Food Chemistry 110 (2008) 1025–1029

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Comparison of different methods for total lipid quantification in meat and meat products

T. Pérez-Palacios *, J. Ruiz, D. Martín, E. Muriel, T. Antequera

Tecnología de los Alimentos, Facultad de Veterinaria UEx, Avda. de la Universidad s/n, 10071 Cáceres, Spain

ARTICLE INFO

Article history:
Received 15 August 2007
Received in revised form 11 February 2008
Accepted 4 March 2008

Keywords:
Lipid extraction methods
Fat content
Meat products

ABSTRACT

This study was aimed to evaluate the efficiency of six extraction methods for the quantification of total lipid content in meat and meat products: standard Soxhlet method (with and without previous acid hydrolysis), continuous Soxhlet method (with and without previous acid hydrolysis), and those methods based in the use of a mixture of chloroform and methanol, and described by Folch, Less, and Sloane (1957) and Bligh and Dyer (1959). Lipid content was determined in nine different meat products with different fat contents and physico-chemical features: cooked turkey breast, fresh pork loin, cooked ham, dry-cured ham, mortadella, beef burger, fresh sausage, dry-cured sausage and salami. The most effective methods for determining fat content in the studied meat products were the method described by Folch et al. (1957) and the Soxhlet with previous acid hydrolysis method. The Soxhlet method without previous acid hydrolysis adequately extracted lipids only in those meat products with very high fat content. The use of the method described by Bligh and Dyer (1959) gave rise to the lowest lipid contents in all the studied meat products.

1. Introduction

The total lipid content of meat and meat products is an important quantity used in numerous studies. Thus, reliable methods for the quantitative extraction of lipids from this type of foodstuffs are of critical importance (Iverson, Lang, & Cooper, 2001). Lipids in muscle foods are a mixture of nonpolar components (mainly acylglycerides and cholesterol), free fatty acids, and more polar lipids, such as phospholipids or sphingolipids (Ruiz, Antequera, Andres, Petron, & Muriel, 2004). Chemical and physical treatments used for lipid extraction must remove them from their binding sites with cell membranes, lipoproteins and glycolipids. Moreover, the solvents used for extracting fat should have a high solubility for all lipid compounds and be sufficiently polar (Smedes & Askland, 1999).

Several methods have been developed for total lipid extraction, the most used in meat and meat products being the Soxhlet method (SOX), which is the official AOAC-recommended method (Association of Analytical Chemists, 1990) and those based in the use of a mixture of chloroform and methanol, and described by Folch et al. (1957) (FOL) and Bligh and Dyer (1959) (B&D). Moreover, new Soxhlet extraction systems offer different modes of extraction which could improve the extraction procedure, reducing the extraction time and the solvent volume.

Since the B&D and FOL methods were published, there have undoubtedly been several modifications to both procedures in order to improve their lipid recovery efficiency in meat and meat products. However, in many publications where these methods have been used, modifications have been neither described nor validated. In other cases, researchers stated that lipids were quantified "according to" one or the other method, but they did not indicate whether any modifications were made implying that the methods were applied basically according to the original procedures (Iverson et al., 2001).

It has been demonstrated that the use of different methods results in different lipid recoveries in biological samples. Indeed, results varied widely due to differences in extraction methodology, as confirmed by an intercomparative study (Baily, Wells, de Boer, & Delbeke, 1994). However, most used lipid extraction methods for muscle foods have been scarcely compared for different types of meat products. In addition, depending on the study, different results have been reported. Thus, some authors have found a better extraction of total lipids with the SOX method than using the B&D in chicken (Manirakiza, Covaci, & Schepens, 2001), while others have found the opposite result studying the fat content of fish muscle (Ewald, Bremle, & Karlsson, 1998), and other researchers found no differences between these two methods when analysing the total lipid content of pork loin (Ragland, Christian, & Baas, 1996). Iverson et al. (2001) compared the B&D and the FOL methods for extracting fat from marine tissue with different fat contents. Their results showed that, for samples containing more

* Corresponding author. Tel./fax: +34 927 257110.
E-mail address: triny@unex.es (T. Pérez-Palacios).

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doi:10.1016/j.foodchem.2008.03.026

than 2% lipids, the B&D method gave rise to significant lower lipid contents than the FOL method, and this underestimation was greater with increasing lipid content of the sample.

Nowadays, lots of meat products with different fat contents and different physical and chemical features (protein network, moisture content, ingredients, additives and so on) are being manufactured. The quantification of total lipids and its characterization is a basic requirement for testing these products (Manirakiza et al., 2001). In order to choose the most appropriate method for extracting lipids in these products, it should be considered that the efficiency of the extraction procedure could be influenced by the nature of the product matrix (Christie, 1993; Young, Frost, West, & Braggins, 2001). In the scientific literature, both the B&D and the FOL methods have been extensively used for lipid quantification in meat and meat products. However, there are not established criteria for choosing the most appropriate one.

The present study was aimed to evaluate the efficiency of different lipid extraction methods (standard Soxhlet with and without previous acid hydrolysis, continuous Soxhlet with and without previous acid hydrolysis, extraction with a chloroform:methanol mixture following the methods described by Bligh and Dyer (1959) and Folch et al. (1957)) for quantification of total lipid content in nine meat products differing in their fat contents and physico-chemical features.

2. Materials and methods

2.1. Sampling

Nine meat products with different composition, processing technology and fat contents were studied in this work. Products were grouped in terms of fat content: low fat content group (1–5%) (cooked turkey breast, fresh pork loin, cooked ham); intermediate fat content group (5–15%) (dry-cured ham, mortadella, beef burger); high fat content group (15–30%) (fresh sausage, dry-cured sausage); very high fat content group (30–50%) (salami). All products were purchased in a local supermarket. Reference values for lipid content of 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) and by the label provided by the manufacturer in the product. Table 1 shows reference values provided by the label, which were in agreement with those provided by the official laboratory. Each meat product (300 g) were ground using a commercial grinder and stored at −80 °C until analysis. Homogeneous samples (5 g) were taken for fat extraction.

2.2. Chemicals

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

2.3. Methods

Six different lipid extraction methods were followed: standard SOX (ST-SOX) (with and without previous acid hydrolysis), continuous SOX (CN-SOX) (with and without previous acid hydrolysis), B&D (Bligh & Dyer, 1959) and FOL (Folch et al., 1957). The weight of the sample was 5 g for the six extraction methods. Six replicates for each meat product were performed in all the tested methods.

2.4. Soxhlet

Standard and continuous SOX extraction methods (with and without previous acid hydrolysis) were performed on a Büchi Universal Extraction System B-811 (Flawil, Switzerland). The ST-SOX method keeps the sample in contact with the solvent for a longer time, while the CN-SOX method implies a higher number of sample washings with the solvent. The used solvent was petroleum ether, fraction 40–60 °C, and the parameters for extraction were those recommended by Büchi laboratories (BÜCHI Labortechnik AG 1998, Flawil, Switzerland). The initial volume of solvent was 120 ml, being necessary to add a little more (40–80 ml) during the extraction process. Extraction was performed for 2 h.

Acid hydrolysis was performed on a Büchi Hydrolysis Unit B-411 (Flawil, Switzerland). Samples (5 g) were boiled in 3 N hydrochloric acid (100 ml) with 5 g of celite during 45 min. Thereafter, the mixture was filtered through celite (5 g) and quartz sand (50 g). The filter (celite and quartz sand) with the retained sample was subsequently washed with 250 ml of water (40 °C). In this way, lipids were retained by the filter. Finally, the glass vessels with the wet celite and quartz sand residue, which contained the lipids, were dried in a conventional oven (100 °C for 6 h).

In both SOX with previous acid hydrolysis methods, the dried glass vessels were placed in the extraction system and lipids were extracted from the celite and quartz sand residue. Both in the ST-SOX and CN-SOX methods without previous acid hydrolysis methods, samples (5 g) were wrapped inside paper envelopes, which

Table 1
Total lipid content (%) in nine different meat products as obtained by six different lipid extraction methods[A]

Fat content	Food	Methods						p	Reference value[H] (%)
		ST-SOX[B]	Hyd ST-SOX[C]	CN-SOX[D]	Hyd CN-SOX[E]	B&D[F]	FOL[G]		
Low	Cooked turkey breast	$0.61^{bc} \pm 0.01$	$0.84^{b} \pm 0.04$	$0.44^{c} \pm 0.01$	$0.78^{bc} \pm 0.03$	$0.67^{bc} \pm 0.04$	$1.38^{a} \pm 0.01$	<0.001	1
	Fresh pork loin	$1.27^{c} \pm 0.02$	$3.21^{a} \pm 0.05$	$1.44^{bc} \pm 0.13$	$3.26^{a} \pm 0.01$	$1.65^{b} \pm 0.01$	$3.41^{a} \pm 0.07$	<0.001	3
	Cooked ham	$1.8^{d} \pm 0.03$	$2.91^{b} \pm 0.01$	$2.33^{c} \pm 0.01$	$2.85^{b} \pm 0.06$	$1.58^{e} \pm 0.06$	$3.91^{a} \pm 0.06$	<0.001	3.5
Intermediate	Dry-cured ham	$4.16^{bc} \pm 0.27$	$6.27^{a} \pm 0.05$	$4.39^{bc} \pm 0.25$	$5.88^{a} \pm 0.22$	$3.29^{c} \pm 0.33$	$5.36^{ab} \pm 0.35$	<0.001	6.4
	Mortadella	$7.72^{b} \pm 0.14$	$10.17^{a} \pm 0.05$	$7.22^{b} \pm 0.04$	$7.95^{b} \pm 0.13$	$6.02^{c} \pm 0.25$	$9.66^{a} \pm 0.23$	<0.001	9.5
	Beef burger	$4.8^{c} \pm 0.27$	$10.27^{a} \pm 0.18$	$4.54^{c} \pm 0.25$	$10.33^{a} \pm 0.56$	$2.51^{d} \pm 0.18$	$8.67^{b} \pm 0.32$	<0.001	10
High	Fresh sausage	$9.89^{b} \pm 0.64$	$20.16^{a} \pm 0.53$	$8.15^{bc} \pm 0.43$	$19.64^{a} \pm 0.20$	$6.33^{c} \pm 0.30$	$19.62^{a} \pm 0.64$	<0.001	19.5
	Dry-cured sausage	$20^{a} \pm 0.53$	$22.56^{a} \pm 0.81$	$21.4^{a} \pm 0.28$	$20.62^{a} \pm 0.81$	$13.51^{b} \pm 0.73$	$22.84^{a} \pm 0.24$	<0.001	25
Very high	Salami	$49.82^{a} \pm 0.25$	$41.67^{b} \pm 1.32$	$49.8^{a} \pm 0.50$	$45.48^{b} \pm 0.77$	$33.25^{c} \pm 0.86$	$49.39^{a} \pm 0.72$	<0.001	48.6

[A] Mean values ± standard error of the mean. Means with different superscripts differ significantly ($p < 0.05$).
[B] Soxhlet standard without hydrolysis.
[C] Soxhlet santandar with hydrolysis.
[D] Soxhlet continuous without hydrolysis.
[E] Soxhlet continuous with hydrolysis.
[F] Bligh and Dyer.
[G] Folch.
[H] Reference values provided by the label of the product.

were subsequently placed into the extraction system in order to directly extract lipids from the samples. The extracted lipids were collected in beakers. Lipid content was then determined gravimetrically after total solvent evaporation.

2.5. Bligh and Dyer method

Extractions following the B&D method were performed as originally outlined by Bligh and Dyer (1959). Briefly, 5 g of sample were mixed with 15 ml of chloroform:methanol (1:2, v/v). The mixture was homogenized for 2 min in a Sorvall Omnimixer homogenizer, centrifuged (10 min, 3000 rpm) and filtered. The residue was rehomogenized with 5 ml of chloroform, centrifuged (10 min, 3000 rpm), filtered and collected together with the previous filtrate. This filtrate was mixed with 5 ml of distilled water and shaken vigorously. The final biphasic system was allowed to separate by centrifugation (10 min, 3000 rpm). The upper aqueous phase was eliminated. The lower chloroformic phase was filtered through anhydrous sodium sulphate and collected. Lipid content was then determined gravimetrically after chloroform was evaporated using a rotary evaporator under vacuum followed by further drying under nitrogen.

2.6. Folch method

Lipid extractions following the Folch et al. (1957) were performed using the original extraction ratio of 20 parts chloroform:methanol (2:1, v/v) to 1 part sample. Briefly, 5 g of sample were mixed with 100 ml of chloroform:methanol (2:1, v/v). The mixture was homogenized, centrifuged (10 min, 3000 rpm) and filtered. Subsequently, 5 ml of distilled water was added to the filtrate and the new mixture was shaken vigorously. The final byphasic system was allowed to separate by centrifugation (10 min, 3000 rpm). The upper aqueous phase was eliminated. The lower chloroformic phase was filtered through anhydrous sodium sulphate and collected. Lipid content was then gravimetrically determined after chloroform was evaporated with a rotary evaporator under vacuum and the solvent was further evaporated under nitrogen.

2.7. Statistical analysis

Differences between lipid content within each meat product by the six studied methods were compared by one way ANOVA using the General Linear Model of SPSS (v.12.0). When a significant effect ($p < 0.05$) was detected, the comparative analyses were conducted using a Tukey test.

3. Results

The six extraction methods significantly differed ($p < 0.001$) in total extracted lipids for the nine analysed meat products (Table 1). For almost all the products, the methods that extracted a higher amount of lipids were the FOL, the CN-SOX and the ST-SOX with previous acid hydrolysis, followed by the CN-SOX and the ST-SOX without acid hydrolysis, and the B&D method showing the lowest levels of total extracted lipids.

The FOL and the SOX with previous acid hydrolysis methods differed in total extracted lipids for some products. In cooked turkey breast, cooked ham and salami, the FOL method extracted significantly ($p < 0.05$) more lipids (1.38%, 3.41% and 49.39%, respectively) than the CN-SOX (0.78%, 2.85% and 45.48%, respectively) and the ST-SOX (0.84%, 2.91% and 41.67%, respectively) with previous acid hydrolysis methods. However, in beef burger, the total lipid content using the FOL method (8.67%) was lower ($p < 0.05$) than that obtained using the CN-SOX (10.33%) and ST-SOX (10.27%) methods with previous acid hydrolysis.

Both SOX methods without previous acid hydrolysis extracted less total lipids than the FOL and the SOX methods with previous acid hydrolysis in most analysed meat products. However, in salami, both the CN-SOX and the ST-SOX methods without acid hydrolysis and the FOL method extracted a higher amount of lipids (49.80%, 49.82% and 49.39%, respectively) than the CN-SOX and the ST-SOX methods with previous acid hydrolysis (45.48% and 41.67%, respectively). In dry-cured sausage, these five methods gave rise to similar lipid contents (22.84%, 20.62%, 22.56%, 21.4% and 20.00% for the FOL, CN-SOX and ST-SOX with and without previous acid hydrolysis methods, respectively).

Differences between the CN-SOX and the ST-SOX methods were only found in two meat products. In cooked ham, the CN-SOX method without previous acid hydrolysis extracted more lipids than the ST-SOX method without previous acid hydrolysis (2.33% *vs* 1.80%). In mortadella, the lipid content obtained when extracting with the ST-SOX method with previous acid hydrolysis was lower than that obtained with the CN-SOX method with previous acid hydrolysis (7.95% *vs* 10.17%).

The B&D method extracted less lipids than any of the other five methods in all the considered meat products except for fresh pork loin, in which the ST-SOX method without previous acid hydrolysis, gave rise to a lower ($p < 0.05$) total lipid content than the B&D method (1.65% *vs* 1.27%).

4. Discussion

There is little scientific information about the more convenient method for lipid extraction in each type of meat product. This is important, since features such as fat and moisture content, the nature of the protein network, the presence of several additives, the technological process or the physical and chemical interactions between lipids and proteins, could influence the performance of each lipid extraction method. Taking the previous discussion into consideration, the CN-SOX and ST-SOX methods with and without previous acid hydrolysis, and the methods described by Folch et al. (1957) and Bligh and Dyer (1959) were carried out for determination of total lipid content in nine meat products whit different features and fat content.

The studied methods which extracted the highest amount of total lipids in the nine meat products were the FOL and the CN-SOX and ST-SOX methods with previous acid hydrolysis. However, some differences were found between these methods which could be related to the features and fat content of the studied meat products, or even to the sample weight. In two products of the low fat content group (cooked turkey breast and cooked ham) the FOL method extracted a higher amount of lipids than both SOX with previous acid hydrolysis methods, while these latter methods gave rise to a similar fat content in the other meat product of this group (fresh pork loin). All these products are whole muscle meat products, in which the muscle structure, including the integrity of muscle fibres, of connective tissue and of other tissues included in meat (such as vascular, nervous and lymph), have not suffered a disruption due to a grinding step. Due to this, the strong links between fat and proteins are not destroyed during elaboration of these products, leading to a more difficult extraction of those lipids strongly linked to proteins, such as some membrane lipids. Both, acid hydrolysis or the use of solvents with different polarities, are aimed to solve this problem. However, it seems that acid hydrolysis was not effective enough in two of these products. The manufacture of cooked turkey breast and cooked ham involves a cooking phase at temperatures usually above 70–72 °C. This leads to protein denature, forming a protein gel network and insoluble

aggregates. These physical modifications could explain the decrease in extraction when using the two SOX methods with previous acid hydrolysis.

The total lipid content of salami using the FOL method was also higher than with both SOX methods with previous acid hydrolysis. Most of the fat content of this product is added lard, and thus, it is easily extracted. The lower amount of lipids extracted with both SOX methods with previous acid hydrolysis could be partly due to the inefficacy of the celite and quartz sand to retain the high amount of fat of this product. In fact, both SOX methods without previous acid hydrolysis were able to extract a similar amount of fat to that obtained with the FOL method, the only difference with the SOX methods with previous acid hydrolysis being the acid digestion and the retention of the digested sample in the celite and quartz sand steps. In this sense, it would be recommendable to adjust sample weights when performing the SOX method with previous acid hydrolysis.

Strangely, a lower amount of total lipids in beef burger was obtained when using the FOL method than using any of the SOX methods with previous acid hydrolysis. This result was not in agreement with those obtained for the other studied meat products. This might be partially due to the ingredients used for burger production. Some of these ingredients, such as carbohydrates and non-meat protein materials, are aimed to improve the connection between the particles of meat obtained after grinding by forming a network. It could be that some of these ingredients could have impaired fat extraction by increasing the strength of the links between the lipids and the rest of the components of the burger, or by physically making the fat less accessible to solvents.

There was a general trend towards higher lipid extraction by the SOX methods when a previous acid hydrolysis was carried out, except for the two products with the highest fat content (dry-cured sausage and salami) (Table 1). In fact, when carrying out the SOX extraction without previous acid hydrolysis, the total lipid content obtained was considerably far from that of the reference method for most meat products. As explained before, the strong links between some lipid components and proteins in muscle foods is the main cause explaining the lower extraction of lipids by the SOX methods without previous acid hydrolysis. On the other hand, the lower amount of lipids determined in salami when performing the SOX method with previous acid hydrolysis could be related to the insufficient capacity of celite and quartz sand to retain such a high amount of lipids, as it has been previously explained. A similar behaviour was observed for dry-cured sausage, which was the product with the second highest fat content (25% fat), although not to the same extent as in salami. This fact strengthens the need of lower sample weights when performing SOX extractions with acid hydrolysis of high fat content muscle foods.

Mortadella was the only product in which differences in the amount of total lipids obtained between the ST-SOX and the CN-SOX methods with previous acid hydrolysis were detected. In addition, the total lipid content of cooked ham obtained by the ST-SOX and the CN-SOX methods without previous acid hydrolysis was also significantly different. Therefore, it seems that both modes of SOX extraction are perfectly valid for lipid extraction in most meat and meat products. However, given that the CN-SOX method needs lower solvent volumes, it might be recommended the use of such method due to environmental and economic reasons.

For all the analysed products, the B&D method showed the lowest amount of extracted lipids, except for those products containig 1–3% fat, in which the obtained amount was lower for the SOX methods without previous acid hydrolysis of the sample. Other authors have also found a worse extraction with the B&D method as compared to the SOX method (Brooks, Ratnayake, Lampi, & Hollywood, 1998). Moreover, Iverson et al. (2001) studying the total lipid content in muscles from fish with different fat contents, have observed differences in total lipid extraction between the FOL and the B&D methods. Indeed, these authors found similar results with both methods in samples below 2% fat content, but increased underestimation of fat content with the B&D method with increasing fat levels, in a similar trend to the one found in our study.

The B&D method is commonly used for extracting total lipids from many different foodstuffs. However, the original B&D method was initially developed for fish samples with lipid contents below 1%, water contents around 80% and a high level of phospholipids. Although the authors (Bligh & Dyer, 1959) stated that their method could be applied to other biological tissues, they advised that samples with a high fat content may require modifications of the method. Even in samples containing 2–10% lipids (which is common for many marine fish and invertebrates), underestimation will still be a significant problem, and this has likely been neglected. Both, in whole animals and in specific tissues, an increase in total lipid content is mainly due to an increase in the triacylglycerol content. The reduced efficiency of the B&D method could be related to the limited solubility of the predominantly non polar lipids, such as triacylglycerols, in the relatively polar solvent solution (chloroform:methanol 1:2 v/v) employed in this method.

Smedes and Thomansen (1996) found that the absorption of the organic phase by the tissue was one of the main causes of incomplete lipid yield when extracting lipids with chloroform:methanol. Relatively constant amounts of the organic phase are absorbed by the tissue; therefore, using greater volumes of organic phase solvents proportionally reduces the fraction of the organic phase that is lost in this way. Thus, investigators which have found reliable results with the B&D method might have modified the extraction procedure using an increased solvent/sample ratio. In this sense, it is important that researchers specify modifications of the procedures, especially the precise solvent/sample ratio used.

Taking the results obtained in this study into consideration, the FOL and the SOX methods with previous acid hydrolysis could be recommended to extract the total lipid content in meat products. Although the SOX methods take shorter analysis time and they are less laborious than the FOL method, when the lipid fraction must be further characterized after extraction, the former methods should not be used, because exposure to heat and acid hydrolysis promote lipid oxidation, phospholipid hydrolysis and other chemical lipid modifications. Therefore, for lipid characterization purposes, the FOL method should be chosen. In order to extract the total lipid content in meat and meat products using the B&D method, the original solvent/sample ratio (3:1) should be optimize.

5. Conclusion

Among the studied methods for total lipid quantification in meat and meat products, the method described by Folch et al. (1957) is suitable for meat and meat products with low, intermediate, high and very high lipid content. The Soxhlet method with previous acid hydrolysis is also proper for lipid quantification in this kind of products, except for meat products with very high lipid content. In this case, the Soxhlet without previous acid hydrolysis should be chosen.

The highly used lipid extraction method described by Bligh and Dyer (1959) underestimate total lipid content in most meat and meat products, being necessary a correct adjustment of the solvent to sample ratio for each meat product.

Acknowledgements

The authors would like to thank Ana Isabel Galaz Mollón and Concepción Fernández Osorio (Laboratorio Agroalimentario y de Residuos de Extremadura) for their excellent technical assistance.

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