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Effect of solvent to sample ratio on total lipid extracted and fatty acid composition in meat products within different fat content

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ABSTRACT

The effect of solvent to sample ratio on total extracted lipids and fatty acid (FA) composition in meat products with different fat contents was evaluated. Total lipids were extracted according to the Folch et al. (1957) method, using a 20:1 ratio of chloroform:methanol (2:1, v/v) to sample (A), and also testing the solvent:sample ratio of 10:1 (B). Higher amounts of total lipids and total FA from neutral lipids were obtained using the A ratio, which could be due to an insufficient chloroform:dry-weight sample proportion which could be insufficient for solubilizing the total amount of lipids. In the polar lipid fraction, the total amount of FA was higher using the B rather than the A ratio, which may be caused by the higher volume of added water when using A than B. When studying the FA composition of different lipid fractions, the volume of both the solvent and the water for total lipid extraction should be considered.

1. Introduction

Lipids are important constituents of meat and processed meat products, influencing both their functionality and sensory properties. The quality characteristics of meat products are strongly linked to their fatty acid (FA) profile (Antequera et al., 1993; Isabel et al., 2003; Ruiz-Carrascal, Ventanas, Cava, Andrés, & García, 2000). In fact, the prediction of some sensory traits in dry-cured ham using the FA profile of the raw meat before the processing has been proposed (Pérez-Palacios, Antequera, Molano, Rodríguez, & Palacios, 2010). Additionally, the proportion of some FA of either the subcutaneous or intramuscular fat, has been proposed to classify pigs fed different diets (natural resources vs. concentrates) (Pérez-Palacios, Ruiz, Tejeda, & Antequera, 2009; Pérez-Palacios et al., 2010).

The measurement of total lipid content and FA composition of biological samples is of interest in different studies. In meat products, lipids mainly comprise nonpolar (primarily triacylglycerol) and polar components (mostly phospholipids). Total lipids and triacylglycerols show similar FA compositions in meat and meat products, because this lipid fraction represents around 85–92% of total lipids (Petrón, Muriel, Timón, Marín, & Antequera, 2004; Tejeda, Gandemer, Antequera, Viau, & Garcia, 2002). Phospholipids are also linked to important meat and meat products quality characteristics, being the substrates in which lipid oxidation develops faster, due to their high proportion of long chain polyunsaturated FA (PUFA) and their close contact with catalysts of lipid oxidation in the aqueous phase of the muscle cell (Ruiz, Muriel, Pérez-Palacios, & Antequera, 2009).

Thus, the solvent or solvent mixture used for lipid extraction must show adequate polarity to extract both polar and non-polar lipids. The most frequently used methods for total lipid extraction in meat and meat products are the Soxhlet method with petroleum ether as solvent, which is the official AOAC-recommended method (Association of Official Analytical Chemist, 1990), and those described by Folch et al. (1957) and Bligh and Dyer (1959), which are based on the use of a mixture of chloroform and methanol. The two latter methods mainly differ in the proportion of chloroform:methanol and in the solvent:sample ratio. Three parts chloroform:methanol (1:2, v/v) to 1 part sample in the Bligh and Dyer (1959) method, and 20 parts chloroform:methanol (2:1, v/v) to 1 part sample in the Folch et al. (1957) method.

Pérez-Palacios, Ruiz, Martín, Muriel, and Antequera (2008) evaluated the efficiency of different methods for the quantification of total lipid content in meat and meat products differing in fat content and physico-chemical features. Results showed that the Soxhlet with previous acid hydrolysis and the Folch et al. (1957) methods are suitable for meat and meat products with low, intermediate, high and very high lipid content, whereas the Bligh and Dyer (1959) method underestimates the total lipid content in most meat and meat products. However, these authors advise not to use the Soxhlet with acid hydrolysis method when performing further analysis of the lipid extracted, as acid hydrolysis causes lipid alterations.

Comparing the Folch et al. (1957) and the Bligh and Dyer (1959) methods in marine tissue samples, Iverson, Lang, and Cooper (2001) found that, for samples containing more than 2% lipid, the Bligh and

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Dyer (1959) method gave rise to significantly lower lipid contents than the Folch et al. (1957) method, and this underestimation was greater with increasing lipid content of the sample. Moreover, Iverson et al. (2001) compared the original solvent:sample ratio of the Folch et al. (1957) method (20 parts chloroform:methanol (2:1, v/v) to 1 part sample) against a modified version using 30 parts chloroform: methanol (2:1) to 1 part sample, finding similar results.

Since the Bligh and Dyer (1959) and Folch et al. (1957) methods were published, there have been several modifications to both procedures. However, in many publications where these methods have been used, the effect of the modifications on lipid recovery and FA profile have not been described.

Considering that in meat and meat products the Folch et al. (1957) method leads to more accurate results than the Bligh and Dyer (1959) one, this work was evaluated whether modifying the solvent to sample ratio in the Folch et al. (1957) method influences the total amount of lipid extracted and the FA composition of the neutral and polar lipids (NL and PL, respectively), in meat and meat products with different lipid content and physico-chemical characteristics.

2. Material and methods

2.1. Experimental design

Nine meat products with different lipid contents and physico-chemical characteristics were selected: fresh loin, dry-cured loin, cooked ham, pork meat, dry-cured ham, mortadella, fresh sausage, frankfurters and dry-cured sausage. All products were obtained from a local store. Lipid content reference values for these nine products were provided by an authorized official laboratory (Official Laboratory for Analysis of Agricultural, Foods and Residues of Extremadura, register number 10–006) (Table 1). 300 g of each meat product were ground using a commercial grinder and stored at −80 °C until analysis.

Total lipids were extracted from each meat product with chloroform:methanol (2:1, v/v), using two different solvent:sample ratios, 20:1 (A) (n = 12), according to the Folch et al. (1957) method, and 10:1 (B) (n = 12). Six out of the twelve replicates of the lipid extracted using each solvent:sample ratio were used for analyzing the fatty acid composition of NL and PL.

2.2. Chemicals

All solvents used were of analytical grade and obtained from Scharlau (Barcelona, Spain) or Panreac (Barcelona, Spain).

2.3. Fat extraction

Five grams of sample were mixed with the volume of chloroform: methanol (2:1, v/v) according to the A and B solvent:sample ratios used. The mixture was homogenized, centrifuged (10 min, 1549 × *g*) and filtered. Subsequently, distilled water (25 or 12.5 ml when carrying out the A or the B ratios, respectively) was added to the filtrate, as described by Iverson et al. (2001), to achieve a final ratio of 8:4:3 chloroform:methanol:water, taking into consideration the water content of the product. The obtained mixture was shaken vigorously. The final byphasic system was centrifuged (10 min, 1549 × *g*), and the upper aqueous phase discarded. The lower chloroformic phase was filtered through anhydrous sodium sulfate and collected. The lipid content was gravimetrically determined after chloroform was evaporated with a rotary evaporator under vacuum and finally under nitrogen.

Table 1
Moisture (%) and reference values for lipid content (%) in the nine different meat products of this study.

	Moisture	Reference values for lipid content
Fresh loin	73.97	2.22
Dry-cured loin	50.50	3.91
Cooked ham	77.73	2.00
Pork	71.68	8.34
Dry-cured ham	46.07	12.47
Mortadella	65.14	13.87
Fresh sausage	43.74	32.26
Frankfurter	61.80	20.45
Dry-cured sausage	29.43	38.80

2.4. Fractionation of lipids

Lipid extracts were separated into lipid classes in NH_2-aminopropyl minicolumns (500 mg) from Varian (Harbor City, CA, USA), as described by Ruiz, Antequera, Andrés, Petron, and Muriel (2004). Briefly, minicolumns were activated with hexane (7.5 ml). Lipids (10 mg) dissolved in 150 ml of hexane/chloroform/methanol (95:3:2, v/v/v) were loaded onto the column. NL were eluted with 5 ml of chloroform, free fatty acids with 5 ml of diethyl ether:acetic acid (98:2, v/v) and PL with 2.5 ml of methanol:chloroform (6:1, v/v) followed by 2.5 ml of sodium acetate in methanol:chloroform (6:1, v/v).

2.5. Fatty acid methyl esters preparation and analysis

Fatty acid methyl esters (FAME) from acyl chains were prepared by acidic trans-esterification in the presence of sulphuric acid (5% sulphuric acid in methanol) (Sandler & Karo, 1992). FAME were analyzed by gas chromatography, using an Agilent 6890 N gas chromatograph, equipped with a flame ionization detector (FID). Separation was carried out on a polyethyleneglycol capillary column (60 m long, 0.32 mm id, and 0.25 mm film thickness) (Supelcowax-10, Supelco, Bellafonte, PA). Oven temperature programming started at 180 °C. Immediately, it was raised 5 °C min^{-1} to 200 °C, held for 40 min at 200 °C, increased again at 5 °C min^{-1} to 250 °C, and held for the last 21 min at 250 °C. Injector and detector temperatures were 250 °C. The carrier gas was helium at a flow rate of 0.8 ml/min. Individual FAME peaks were identified by comparing their retention times with those of standards (Sigma, St. Louis, MO).

2.6. Statistical analysis

The effect of modifying the solvent:sample ratio on total lipid extracted as well as on the FA composition of NL and PL in the meat and meat products was compared by One-Way Analysis of Variance (ANOVA) using the General Linear Model of SPSS (v.12.0).

3. Results and discussion

3.1. Effect of solvent to sample ratio on total lipid extracted in meat products

Fig. 1 shows the proportions of total lipids extracted in nine different meat products using chloroform:methanol (2:1, v/v) and A and B solvent to sample ratios, as compared to the reference values for the same products, which were considered 100%. Significantly higher proportions of total lipids were extracted using the A method compared to the B one ($p<0.05$ for fresh sausage, $p<0.01$ for dry-cured sausage, frankfurter and fresh loin, and $p<0.001$ for mortadella, dry-cured ham, pork, cooked ham and dry-cured loin); thus, using the A ratio the proportions of total lipids obtained were higher than 90% of the reference values for most meat products, and lower than 60% for all the products when using the B ratio.

Studying marine tissues, Iverson et al. (2001) found that the Folch method, using 7.5:1 solvent:sample ratio, tended to underestimate lipid content as fat content increased in the product. Thus, in marine