strength), except $CaCl_2$, significantly decreased moisture loss in raw and cooked processed meats. Similarly, Ibañez, Quintanilla, Gil, Astiasarán, & Bello (1996) found that lower drying over the same time occurred in dry-fermented sausages with 1.5% added NaCl and 1% KCl compared to those with 3% of added NaCl.

5.2.2. Effect of salt reduction and KCl addition on instrumental color

The simultaneous reduction of NaCl and fat resulted in a significant decrease of lightness (L*) and an increase of redness (a*) (L *vs.* CT batch). In general terms, the addition of 0.64% KCl or sunflower oil (1.5% and 3%) to S batch did not significantly modify the instrumental color parameters. However, the L-KCl batch with NaCl reduction and 0.64% KCl showed higher L* values than the L batch with only NaCl reduction. Gimeno, Astiasarán, & Bello (1999) reported higher lightness in dry-fermented sausages with a mixture of added NaCl (1%), KCl (0.55%) and $CaCl_2$ (0.74%) compared to those containing only added NaCl (2.6%), which agrees with our results in this case. Likewise, the yellowness of S-KCl batches tended to increase with the addition of sunflower oil, being not significantly different to the yellowness of control batch when 3% of sunflower oil was added.

5.2.3. Effect of salt reduction and KCl addition on flavor

The sensory differences for saltiness and bitterness associated to KCl in small caliber non-acid fermented sausages have been discussed in section 4.1.3. In the three Papers, the differences and similarities among batches observed for these attributes were related to differences in the fat content.

Gimeno, Astiasarán, & Bello (1998) reduced the salt content in dry-fermented sausages from 2.6% to 1.0% using a mixture of 1.0% NaCl, 0.55% KCl, 0.23% $MgCl_2$, and 0.46% $CaCl_2$. The reduction in the total sodium content of the sausages was from 1.88% to 0.91% (48% sodium reduction). These authors reported reductions in the salty taste of dry fermented sausages at that level of salt reduction. Nevertheless, the NaCl and sodium reduction was lower in our case. For this reason, no significant differences for saltiness were observed among batches in our studies. In Paper II, the scores for saltiness obtained in the sensory analysis were in the range of 2.7–2.8. In Paper III, these scores ranged from 2.6 to 3.0. Thus, the saltiness of small caliber non-acid fermented sausages with 30% salt reduction (Paper II) was similar to the saltiness in sausages with 16–22% salt reduction (Paper III).

Scores for bitterness in Paper II depended on the content and type of fat because all batches included the same equimolar substitution of NaCl by KCl, as explained in section 4.1.3. Therefore, the simultaneous effect of the NaCl and fat reduction and the effect of the addition of 0.64% KCl to the reduced fat and NaCl sausages is only discussed for Paper III. Regarding the sensory analysis in this paper, only the reduced NaCl batches with added KCl and sunflower oil were significantly bitterer than the batches without KCl. Nevertheless, the addition of 0.64% KCl tended to increase bitterness also in batches without sunflower oil, as expected, even though this increase was not significant.

When KCl is used in a high percentage (> 40% of NaCl substitution) a decrease in aroma and taste has been reported in fermented sausages (Campagnol, dos Santos, Wagner, Terra, & Pollonio, 2011; Guàrdia, et al., 2008). In our case, no significant differences for the rest of flavor attributes were observed among batches, as the substitution of NaCl by KCl was lower than 40% (16–22%).

In conclusion, acceptable saltiness and low bitterness were obtained in reduced fat small caliber non-acid fermented sausages with 1.4% added NaCl and 0.36% NaCl equimolar substitution by a mixture of KCl and K-lactate (salt reduction of 30% with respect to a standard *fuet* type sausage with 3.68% NaCl). Likewise, similar results were observed in reduced fat small caliber non-acid fermented sausages with 1.5% added NaCl with or without 0.5% equimolar substitution by KCl (Na reduction of 16–22% with respect to a standard *fuet* type sausage with 1.45% Na content).

5.2.4. Effect of salt reduction and KCl addition on instrumental and sensory texture

Instrumental hardness, chewiness and cohesiveness increased with the simultaneous reduction of NaCl and fat (L, S batches in comparison with the CT batch). According to Ruusunen & Puolanne (2005) salt favors gel formation in fermented sausages and leads to the desirable texture. In salami-type fermented sausage a decrease of NaCl from 2.5% to 2.25% resulted in less firm sausages (Petäjä, Kukkonen, & Puolanne, 1985). In Paper III, this effect was not appreciable most likely because the effect of the fat reduction had a higher impact on the texture properties than the salt reduction effect.

Concerning sensory texture, the simultaneous reduction of NaCl and fat increased hardness and elasticity, whereas decreased crumbliness and fat mouthfeel (CT *vs.* L batches). Gelabert et al. (2003) and Guàrdia et al. (2008) reported similar results for sensory hardness in small caliber fermented sausages with 40% and 50% NaCl substitution by KCl, respectively, whereas no significant differences were reported for other sensory texture attributes. However, these studies did not include any fat reduction in the formulation of the sausages. Thus, the results observed for sensory texture in Paper III were more dependent on the fat reduction and addition of sunflower oil than on the NaCl reduction.

The addition of 0.64% KCl barely modified the instrumental and sensory texture parameters of the sausages by. There was only a significant increase of TPA chewiness in the leanest batch (S *vs.* S-KCl batch). KCl has been reported to increase hardness and cohesiveness in cured meat products (Vignolo, Pesce de Ruiz, & Oliver, 1988). However, the addition of 0.64% may have not been high enough to produce a significant increase in our study.

5.3. Consumer acceptability of reduced fat and salt non-acid fermented sausages

The acceptability study carried out in Paper III included eighty-four Spanish consumers representing different socio-demographic levels. The consumer study was carried out at home (home-use test; HUT) and specific and simple indications were provided to consumers. This test, as the name implies, requires that be conducted in the participants' own homes. First, participants were pre-recruited and screened for their eligibility to participate in the test. The major advantage of the HUT is that products are tested under actual home-use conditions (Resurrección, 1998) and the main disadvantages were related with the time needed for samples distribution and collect the participant's responses. In addition, little can be done to exert any control over the testing conditions once the product is placed in the home of the respondent (Resurrección, 1998).

In the present work, acceptability was only referred to as a hedonic evaluation of the *fuets* because consumers had no additional information about the reduced fat and salt content of the products, and neither about the addition of sunflower oil as fat substitute. Bølling et al. (2010) showed that nutritional information affects the consumers' acceptance. Therefore, the acceptability may change if consumers were informed about the fat and salt levels of the different batches (Shepherd, Sparks, Bellier & Raats, 1991).

No significant effect of the socio-demographic variables (age, gender, place of residence and education level) on the consumer acceptability was detected (results not shown). On the contrary, the simultaneous reduction of fat (from 33.22% to 15.09%) and salt (from 2.5% to 1.5%) led to a significant increase on the consumer acceptability (CT vs. S batch). Olivares et al. (2010) also reported higher consumer acceptability in fermented sausages with 16.5% of fat content in raw mixture (this content was half of the usual fat content in fermented sausages) when compared with those with higher fat content (19.3%).

The addition of 0.64% KCl did not result in significant differences on consumer acceptability, likely because this amount of KCl was not enough to be detected by consumers. This amount was lower than 1.4% used in the study of Guàrdia et al. (2008) who achieved a reduction of 50% of the NaCl in fermented sausages by molar substitution with KCl (1.4%) without changing the acceptability of the product. In addition, KCl decreased a_w conferring a positive effect on food safety. This result suggests that it could be possible to reduce the NaCl content and increase the amount of KCl without decreasing consumer acceptability and improving food safety in reduced fat *fuet* type sausages.

Regarding sunflower oil addition, in overall, there was a slight tendency to decrease acceptability as sunflower oil addition increased (S-KCl *vs.* S-KCl-1.5SO and S-KCl-3SO). According to the sensory evaluation carried out by trained panelists, some sensory attributes of batches with sunflower oil were similar to the CT batch that showed lower consumer acceptability, i.e., oil flavor, hardness, elasticity, chewiness. Similarly to the panelists, consumers could notice these attributes that determined the tendency to decrease the acceptability of sausages with sunflower oil. In this regard, it would be interesting to perform a future study in order to determine the maximum level of sunflower oil addition at which the positive sensory attributes observed could be kept without affecting consumer acceptability.

6 CONCLUSIONS

- It is possible to obtain reduced fat non-acid fermented sausages containing less than 12.5% of final fat content with a satisfactory overall sensory quality.

- Reduced fat sausages with 5% pork diacylglycerols used as pork backfat substitute show similar properties to those of reduced fat sausages with 5% pork backfat, suggesting that they could be a good alternative to produce healthier reduced fat non-acid fermented sausages.

- By using 5% sunflower oil as pork backfat substitute in non-acid fermented sausages, desirable ripened odor and flavor attributes and improved texture, defined by lower hardness and chewiness and higher crumbliness, can be obtained.

- Reduced sodium small-caliber non-acid fermented sausages with reduced fat content (20%) and with acceptable sensory characteristics can be obtained by adding to the pork shoulder lean (8% fat content) during the grinding either 3.3% backfat (3% fat content) or 3% sunflower oil, both previously finely comminuted with lean.

- Sunflower oil is confirmed to be suitable for partial pork backfat substitution in very lean fermented sausages, conferring desirable sensory properties similar to those of sausages with standard fat content.

- The sensory quality of reduced sodium and fat small caliber non-acid fermented sausages is maintained after three-month storage in modified atmosphere at +3 °C.

- The addition of sunflower oil can improve the appearance and texture attributes by achieving values closer to those of the control batch but by modifying the oil flavor.

- The addition of 0.64% KCl to small-caliber non-acid fermented sausages with simultaneous reduction of fat and salt content decreases water activity and do not have negative effects on either sensory attributes or consumer acceptability.

- Regarding consumer acceptability, the simultaneous reduction of fat (from 20% to 10% and 7%) and salt (from 2.5% to 1.5%) content is acceptable to the consumers. The addition of sunflower oil shows a tendency to decrease acceptability.

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