Cooking effect on fatty acid profile of pork breakfast sausages enriched in conjugated linoleic acid by dietary supplementation or direct addition

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The effectiveness of increasing CLA in pork products through animal dietary supplementation or direct addition in the product formulation has been studied, and the effect of grilling on dry matter and fat contents and fatty acid composition has been analysed. Sausages made with meat and back fat from pigs with CLA dietary supplementation had the highest saturated fatty acid content. Sausages from dietary supplementation and direct addition had CLA levels between 6% and 7% of total fatty acids. Moisture and fat contents decreased and increased respectively after cooking for the three sausage types (control, dietary supplementation, direct addition). Grilling had little effect on fatty acid levels, especially for sausages with direct addition in the product formulation. In general, saturated fatty acids increased and poly-unsaturated fatty acids decreased due to the increase of C16:0 and to the decrease of C18:2 n–6 and C18:3 n–3 fatty acids. Added CLA, both from animal dietary supplementation or direct addition, remained at similar levels in cooked sausages to those found in raw sausages.

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1. Introduction

The relationship between diet, health and lifestyle is now a key focal point for consumers, researchers and policy makers alike as we witness an increase in obesity and the rise of diet-related chronic diseases (Swinburn, 2009). A key objective in the European Technology Platform on Food for Live focuses on ensuring that the healthy choice is an easy choice for consumers. The meat industry is addressing this demand by adopting strategies to produce meat products which have more beneficial ingredient profiles in a value-added manner. One such strategy includes the incorporation of functional lipids into existing meat products to provide a healthier version of an existing product.

Conjugated linoleic acid (CLA) is a collective term for a group of octadecenoic acids that are geometric and positional isomers of linoleic acid (C18:2) (Pariza, Park, & Cook, 2001). CLA has been shown to have a variety of biological effects (Hur, Park, & Joo, 2007). Several health benefits, such as anticancer, anti-oxidation, anti-atherosclerosis and improving immuno-responses (Belury, Nickel, Bird, & Wu, 1996; Lee, Kritchevsky, & Pariza, 1994; Miller, Stanton, & Devery, 2001; Pariza & Hargraves, 1985; Park et al., 1999) have been reported for CLA. These substances have been found in the meat and milk of ruminants, where they are mainly formed by biohydrogenation of grass derived fatty acids. Pork contains only small amounts of CLA because pig is a mono-gastric animal (Chin, Liu, Storkson, Pariza, & Ha, 1992).

Interest in dietary supplementation with CLA for pigs increased during the last decade due to its potential to improve productive, carcass and meat quality traits and, at the same time, for obtaining meat and meat products enriched in CLA (Marco et al., 2009; Martín, Antequera, Muriel, Andrés, & Ruiz, 2008a; Schmid, Collomb, Sieber, & Bee, 2006). A second approach for increasing CLA in meat products is its direct addition as an ingredient during the manufacturing process (Hah et al., 2006; Martín, Ruiz, Kivikari, & Puolanne, 2008b). In addition to the healthy benefits of CLA, its addition into products provides a strategy for partial replacement of saturated fatty acids in the diet by unsaturated fatty acids (Martín et al., 2008a).

While strategies can be enacted to improve the ingredient profile of foodstuffs the cooking method can have an impact on the levels of the beneficial ingredient in the product which is ready to consume. Some studies focused on the influence of processing and cooking on CLA content in meat products that naturally contain CLA such as beef (Ma, Wierzbicki, Field, & Clandinin, 1999; Shantha, Crum, & Decker, 1994) or lamb meat (Badiani et al., 2004). However, little work has been presented which assesses the impact of cooking on the fatty acid profiles of CLA supplemented meat products.

The aim of the present study was to study the effects of grilling on the chemical and fatty acid composition of pork products enriched in CLA through animal dietary supplementation or through direct addition in the product formulation. In addition this study...
aims to compare the useful of CLA dietary supplementation or direct addition into breakfast sausages.

2. Material and methods

2.1. Treatments

Sausages were manufactured according to three different protocols (control, dietary supplementation and direct addition in the formulation) using Boston Butt (Musculus infraspinatus, M. supraspinatus, M. subscapularis and M. serratus ventralis) and back fat removed from the pigs 24 h after slaughter. The CLA supplement used for both, diet and formulation supplementation, was Luta-CLA 60 (BASF, Germany), which consists of 56% (w/w) of the two main CLA isomers (t9,c11 and c10,t12) dissolved in a base of limoleic acid.

Dietary supplementation with CLA was carried as reported in Marco et al. (2009). Ten female pigs of approximately 40 kg live weight were selected and their diet was supplemented with 2.0% of CLA (3.57% of total oil added per tonne of feeding). Pigs were fed ad libitum from hopper feeders. The feeding trial lasted for 8 weeks, after which the animals were slaughtered having reached live weights of approximately 95 kg.

Pork breakfast sausages with CLA added in the formulation were prepared with meat and back fat from pigs with non-CLA enriched diets and live weights of approximately 95 kg, where 2% Luta-CLA (1.12% of CLA), substituting the same weight of back fat, was added to the mixture during the manufacturing process.

As a control, another treatment was prepared using meat and back fat from pigs fed with a non-CLA enriched diet and with no added CLA.

2.2. Sausage manufacture

Sausages were manufactured with the following formulation (w/w), 44.2% of lean meat, 18.7% of back fat, 2.5% seasoning, 7.0% rusk and 27.5% water. Prior to manufacture, back fat was chopped whilst frozen for 1 min at chopping speed 2 and bowl speed 2 (2, 2) using a bowl chopper (Fatosa C-35-2Z, Fatosa S.A., Sabadell, Spain) and then refrigerated. Diced lean meat, seasoning, overnight-hydrated rusk and 1/5 of the ice water were introduced into the bowl chopper and blended at speed (1, 1) for 20 s. The chopped fat was then added to the bowl along with the remaining ice water. All ingredients were then chopped for 2 min at speed (1, 1) and the mix was stuffed into collagen casings of 16 to the lb. The process was made by triplicate with meat and back fat from each animal (batch). For each batch, the sausages were then vacuum packed in bags containing six sausages, and stored at −20 °C.

Prior to cooking, raw samples were taken from all the treatments for subsequent analysis. Sausages were grilled for 30 min at 200 °C using a domestic oven grill (B-AH51-7 SIEMENS-Electrogerate, GmbH Germany), minced (R301 Ultra Robot Coupe, Robot Coupe UK Ltd., Middlesex, UK), vacuum packed and frozen for subsequent analysis. All results are expressed as the mean of six replicates of each treatment.

2.3. Analysis of fat and dry matter contents

Fat and dry matter contents were analysed with Smart Trac (CEM SMART Trac™ Fat and Moisture Analyzer, CEM Corporation, Matthews, USA), using a combination of microwave drying technology and Nuclear Magnetic Resonance (NMR). Minced samples (two replicates of 3 g) were dried in the microwave to calculate their dry matter content using the difference of weight before and after drying. Dried samples were placed in the NMR analyzer to calculate their fat content.

2.4. Fatty acid analysis

The total fatty acids were extracted, methylated and analysed with an adaptation of the method described by Aldai, Osoro, Bar- ron, and Nájera (2006), which has been reported to be highly effective for poly-unsaturated analysis (Juárez et al., 2008). Separation and quantification of the fatty acid methyl esters was carried out using a gas chromatograph (GC, Varian Star 3400CX, Varian Associates Inc., California, USA) equipped with a flame ionisation detector automatic sample injector, and using a BPX-70 capillary column (120 m, 0.25 mm i.d., 0.2 μm film thickness, SGE, Australia). Tricosanoic acid methyl ester (C23:0 ME) at 10 mg/ml was used as an internal standard. Individual fatty acid methyl esters were identified by comparing their retention times with those of authenticated standards from Sigma (Sigma Chemical Co. Ltd., Poole, UK). Fatty acids were expressed as a percentage of total fatty acids identified and grouped as follows: saturated (SFA), mono-unsaturated (MUFA) and poly-unsaturated (PUFA) fatty acids. PUFA/SFA ratio and total CLA and 9-desaturase activities were calculated.

2.5. Statistical analysis

Statistical analyses were performed using Statistica 7.0 for Windows (StatSoft Inc., 2006). The effects of the different treatments (control, CLA dietary supplementation and CLA addition in the formulation) and cooking process as well as the interaction between them were studied using analysis of variance (multifactor ANOVA).

3. Results and discussion

In general, heat is applied to meat products in different ways to improve its hygienic quality by inactivation of pathogenic microorganisms to enhance its flavour and taste, and increase shelf life (Bogmår, 1998; Polkorný’, 1999). During cooking, physicochemical reactions modify the food nutritional value. Cooking induces water loss in the food, increasing its lipid content, while only some fat is lost (García-Arias, Álvarez Pontes, García-Linares, García-Fernández, & Sánchez-Muniz, 2003; Yearman & Homayouni, 2009).

In all the types of sausages, fat (P < 0.01) and dry matter (P < 0.001) contents increased after cooking with no interaction (P > 0.05) between cooking and treatment observed (Table 1). If expressed on dry matter basis, fat content of control, diet and formulation sausages decreased (P < 0.001) from 55.7%, 57.2% and 54.3% to 49.9%, 52.2% and 50.0% respectively, due to cooking losses. However, this was accompanied, by higher decreases in moisture content in all treatments following cooking, resulting in an apparent increase of fat content. This has been reported by other authors: for example Baggio and Bragagnolo (2006) in sausages, meat balls and hamburgers and by Dreeling, Allen, and Butler (2000) in beefburgers. Sheard, Wood, Nute, and Ball (1998) noted a similar effect in pork loin chops.

Fatty acid profiles of the sausages (Tables 2 and 3) were in line with those profiles reported elsewhere for pork products (Baggio & Bragagnolo, 2006; Lauridsen, Mu, & Henckel, 2005; Lo Fiego, Macchioni, Santoro, Pastorelli, & Corino, 2005; Pereira, Tarley, Matsushita, & Souza, 2000). The interaction between the studied factors (cooking and treatment) was significant (P < 0.05) for SFA and PUFA indices and for PUFA/SFA ratio (Table 2), as well as for several individual fatty acids (Table 3). Therefore cooking impacted in different ways depending on the type of sausage.

When the fatty acid profiles of the different types of sausages are compared, the levels of SFA of sausages from pigs with CLA dietary supplementation showed higher levels (P < 0.001) in comparison to those from the other two types. In this context, Dugan,
Table 1
Cooking effect on fat and dry matter contents (%) of control sausages and sausages with added CLA.

<table>
<thead>
<tr>
<th></th>
<th>Control Diet Formulation</th>
<th>SEM</th>
<th>Cooking Treatment xTreatment</th>
</tr>
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<tbody>
<tr>
<td>Fat</td>
<td>Raw Cooked</td>
<td>Raw Cooked</td>
<td>Raw Cooked</td>
</tr>
<tr>
<td></td>
<td>Raw</td>
<td>Cooked</td>
<td>Raw</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.038</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter</td>
<td>34.334c</td>
<td>39.956c</td>
<td>34.18d</td>
</tr>
<tr>
<td></td>
<td>ns</td>
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SEM: standard error mean. ns: p > 0.05; *: p < 0.01; ***: p < 0.001.

Aalhus, and Kramer (2004) observed how CLA dietary addition increased SFA in pig meat and fat, increasing marbling and fat firmness. The increase of SFA in this study was accompanied by a decrease of MUFA (p < 0.001) in sausages with dietary supplementation. Schöne et al. (2003) reported that same effect, particularly in the back fat of pigs fed with CLA. According to Schöne et al. (2003), the shift from the MUFA to the SFA could result from the diminution of the Δ9-desaturase activities by the CLA, as observed (Table 2) for sausages with CLA dietary addition (p < 0.001). No cooking effect (p > 0.05) was observed for Δ9-desaturase activities from any treatment. However, as expected, PUFA levels of the sausages with dietary supplementation and direct addition were much higher (p < 0.001) than those of the control sausages, as a result of the added CLA.

The PUFA/SFA ratio for all the treatments was over 0.45, the minimum recommended by the British Department of Health (1994), showing cooking had no negative effect on this parameter. The highest ratio (p < 0.001) was that from CLA direct addition. CLA dietary supplementation led to an increase of both PUFA and SFA levels as compared to meat and fat from pigs with regular diet. Nevertheless the final PUFA/SFA ratio was greater than that from the control sausages.

Sausages enriched with CLA (diet and formulation) had CLA levels between 6% and 7%, whereas CLA levels of control sausages were very low (0.1% of total fatty acids). Cooking had no impact on the percent CLA present in all products. As mentioned earlier pork may content low amounts CLA, depending on the diet, as reported by Dhiman, Olson, MacQueen, and Pariza (1999) and Raes, De Smet, and Demeeyer (2004) and reviewed by Chilliard, Ferlay, and Doreau (2001). From a nutritional point of view, the increase in PUFA and SFA levels would be positive. Therefore, the simplest way to increase CLA levels in breakfast sausages is the direct addition of the added CLA.
tion during the manufacturing process, as this method would be cheaper and does not result in increased SFA. It is important to note though that an increase in PUFA could lead to variations in the texture and flavour of sausages as seen in other similar emulsion type products; Turkish sausages (Yildiz-Turp & Serdar Argoğlu, 2008), and emulsion type sausages (Hah et al., 2006). Nevertheless, other authors have increased the n − 3/n − 6 ratio of sausages without significant variations in texture or sensory evaluation (Cáceres, García, & Selgas, 2008). Finally, CLA dietary supplementation has been shown to be able to get high CLA levels in sausages, while increasing PUFA and SFA and decreasing MUF A levels. Also PUFA/SFA level was greater than the minimum recommended.

Cooked control sausages had higher SFA (P < 0.05) and lower PUFA (P < 0.05) contents, as well as lower PUFA/SFA ratio (P < 0.05) compared to raw ones (Table 2). The increase of SFA was related to the increase (P < 0.001) of C16:0 (Table 3). The decrease of PUFA was due to the decrease of C18:2 n − 6c (P < 0.05) and C18:3 n − 3 (P < 0.001). In the literature reduced PUFA on cooking has been related to triglycerides unsaturated fatty acid drip losses (Cobos, Vega, & Díaz, 2008; Scheeder et al., 2001). While the overall MUF A levels were not affected by cooking (P > 0.05), the levels of C16:1 were higher (P < 0.05) and those of C17:1 lower (P < 0.05) in cooked than in raw control sausages.

One fatty acid from each type (SFA, MUFA and PUFA) was affected by cooking in sausages made from pigs with CLA dietary supplementation. An increase was observed for C14:0 (P < 0.05) and C18:1 n − 9c (P < 0.001), while C18:2 n − 6c levels decreased (P < 0.01) after cooking. Therefore, cooked sausages showed again higher levels of SFA and MUFA (P < 0.05) than raw ones. Increases in PUF A after cooking have been observed in other studies. Maranesi et al. (2005) observed an increase in PUFA in lamb rib-loins after broiling and microwaving followed by final grilling. Some authors (Gerber, Scheeder, & Wenk, 2008; Igene, Pearson, & Gray, 1981; Rodríguez-Estrada, Penazzi, Caboni, Bertacco, & Lercker, 1997) have found an increase in PUFA levels of meat and meat products after cooking due to the lipid losses, containing mainly triacylglycerols of adipose tissues with relatively more SFA than PUFA, as suggested by Ramamurti (1986). However, in those studies, PUFA levels were much lower (1–6%) than those found in the sausages of the present study (21–29%). It is interesting to note that in a recent study, Sarriès, Murray, Moloney, Troy, and Beriaín (2009) found no changes in the relative distribution of fatty acids upon cooking (140 ºC for 30 min) in beef from animals with diets designed to enhance the concentration of CLA in tissue. Unlike the other two types of sausages, no cooking effect (P > 0.05) was observed between the levels of any measure of fatty acid content when CLA was added during manufacturing process. This suggests that the oil added during manufacturing might help stabilise the presence of fatty acids during cooking.

No significant differences were found in CLA levels (total CLA and CLA isomers) between raw and cooked sausages within each treatment (P > 0.05). Maranesi et al. (2005), also reported no differences in total CLA levels before and after microwaving and broiling lean meat from rib loins. Therefore, CLA addition, either by dietary supplementation or direct addition in the formulation, resulted in similar levels to that added to the raw sausages after grilling.

4. Conclusions

The results from this study show that pork products can be modified to provide a significant increase in a functional lipid, which can have positive influences on health. Moreover, grilling had no clear effect on fatty acid levels in sausages with CLA added during manufacturing process. Grilling has a small but significant effect in sausages made with meat and back fat from pigs with a dietary CLA addition. Total CLA levels were not affected by grilling. Therefore, since CLA levels have been found to be stable during cooking, texture and sensory properties of both types of sausages should be evaluated in future studies to clarify the optimal process to increase CLA levels in pork breakfast sausages.

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Igene, J. O., Pearson, A. M., & Gray, J. I. (1981). Effects of length of frozen storage, drip losses (Cobos, Veiga, & Díaz, 2008; Scheeder et al., 2001). Nevertheless, other authors have increased the

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