Abstract

Introduction and objectives: the aim of this study was to assess the fatty acid profile of two cured meat products of similar manufacturing processes and characteristics, dry-cured ham (JA) and cecina (CE), a type of dry-cured beef. The obtained results were discussed in terms of the effects that each singular fatty acid, when consumed, could have on human health.

Materials and methods: for this purpose, 10 samples of 100 g of JA and CE were obtained in local food stores in León, Spain. Lipids were extracted and transesterified, then a gas chromatography-mass was used to analyze the samples.

Results and discussion: results for fatty acid profiles for JA and CE showed significant differences (p < 0.01), with these values for main lipids fractions, saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA), respectively: 42.86%, 43.27% and 13.87 for JA and 46.87%, 46.96% and 6.20% for CE. SFA and MUFA percentages were slightly higher in CE at the expense of PUFA, specifically in the n-6 series, where values of 11.06% in JA and 3.91% in CE were obtained. In both products, the most prevalent fatty acid was an unsaturated fatty acid, oleic acid, with percentages of 37.28% in JA and 38.48% in CE. Other fatty acids with higher percentages, with respect to total fat, were two saturated fatty acids: palmitic acid, 20.63% in JA and 22.95% in CE, and stearic acid, 18.65% in JA and 17.14% in CE.

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Key words: Fatty acids. Meat products. Health.
Introduction

The study of the composition of dietary fats is currently a topic of great interest, especially because of the effects that fat consumption has on health. This interest is not only because of the classic relationship between the type of fat and disease risk; it is also due to the potential benefit of replacing some fatty acids in dietary fats with others. It is scientifically accepted that the relationship between health and fat intake depends more on the quality of the fats than the amount of ingested fat. This is due to the biological effect that the predominant type of fatty acid in the diet has on organic systems and their functions. For instance, experimental studies have shown a protective role of Omega-3 (n-3) in cardiovascular health: it has an anti-inflammatory, antithrombotic and anti-arrhythmic effect. Furthermore, the health benefits of CLA (Conjugated Linoleic Acid), including weight management, anti-cancer, possible therapeutic effects to insulin resistance, anti-atherosclerosis and immune system modulation have been shown.

The terms “fats” or “lipids”, often used as synonymous, define a chemically diverse group of natural molecules which have in common that they are soluble in alcohol and ether but insoluble in water. They include fats themselves, such as waxes, sterols, fat-soluble vitamins, phospholipids and others. From a qualitative and quantitative point of view, triglycerides are the most important component of the lipid fraction of foods. Triglycerides are esters derived from glycerol and three fatty acids. Further, fatty acids are part of complex lipids and can be esterified with cholesterol.

Fatty acids are molecules of great biological interest because of their digestive, metabolic and structural essential functions. A fatty acid (FA) is a carboxylic acid with an aliphatic tail which, in fatty acids of biological interest, has an even number of carbon atoms. They present a great variability in the length, the degree of unsaturation, and the isomeric configuration of the aliphatic chain, which have an important effect on the biological functions of the fatty acids. Therefore, as it has been said before, the biological effect of the dietary fats will vary depending on the type of fatty acid that is most prevalent in regularly ingested foods.

Spain is a country with a long tradition of manufacturing and consuming a large variety of meat products. Cured-ripened meats are a type of meat product which are dry-salted and air-dried until they achieve their organoleptic and stability characteristics. Within this group, there are meat products which are made of an anatomically identifiable piece of meat, mainly dry-cured ham and cecina. Spanish dry-cured ham (JA) is a typical product made of pork legs, whose manufacturing process involves a salting and drying process and it is also sometimes smoked. Cecina (CE) is a salted, smoked and dried beef product, typical of western Spain, whose manufacturing process is very similar to that used in the elaboration of dry-cured ham. Both JA and CE are part of the eating habits of the Spanish population because of their special organoleptic properties and convenience, and because they belong to their food heritage, among other reasons. Fatty acid profiles of these meat products could be of interest as they also provide a part of the total amount of dietary fatty acids.

Objectives

In view of this context, the fatty acid profiles of JA and CE, obtained during a microbiological study about the effects that JA and CE fats could have on the growth of some foodborne pathogens, could be relevant to human health studies. The aim of this study was to assess the fat composition and possible differences between fatty acid profiles of two meat products of similar manufacturing process and characteristics, dry-cured ham and cecina, which are often consumed in some regions of Spain, and relating these fatty acid profiles with scientifically established effects that fat consumption can have on human health.

Materials and Methods

Samples of dry-cured ham, from white pigs, and cecina, from cow, were purchased for fat extracting and fatty acid analyses. For this purpose, ten samples of 100 g for each meat product, JA and CE, were acquired from different local food stores in the province of León. They were carried to the laboratory using common bags where they were stored at 3°C until being analyzed.

JA and CE lipids were extracted by the De Jong and Badings Method (1990) using diethyl ether and hexane. The lipids extracts were stored at –30°C until further analysis. Fatty acid methyl esters (FAME) were prepared by transesterification in situ according to the Carrapizo, et al. Method (2000). The FAME were analyzed on a Hewlett-Packard chromatograph (Model 6890, Hewlett-Packard, Wilmington, DE) equipped with an automatic injector (Model 7683, Hewlett-Packard) and a mass selective detector (Model 5973, Hewlett-Packard). Helium was used as a carrier gas at a flow rate of 1 mL/min. Samples (1 μL) were injected by split injection (split ratio 10:1). Undecanoic acid (C11:0) was added as an internal standard. The FAMEs were separated using a Teknokroma TR-CN100 capillary GC column (60 m × 0.25 mm i.d. × 0.20 μm film thickness; Teknokroma Analítica S.A, Barcelona, Spain). The injection and detector temperatures were 230°C. The temperature program was as follows: the initial temperature was maintained at 50°C for 1 min after injection, then programmed to increase by 15°C/min to 200°C, maintained there for 3 min, and then programmed to increase by 2°C/min to 220°C, and it was maintained for 5 min. Identification of FAME was supported from the retention times by using standards.
of methyl esters (Supelco 37 component FAME mix and commercial preparation of cis-9,trans-11 CLA (c9t11) and trans-10,cis-12 CLA (t10c12) as the principal isomers of CLA, Supelco, Bellefonte, PA). The peak areas in the chromatogram were calculated and normalized using response factors. All results concerning the fatty acid composition are expressed as a percentage, FAME g/100 g of lipids. The repeatability of the method was between 85% to 99% for those FAME with a concentration of over 1%.

Data were analyzed using the statistical program SPSS for Windows (Version 21; SPSS INC., Chicago, IL, USA). Measures of central tendency and dispersion were calculated. The Kolmogorov-Smirnov test was used to test the normality of the sample distribution and Levene’s test was performed to assess the equality of variances. The Mann-Whitney U test for independent samples was applied for statistical determination of differences between fatty acids of dry-cured ham and cecina. Differences were considered significant at the level of p<0.01.

Results and discussion

With the chromatography technique used, 22 different fatty acids were found in analyzed fat extracts of JA and CE (Table I). FA values were expressed as a percentage of the total amount of fatty acids detected. In both meat products, all detected FAs, except for lauric acid, which has 12 carbon atoms and was present in a low amount, could be classified as a long chain and a very long chain FAs. Most of the fatty acids present were carboxylic acid with an even number of carbon atoms, as FAs of biological interest usually are. Only three types of FAs with an odd number of carbon atoms were found in low amounts: C15:0, pentadecylic acid; C17:0, margaric acid and C17:1.

Our results for fatty acid composition of analyzed JA samples showed approximate mean percentages of saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA), of about 43% of the total FA, and 13.87% of polyunsaturated fatty acid (PUFA) (Table I). Other authors13-15 reported similar, but not identical, values for dry-cured Serrano-type ham with commercial feed. In both meat products, all detected FAs, except for lauric acid, which has 12 carbon atoms and was present in a low amount, could be classified as a long chain and a very long chain FAs. Most of the fatty acids present were carboxylic acid with an even number of carbon atoms, as FAs of biological interest usually are. Only three types of FAs with an odd number of carbon atoms were found in low amounts: C15:0, pentadecylic acid; C17:0, margaric acid and C17:1.

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With respect to each singular FA detected in JA samples (Fig. 1), the most abundant FA was oleic acid, C18:1ω9, which is common in this type of product. In our results, it represented the 37.28% of the total FA of the JA. More abundant saturated fatty acids in our samples were palmitic acid (20.36%), followed by stearic acid (18.65%). Other surveys13-15 reported that these FA are

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Dry-cured ham</th>
<th>Cecina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric (C12:0)</td>
<td>0.17 ± 0.06</td>
<td>0.13 ± 0.06</td>
</tr>
<tr>
<td>Myristic (C14:0)</td>
<td>2.01 ± 0.25</td>
<td>3.74 ± 0.86</td>
</tr>
<tr>
<td>Myristoleic (C14:1)</td>
<td>0.11 ± 0.03</td>
<td>0.95 ± 0.29</td>
</tr>
<tr>
<td>Pentadecanoic (C15:0)</td>
<td>20.63 ± 0.71</td>
<td>22.95 ± 1.58</td>
</tr>
<tr>
<td>Palmitic (C16:0)</td>
<td>4.48 ± 0.71</td>
<td>7.18 ± 0.73</td>
</tr>
<tr>
<td>Margaric (C17:0)</td>
<td>0.83 ± 0.20</td>
<td>1.95 ± 0.31</td>
</tr>
<tr>
<td>Heptadecenoic (C17:1)</td>
<td>0.78 ± 0.24</td>
<td>ND</td>
</tr>
<tr>
<td>Stearic (C18:0)</td>
<td>18.65 ± 2.51</td>
<td>17.14 ± 2.36</td>
</tr>
<tr>
<td>Oleic (C18:1)</td>
<td>37.28 ± 2.20</td>
<td>38.48 ± 2.20</td>
</tr>
<tr>
<td>Linoleic (C18:2n6)</td>
<td>10.71 ± 1.34</td>
<td>3.74 ± 0.70</td>
</tr>
<tr>
<td>Arachidic (C20:0)</td>
<td>0.40 ± 0.15</td>
<td>0.23 ± 0.08</td>
</tr>
<tr>
<td>Linolenic (C18:3n3)</td>
<td>1.35 ± 0.69</td>
<td>0.83 ± 0.25</td>
</tr>
<tr>
<td>CLA</td>
<td>0.33 ± 0.19</td>
<td>1.02 ± 0.49</td>
</tr>
<tr>
<td>Gondoic (C20:1)</td>
<td>0.45 ± 0.24</td>
<td>0.33 ± 0.10</td>
</tr>
<tr>
<td>Eicosadienoic (C20:2)</td>
<td>0.68 ± 0.08</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td>Eicosapentanoic (C20:3n3)</td>
<td>0.14 ± 0.04</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td>Erucic (C22:1)</td>
<td>0.17 ± 0.08</td>
<td>0.14 ± 0.06</td>
</tr>
<tr>
<td>Docosahexanoic (C22:6n3)</td>
<td>0.06 ± 0.03</td>
<td>ND</td>
</tr>
</tbody>
</table>

Fatty acids groups

- SFA | 42.86 ± 2.84 | 46.87 ± 3.05 |
- MUFA | 43.27 ± 2.46 | 46.93 ± 2.37 |
- PUFA | 13.87 ± 1.21 | 6.20 ± 1.08 |
- MUFA+PUFA/SFA | 1.33 ± 0.16 | 1.13 ± 0.13 |
- PUFA/SFA | 0.32 ± 0.04 | 0.13 ± 0.03 |
- n-6 | 11.06 ± 1.37 | 3.91 ± 0.69 |
- n-3 | 1.79 ± 0.66 | 1.20 ± 0.29 |
- n-6/n-3 | 6.18 ± 6.03 | 3.26 ± 0.98 |

Data expressed as means in percentage over total detected and identified fatty acids ± standard deviation. * and different superscripts indicate statistically significant differences between fatty acids of dry-cured ham and cecina (p<0.01). ND: not detected.
the most abundant as well, but with other percentages, for instance 23.51% of C16:0 and 15.03% of C18:0 in data for the concentrate feeding system in the Bermúdez, et al. study. Within the PUFA, the main n-6 FA was linoleic acid, which represented 10.71% of the total FA. The most prevalent n-3 FA was linolenic acid with 1.35%.

The fatty acid composition of our CE samples’ fat extracts presented almost the same values for SFA and MUFA, about 46% of the total of FA. The percentage for PUFA was 6.20% and the Omega-6/Omega-3 ratio obtained for this product was 3.26. Few data were found about the fatty acid profile of cecina. One of the most extensive studies on this product was the academic dissertation of Molinero16, in which these percentages over the total amount of FA were reported (as means of three different meat pieces that can be made cecina): 44.96% of SFA, 50.03% of MUFA and 5.01% of PUFA. Values reported for the Omega-6/Omega-3 ratio by Molinero varied for different pieces of cecina as well, with a mean value of 5.46, in the middle of a range of 3.54 to 7.33.

With regard to single composition of FA in CE samples (Fig. 1), oleic acid represented the highest percentage of analyzed fat extracts: 46.56%. More numerous SFAs were palmitic acid, 27.51%, and stearic acid, 13.79%. With respect to PUFA, linoleic acid represented 3.74% of the FA and linolenic acid was 0.83%. In the same study mentioned before16, which also occurs in the JA results, their values are similar to ours, but not identical. Oleic acid represented the biggest difference found since the percentage for this FA was 46.53%. Other data were: 44.96% for SFA, 50.03% for MUFA and 5.01% for PUFA.

After describing the FA composition of these two cured meat products, JA and CE, the main objective of this paper was to compare FA composition of JA versus CE fats from the standpoint of the influence that their consumption could have on health, especially because of the significant roles that FAs have in causing and prevention of cardiovascular disease (CVD). Three main fatty acid fractions, SFA, MUFA and PUFA, presented significant differences (p<0.01) between JA and CE.

The MUFA fraction was slightly higher in CE fat than in JA, with four units of difference between them (Table I). Within the MUFA, oleic acid was the main FA of this fraction and of all fat in the JA and CE extract (p>0.01). It represented more than a third of the total of fat in both products. From an informative point of view, a fact wanted to be highlighted is that the CE has the same amount of oleic acid than the JA, in which this particular characteristic it is more known. Numerous studies have been done, and there are many reports about the effects that consumption of monounsaturated fatty acids has on health. These studies show that there is solid scientific evidence showing that MUFA appears to have a neutral effect on cholesterol levels or to be lightly hypocholesterolemic17. However, an additional aspect of interest that is firmly established is that, although they do not lower total cholesterol levels, since they decrease LDLC (Light Density Lipoprotein Cholesterol) and they increase HDLC (High Density Lipoprotein Cholesterol)18-19. It has been suggested, based on observational studies, controlled clinical trials and other studies, that high concentrations of circulation HDLC will help to prevent cardiovascular disease, CVD20. In the review about FA and CVD, Lecerf reported another health effect that MUFA could have as an element in decreasing the susceptibility of LDLC to oxidation and some antiatherogenic effects. Studies about the individual effect of oleic acid have been done as well17. They report that C18:1 are hypocholesterolemic compared with C12:0. C16:0 fatty acids.

Regarding saturated fatty acids, the percentage of SFA in CE was slightly higher than in JA. This was the case in the MUFA fraction but in reverse, the difference is about four units (Table I). Between the SFA fraction, two FAs are the majority fats: C16:0 and C18:0. The cholesterol-raising effect of SFA is largely accounted for by C12:0, C14:0 and C16:0, but the effect of stearic acid, C18:0, it is not so clear. The stearic acid is absorbed by the gut and conducted to the liver and, once there, the excess is simply converted to C18:1 via
a desaturase enzyme and then recirculates as oleic acid which does not elevate plasma cholesterol concentrations, as has been discussed before. Some studies have observed that stearic acid has a neutral or even cholesterol-lowering effect when compared with other SFAs\textsuperscript{22,23}. One of them, from a systematic review, has presented these results: in comparison with other saturated FAs, stearic acid lowered LDLC and has a neutral effect with respect to HDLC. More research about the impact of these FAs reported that stearic acid and oleic acid similarly affect markers of hemostasis in healthy men with a controlled diet\textsuperscript{24}. But, on the other hand, Hu, et al\textsuperscript{25}, in their Nurses’ Health Study based on more than 80,000 women, concluded that: “a distinction between stearic acid and other SFAs does not appear to be important in dietary advice to reduce coronary heart disease risk”. They based this inference on the association among stearic acid and other SFAs in habitual diets.

With respect to PUFA, in this fraction, the biggest difference between fat extracts of JA and CE was found. The percentage of linoleic acid (LA) in ham, of the Omega-6 series (n-6), was almost three times higher than in cecina. With regard to α-linolenic acid (ALA), of the Omega-3 series (n-3), this FA was present in similar percentages in both products, and slightly more in the JA samples. Despite the fact that conversion of ALA to EPA (Eicosapentaenoic Acid) and DHA (Docosahexaenoic Acid) is inefficient by humans, it has generated less scientific interest than EPA or DHA\textsuperscript{26}. However, ALA intake was associated with a reduced risk of coronary heart disease, diabetes and metabolic syndrome\textsuperscript{27}. As a result of these values, the ratio between n-6/n-3 was 6.18 in JA and 3.26 in CE. About this ratio, there is a difference between our results and values of other authors that is worth discussing. For instance, in Bermúdez, et al., the values for the n-6 series in JA is about 14.24 for concentrating feeding, but they obtained lower values, 9.04 and 8.88 for other types of feeding. In CE, the mean value of Molinero is 5.46, with differences as well between the cut of meat. These values are different and higher than ours, but they maintain a trend similar to the one that we observed, which is that the ratio of n-6/n-3 is bigger in JA than in CE, approximately double. The n-6/n-3 ratio in current Western diets is a topic of concern now, because it has been established that it has evolved from approximately 1 (the hunter-gatherer’s diet from our ancestors) to a 15/1-16.7/1\textsuperscript{28-29}. The high ratio has been linked to the pathogenesis of many diseases such as CVD, cancer and inflammatory diseases. On the other hand, a low n-6/n-3 ratio has a positive effect, reducing the risk of many chronic diseases\textsuperscript{28}. There is a recommended ratio of n-6/n-3, although it is not well defined yet, that is about 5/1\textsuperscript{30}, but some authors consider that this quotient has low utility and the absolute contribution of n-3 to the diet is more important to guarantee a sufficient amount. Our results for n-6/n-3 ratios in JA, 6/18/1, and CE, 3.26/1, present suitable values, near the recommended 5/1, but it is difficult to evaluate the contribution of these ratios to the total n-6/n-3 diet ratio. On the other hand, it could be affirmed, based on our results and in values obtained by other researchers, that contribution of CE lipids to the total amount of n-6 and n-3 series, especially to n-6, of a normal diet, is lower than the JA lipids.

Other FAs of recent interest, such as long chain Omega-3 PUFA, EPA and DHA, were present in very low amounts in both products. EFSA recommend that an intake of 250 mg per day of long-chain Omega-3 fatty acids are sufficient for the maintenance of normal cardiac function\textsuperscript{31}, therefore, even in small amounts, both products contribute to the necessary levels of these fatty acids. In addition to the above-mentioned cardiovascular benefits of Omega-3, it also has a small hypotensive effect in normotensive and hypertensive patients\textsuperscript{32}, so it would be interesting to see if could partly offset the hypertensive effect of salt.

Another point of this discussion is about values of conjugated linoleic acid (CLA), which were 0.33% for JA and 1.02% for CE. CLA is a group of positional and geometric isomers of linoleic acid that have double bonds in a conjugated position. These substances have been a point of attention since Pariza’s group found that CLA exerted antimutagenic activity\textsuperscript{33}, among other properties, such as antiobesity, antidiabetes, enhancement of immune function and antihypertension, which have been attributed to CLA in experimental animal models\textsuperscript{34,35}. CLA abounds in meat of ruminant animals and dairy products, because it is an intermediate on the biohydrogenation of PUFA by a bacterial enzymatic process, achieved mostly in the rumen but not limited to it. The amount of CLA in animal meats is very low, in the range of 2-5 mg/g of the total fat. With respect to the products of interest in this article, JA and CE, the content of CLA in pork and beef meat has been measured by some authors, such as Koba and Yanagita\textsuperscript{36}, to be 0.6 mg/g fat for pork and 4.3 mg/g fat for beef. This corresponds to values observed in our study, where the percentage of CLA in CE was larger than in JA, and also had a low amount of fat, but that could contribute to these potential health benefits when these products are part of a balanced and varied diet.

Finally, from a global perspective and as a summary and conclusion, FA profiles obtained for these meat products, dry-cured ham and cecina, present significant differences for main fatty acids fractions such as SFA, MUFA and PUFA, and for most detected single fatty acids. With respect to global effects that fat consumption of JA and CE could have on health, statements beyond exploring the effects of each singular FA or fraction of fat, as mentioned in previous paragraphs, are beyond the scope of this study. However, a related article\textsuperscript{32} can be cited, based on a randomized, controlled trial of healthy people, in which significant differences of serum lipids were not found between pork and veal diet consumption.
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