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Pressurized solvent extraction of total lipids in poultry meat

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Abstract

Pressurized solvent extraction is an innovative method for rapid extraction of analytes. It uses liquid solvents at high temperature (from 60 to 200 °C) and pressure (from 3.5 to 20 MPa), through a stainless steel extraction cell containing a solid sample. In this study, an ASE instrument was used for the quantitative determination of the fat content in homogenised poultry meat samples. The recovery of total lipids obtained by ASE extraction was compared to those obtained by conventional methods, such as the Folch method and acid hydrolysis, followed by Soxhlet extraction. Two different solvent mixtures (chloroform/methanol and *n*-hexane/2-propanol) were tested at various temperatures and pressures and with different sample preparations. The composition of the extracts from each method were determined and compared by thin-layer chromatographic (TLC) analysis; a CGC analysis of the fatty acid composition of the extracts was carried out.

Keywords: ASE; Extraction; Folch; Meat; Poultry; Soxhlet

1. Introduction

Not considering some recently developed spectroscopic methods, using FT-IR, FT-NIR, or NMR, the analysis of lipids from meat, both for qualitative or quantitative purposes, requires a preliminary extraction step. The ''ideal'' extraction method should be quantitative, non-destructive, short time and less solvent-consuming. Such a procedure is hard to imagine, considering that lipids include simple lipids, primarily triacylglycerols, easily soluble in non-polar organic solvents, such as *n*-hexane or supercritical carbon dioxide, and complex lipids, such as phospholipids, glycolipids, partial glycerides and free fatty acids. Hence, complex lipids are harder to free from the other components of the tissues than simple lipids. The complex lipids are tightly held by hydrophobic bonds, Van der Waals forces, and hydrogen or ionic bonding. Therefore, such matrices can be extracted only by polar solvents that can overcome these interactions.

Various procedures and solvent combinations have been employed to quantitatively extract lipids, such as the traditional or modified Folch procedure (Folch, Lees, & Stanley, 1957), which employs chloroform/methanol (2:1 v/v), or the Hara and Radin method (Hara & Radin, 1978; Radin, 1981), which employs *n*-hexane/2-propanol (3:2 v/v). The latter solvent mixture is less efficient (it does not quantitatively extract complex lipids such as gangliosides); otherwise it is less toxic. Another rapid extraction procedure, used for samples containing 80% water, employs a chloroform/methanol/water mixture to separate the lipids from all the non-lipids and it is commonly called the Bligh and Dyer method (Bligh & Dyer, 1959). Overall, the quantitative determination of total lipids can require a digestive step, which is time-consuming, but it is often necessary for dairy and meat products to free bound lipids prior to solvent extraction (Barbano & Clarck, 1988).

Supercritical fluid extraction (SFE), employing supercritical carbon dioxide, can reduce the use of toxic organic solvents but, in many cases, quantitative extraction of the most polar components requires the use of a traditional solvent (such as methanol or ethanol) as polar modifier (cosolvent).

In this paper, the extraction of total lipids of poultry was facilitated using an ASE (Dionex Corporation) instrument, and the results were compared to those of a modified Folch procedure and those from an acid

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hydrolysis, followed by a traditional solvent extraction, using a Soxhlet apparatus.

2. Materials and methods

2.1. Samples and solvents

Twenty Cobb chickens, donated by Cafar (Rimini, Italy), were butchered (by the manufacturing firm) when they were 45–53 days old. All the solvents were of analytical grade and were supplied by Carlo Erba (Milan, Italy).

2.2. Samples preparation

The homogenised muscle and the skin of 20 chicken hip samples, previously deprived of the thighs and boned, were separately collected in plastic bags, and frozen at −18 °C, until the day before the lipid extraction, when they were defrosted overnight, at 4 °C.

2.3. Modified Folch procedure

Homogenised tissue (10 g) was progressively added to small amounts of a chloroform/methanol 2:1 (v/v) mixture (up to 200 ml), with vigorous shaking, and then the extraction was carried on for a further 2 h, using an electromagnetic stirrer. The mixture was filtered and the filter was re-washed with fresh solvent and pressed. Fifty millilitres of 0.88% potassium chloride were added and the mixture was shaken. The aqueous layer (upper) was removed by aspiration and the washing procedure was repeated. The extract was then dried by adding anhydrous sodium sulphate, which was filtered again, before the solvent was removed using a rotary evaporator. The extract was then placed in a desiccator overnight and weighed (Folch, Lees, & Stanley, 1957; Ways & Hanan, 1964).

2.4. Extraction by acid hydrolysis

Homogenised tissue (2.5 g) was added to 100 ml of a 3 N aqueous solution of hydrochloric acid. The mixture with a few added pumice stones, was boiled, and refluxed for 1 h. Sufficient amounts of infusorial earth were added to retain the lipids. After refluxing, the solution was filtered through two Whatman 41 filters (Maidenstone, UK), in series, and washed with distilled water until neutrality. The filter and the sample on it were then left in an oven at 100 °C for 1 h and extracted, in a Soxhlet-type apparatus, with 200 ml of diethyl ether. The solvent was removed using a rotary evaporator. The extract was stored in a desiccator overnight and weighed (Method 8.2, G.U. no. 15/29, 18/1/1984).

2.5. Extraction by ASE

From 5 to 5.5 g of homogenised tissue were exactly weighed on a thin layer of Hydromatrix (Varian SpA, Torino, Italy), in a mortar and treated with approximately 3–4 g of Hydromatrix. Using a pestle, the sample was ground and placed in a 22 ml steel extraction cell, previously prepared with a cellulose filter and a 1-cm layer of Hydromatrix; the void volume, on the top of the column, was filled with Hydromatrix. The loaded cell was then mounted in the carousel of a Dionex ASE 200 Accelerated Solvent Extractor (Salt Lake City, Utah, USA) and then extracted. The extract was collected in a 60-ml glass vial and dried by adding anhydrous sodium sulphate. It was then filtered before the solvent was removed in a rotary evaporator Finally 1–1.5 ml of anhydrous ethanol were added to make it easier to evaporate the water. The extract was then kept in a desiccator overnight and weighed. Alternatively, to clean the chloroform-methanol extract, before the lipid analysis, the sample was shaken with one-fourth its volume of a saline solution (0.88% potassium chloride in water) (Folch, Lees, & Stanley, 1957). The upper layer was then removed, anhydrous sodium sulphate was added (and left overnight); the extract was then filtered and evaporated as previously described.

2.6. Conditions of ASE extraction common to all the samples

Flushing (fresh solvent added between each cycle as a percent of the cell volume): 60%; purge: 120 s; cycles: 2; total solvent collected: 52–55 ml.

2.7. Conditions of each single extraction

The conditions (solvent mixture, time of two static cycle, temperature and pressure) used for each extraction method (ASE 1-10) are listed in the Table 1.

2.8. Capillary gas chromatographic (CGC) analysis

Fatty acid methyl esters, prepared by sodium methoxide-catalysed transesterification (Christie, 1989), were analysed using a Carlo Erba Mega 5300 capillary gas chromatograph (Milan, Italy) equipped with a 30 m×0.25 mm i.d. SP2330 fused silica capillary column (Supelco Inc., Bellafonte, PA). The injector and detector temperatures were 240 °C; helium was the carrier gas. The temperature of the oven was programmed from 120 to 220 °C at 4 °C/min.

2.9. Statistical analysis

Statistical analysis was effected by the software SPSS r.11.0.0; the comparison of the values was carried out

Table 1
Analytical conditions used for the ASE 1–10 extraction methods

	Conditions of ASE extractions			
	Solvent mixture	Time (min)[a]	Temperature (°C)	Pressure (MPa)
ASE 1	*n*-hexane/2-propanol 3/2	10	60	15
ASE 2	*n*-hexane/2-propanol 3/2	10	100	15
ASE 3	chloroform/methanol 2/1	5	60	15
ASE 4	chloroform/methanol 2/1	5	80	15
ASE 5	chloroform/methanol 2/1	5	100	15
ASE 6	chloroform/methanol 2/1	10	120	15
ASE 7	chloroform/methanol 2/1	10	60	20
ASE 8	chloroform/methanol 2/1	10	80	20
ASE 9	chloroform/methanol 2/1	10	100	20
ASE 10	chloroform/methanol 2/1	10	120	20

[a] Time of two static cycles.

by HSD (Honest Significant Difference), Tukey test ($P < 0.05$) and by linear correlation analysis.

3. Results and discussion

Table 2 lists the results of the different extraction procedures of five samples of deboned and homogenised poultry hips. The first two rows list the data obtained by the modified Folch extraction and the hydrolytic procedure, respectively. The other rows list the mean values of the lipid content obtained by the ASE extractions, using different extraction pressures, temperatures, times, and solvent mixtures (ASE 1–10).

The recoveries from a mixture of *n*-hexane/2-propanol 3:2 (v/v) for the extraction of sample 5, at 60 and 100 °C (ASE-1 and ASE-2, respectively) were significantly lower than those obtained by the modified Folch and the acid hydrolytic procedures. Such low lipid recoveries are probably due to the permeation of this mixture, particularly through the muscle tissue and its inability to provide sufficient solvation to overcome the interactions between the lipids and the other muscle components. The same pressure value (15 MPa) was also used for the extraction using chloroform/methanol 2:1 (v/v) (ASE-3 from ASE-6) and different temperatures from 60 °C (ASE-3) to 120 °C (ASE-6) were tested. Moreover, with these extraction procedures, the mean values were found to be significantly lower than with the modified Folch method or the acid hydrolytic procedure. The conditions used for ASE-7 permitted a yield approaching that of the modified Folch procedure. This result was further improved by increasing both the cycle time and the pressure employed. A fixed higher pressure (20 MPa) and higher temperatures of 80, 100, and 120 °C (from ASE-8 to ASE-10) were able to quantitatively extract the lipid as in the modified Folch and the acid hydrolytic procedures. The higher recoveries can be explained by considering the harsher stripping but this may also be due to higher extraction rate of non-lipid contaminants, such as sugars, amino acids and salts. To remove these molecules, as suggested by Folch (Folch, Lees, & Stanley, 1957), a saline solution could be added before the weighing step. With regard to this cleaning step, it is pertinent to emphasise that some trace components, such as glycolipids, can be partially lost using this washing procedure. It is possible to note that for 1 and 4 samples the yields of extraction obtained by ASE-10 were equivalent only to the modified Folch method, but for the other three samples (2, 3 and 5) there were no significant differences between acid hydrolytic, modified Folch and ASE-10 procedures.

Table 2
Total lipids (%) (w/w) of five samples of boned and homogenised poultry hips, extracted by the modified Folch method, the acid hydrolytic procedure and different ASE methods

	Samples				
Procedures	1[a]	2	3	4	5
Modified Folch	15.5a,b	15.6a	15.0a	14.7a,b	14.6a
Acid hydrolysis	17.7a	17.7a	17.4a	15.8a	15.9a
ASE-1	–	–	–	–	6.6c
ASE-2	–	–	–	–	7.5c
ASE-3	–	–	–	–	7.2c
ASE-4	–	–	–	–	7.1c
ASE-5	–	–	–	–	8.1b,c
ASE-6	10.3c	12.3b	7.7b	8.4c	–
ASE-7	–	–	–	–	13.0a,b
ASE-8	–	–	–	–	14.8a
ASE-9	–	–	–	–	14.7a
ASE-10	14.1b	16.2a	15.3a	14.4b	15.6a

Different letters in the same row indicate significantly different values ($P < 0.05$).

[a] g of fat/100 g of boned and homogenised meat.

Based on the above results, the ASE-10 method was used for the quantitative extraction of all of the poultry samples analysed.

Table 3 lists the data for the extractions of 20 boned and homogenised poultry hips by the modified Folch procedure, by the hydrolytic method, and by the developed ASE-10 method. As expected, the mean weights of the lipids for the hydrolytic method were the highest because this procedure also removes complex and polar lipids, such as phospholipids, glycolipids or lipoproteins, that the other methods are not able to extract. However, only for five samples (1, 9, 10, 12, 13) was the fat extracted by ASE-10 significantly lower than that obtained by acid hydrolysis. In the other cases, the values achieved by ASE-10 were not significantly different from those achieved by the Folch method.

From the TLC analyses of the different extracts, it was evident that the qualitative composition of the extracts were comparable. It appeared that the free fatty acids were better extracted at higher pressure and temperatures. The fatty acid composition (Table 4), by CGC analysis of the methyl esters, indicated the same recoveries of the polyunsaturated fatty acids by both the Folch procedure and the ASE-10 method, confirming the possibility of using the ASE-10 method instead of the Folch procedure. Although this might be adopted as a preparative step, for many purposes, less drastic temperature conditions might be used, to limit the destruction of thermolabile molecules. To prevent lipid autoxidation, nitrogen should be used to pressurize the steel cells.

The correlations ($P<0.05$) among the lipid content of the twenty samples (which were in duplicate) obtained by the three extraction methods ($n=40$) were calculated: the correlation between the Folch modified procedure and the ASE-10 method was higher ($r=0.94$) than that between the acid hydrolysis procedure and the ASE-10 method ($r=0.88$); these data confirm the validity of using ASE-10 instead of the Folch modified procedure, at least for quantitative purposes.

Table 3
Total lipids (%) (w/w) of 20 samples of boned and homogenised poultry hips, extracted by the modified Folch method, the acid hydrolysis procedure and the ASE-10 method[a]

Samples	Modified Folch	Acid hydrolysis	ASE-10
1	15.5a,b[b]	17.7a	14.1b
2	15.6a	17.7a	16.2a
3	15,0a	17.4a	15.3a
4	14.7a	15.8a	14.4a
5	14.6b	15.9a	15.6a
6	17.2a	18.3a	17.6a
7	12.7a	13.5a	11.6a
8	12.8a	13.0a	11.0a
9	20.8a,b	22.7a	20.5b
10	17.1a,b	18.2a	16.4b
11	16.9a	17.6a	17.0a
12	13.1a,b	13.8a	11.7b
13	18.8a,b	17.9a	17.4b
14	16.0a	18.7a	15.4a
15	18.1a	17.9a	18.1a
16	15.8a	17.5a	15.0a
17	15.2a	15.9a	15.3a
18	18.7a	18.5a	19.0a
19	16.5a	17.3a	16.0a
20	17.3a	18.7a	18.3a
Yield[c]	93.8	100	91.7

Different letters in the same line indicate significantly different values ($P<0.05$).

[a] The conditions for the ASE-10 method are listed in Table 1.

[b] g of fat/100 g of boned and homogenised meat.

[c] Calculated setting the yield for the hydrolytic procedure at 100%.

Table 4
Fatty acid composition of the total lipids from sample 5, extracted by the modified Folch method, the ASE-7, the ASE-10 and the acid hydrolytic procedure (weight% of the total)

	Sample 5			
Fatty acids	ASE-10[a]	ASE-7[b]	Modified Folch	Acid hydrolysis
C12:0	0.1	0.2	0.2	0.1
C14:0	1.2	1.2	1.3	1.1
C14:1	0.3	0.3	0.3	0.3
C15:0	0.1	0.1	0.2	0.1
C16:0	24.4	24.6	25.7	24.2
C16:1-t[c]	0.5	0.5	0.4	0.5
C16:1 (n-9)	5.7	5.8	5.9	5.6
C16:1 (n-7)	0.1	0.1	0.2	0.1
C17:0	0.3	0.3	0.3	0.3
C17:1 (n-7)	0.2	0.3	0.2	0.3
C18:0	6.3	6.2	6.1	5.8
C18:1-t	0.5	0.5	0.4	0.6
C18:1 (n-9)	37.7	37.8	37.0	40.2
C18:1	2.2	2.2	2.2	2.2
C18:2-t	tr[d]	tr	tr	tr
C18:2 (n-6)	17.5	17.4	17.2	16.5
C18:3 (n-6)	0.2	0.2	0.2	0.1
C18:3 (n-3)	1.2	1.2	1.1	1.2
C20:0	tr	tr	tr	tr
C20:1	0.4	0.4	0.4	0.4
C20:2	0.2	0.2	0.2	0.1
C20:3 (n-3)	0.2	0.2	0.1	tr
C20:4 (n-6)	0.4	0.4	0.4	0.2
C22:2	tr	tr	tr	tr
C22:4	0.1	tr	0.1	0.2
C22:5	tr	tr	tr	tr
C22:6	tr	tr	tr	tr
Total	100.0	100.0	100.0	100.0
Uns/Sat	1.98	1.96	1.87	2.04

[a] ASE 10: chloroform/methanol 2:1 (v/v), 2 static cycles of 10 min each, $T=120$ °C and $P=20$ MPa.

[b] ASE 7: chloroform/methanol 2:1 (v/v), 2 static cycles of 10 min each, $T=60$ °C and $P=20$ MPa.

[c] Tentative identification.

[d] Traces < 0.1%.

4. Conclusions

An ASE method for the extraction and the quantification of poultry fat was investigated. The solvent mixture employed was the same as used for the traditional Folch procedure, chloroform/methanol 2:1, at a pressure of 20 MPa, using two static cycles of 10 min each. The consumption of solvents was reduced from a half to one third, relative to those required by the traditional Folch method. Hence, the time of extraction was reduced to 1 h, including washing and solvent evaporation. The recovery was 97.8% of that by the modified Folch procedure when calculated as the mean of 20 samples. The ASE instrument required about an 80 l of nitrogen for each extraction (2 cycles of 10 min each, 120 s of purge). To obtain a quantitative extraction, it was better to grind the homogenised tissue with a material having a high particle size, such as Hydromatrix, to permit the permeation of the solvent, rather than use a powdery product, such as Celite. A high correlation between the modified Folch and ASE-10 total lipid extraction procedures was found. The fatty acid compositions (as methyl esters) of the poultry lipid, extracted by the three extraction methods and analysed by CGC, were found to be equivalent.

References

Barbano, D. M., & Clark, J. L. (1988). Comparison of babcock and ether extraction methods for determination of fat content of milk: collaborative study. *Journal of the Association of Office Analytical Chemists*, *71*, 898–903.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*, 911–917.

Christie, W. W. (1989). *Gas chromatography and lipids a practical guide*. Scotland: The Oily Press.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, *226*, 497–509.

Hara, A., & Radin, N. S. (1978). Lipid extraction of tissues with a low-toxicity solvent. *Analytical Biochemistry*, *90*, 420–426.

Radin, N. S. (1981). Extraction of tissue lipids with a solvent of low toxicity. *Methods in Enzymology*, *72*, 5–7.

Ways, P., & Hanan, D. J. (1964). Characterization and quantification of red cell lipids in normal man. *Journal Lipid Research*, *5*, 318–328.