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Rapid microwave assisted extraction of meat lipids

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ABSTRACT

A new method for microwave assisted extraction (MAE) of meat lipids using a non-halogenated solvent and at low temperature was developed. The effect of microwave irradiation on lipid oxidation during extraction was verified by conjugated dienes, peroxide index, volatile compound (hexanal, pentanal and propanal) and fatty acid analyses. The method showed to be precise and accurate at comparison with Folch extraction and by validation with standard reference material. No changes occurred in the fatty acid composition and no lipid oxidation products were detected. The optimized and validated method was applied to meats with different lipid contents. The results showed that MAE can be used to study lipids from meat samples without the risk of chemical changes during the extraction process, allowing for automation, precision, accuracy, reduction in extraction time, lower cost, reductions in sample size and solvent consumption, hence producing fewer residues for the environment.

1. Introduction

Total lipid content of meat is an important parameter used in biochemical, physiological and nutritional studies. Thus, reliable methods for the quantitative extraction of lipids from this type of food matrices are of critical importance (Iverson, Lang, & Cooper, 2001). Lipids in muscle foods are a mixture of nonpolar components (mainly acylglycerides and cholesterol, which are easily soluble in nonpolar organic solvents) and complex lipids, such as phospholipids, glycolipids, partial glycerides and free fatty acids. Complex lipids are more difficult to extract than simple lipids since they are linked by hydrophobic, van der Waals and hydrogen bonds or by ionic bonds; therefore, the use of polar solvents is mandatory (Ruiz, Antequera, Andres, Petron, & Muriel, 2004; Toschi, Bendini, Ricci, & Lercker, 2003). Thus, the solvent or solvent mixture used for lipid extraction must show adequate polarity to extract both polar and nonpolar lipids (Pérez-Palacios, Ruiz, Ferreira, Petisca, & Antequera, 2012).

Several methods have been developed for total lipid extraction, being the Soxhlet method the official AOAC-recommended method (AOAC, 1997), and the methods described by Folch, Lees, and Stanley (1957) and Bligh and Dyer (1959), which use a mixture of chloroform and methanol, the most used methods for lipid extraction from meat and meat products. However, some problems are associated with these conventional extraction techniques since they are labor intensive, time consuming, difficult to automate, use toxic solvents and often require a post-extraction clean-up step.

An extraction technology should be versatile, relatively simple, safe and of low cost (Letellier & Budzinski, 1999). Thus, the development of new extraction procedures that overcome the limitations imposed by the conventional methods is desirable. Microwave assisted extraction (MAE) has potential to extract compounds from diverse materials by combining the action of microwave energy with solvents. This technology is passive to automation, decreases the extraction time and reduces the consumption of organic solvents, consequently reducing laboratory residues and sample preparation costs, as well as improving extraction efficiency (Paré, Bélanger, & Stafford, 1994; Kwon, Lee, Bélanger, & Pare, 2003; Regueiro, Llompart, García-Jares, & Cela, 2006).

Different compounds have already been obtained by MAE, such as saponins (Kwon et al., 2003; Hu, Cai, & Liang, 2008), phenolic compounds (Beejmohun et al., 2007; Sun, Liao, Wang, Hu, & Chen, 2007; Terigar et al., 2010) lignin (Li, Sun, Xu, & Sun, 2012), pectin (Wang et al., 2007), polycyclic aromatic hydrocarbons (Pena et al., 2006) and organic acids (Papadakis & Polychroniadou, 2005). Few reports about MAE lipid extraction are found in the literature. Most of these studies are about lipid MAE from microalgae or other matrices to produce

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biodiesel (Iqbal & Theegala, 2013; Dai, Chen, & Chen, 2014); however, reports on green coffee oil extraction, quantification of diterpenes (Tsukui et al., 2014) and lipid extraction from fish (Ramalhosa et al., 2012) are found in the literature. Concerning the latter study, despite the high fish lipid extraction efficiency, the method uses high solvent volume (30 mL) and temperature (90 °C). Moreover, the evaluation of lipid degradation, which could have been influenced by the conditions applied in such method, was not evaluated. Other systems using microwave energy, such as the microwave-integrated Soxhlet extraction system which is based on the same principles as a conventional Soxhlet extractor but modified to facilitate accommodation of the sample cartridge compartment in the irradiation zone of a microwave oven (Luque-García & Luque de Castro, 2004), were used for lipid extraction from olive (Virot, Tomao, Colnagui, Visinoni, & Chemat, 2007) and bakery products (Priego-Capote & Luque de Castro, 2005). The disadvantages of this system are that it allows the extraction of only one sample at a time and uses a large solvent volume, in a similar way to the Soxhlet extraction. Focused open vessel microwave assisted extraction was used for lipid extraction from fish (Batista, Vetter, & Luckas, 2001). This system is less safe and loss of compounds can occur during extraction because it consists of an open tube. However, none of these studies verified if degradation of lipid compounds occurred during extraction.

Based on the exposed above, the objectives of the present study were to develop and validate a MAE method for meat lipids using a non-halogenated solvent at low temperature. To verify if the energy applied during MAE can generate lipid oxidation products, the extracted lipids were submitted to analysis of conjugated dienes, peroxide index, volatile compounds and fatty acid composition. The MAE method was optimized by means of a central composite design (CCD) using chicken breast as food matrix. The validated method was then applied to several meat samples with different lipid contents, namely chicken leg, chicken thigh, fresh ham, pork loin, eye round and beef hump.

2. Material and methods

2.1. Samples

Six hundred grams of chicken breast, chicken leg, chicken thigh, fresh ham, pork loin, eye round and beef hump were acquired in the local market (Campinas, São Paulo, Brazil). After removing the superficial fat, each sample was homogenized in a food processor (Philco, Brazil) for 1 min. Chicken breast was used for method development and validation, and the other meat samples for application of the validated method. Moisture content was determined by AOAC (1997). The standard homogenized meat reference material SRM 1546 was obtained from NIST (Gaithersburg, MD, USA).

2.2. Reagents

Boron trifluoride (BF_3) in methanol (13–15%), mixture of 37 fatty acid methyl ester standards from 4:0 to 24:0 (FAME MIX, ref. 47885-U, Sulpeco Co., Bellefonte, CA, USA), methyl esters of undecanoic (99% purity) and tricosanoic (99% purity) acids, 2-heptanone (99% purity), hexanal (98% purity), pentanal (97% purity) and propanal (97% purity) were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade hexane was acquired from JT Baker (Phillipsburg, NJ, USA). Analytical grade chloroform, methanol, hexane, ethyl acetate, isopropanol, iso-octane, sodium hydroxide (NaOH), sodium chloride (NaCl), barium chloride, ferrous chloride, ferrous sulfate, hydrochloric acid and ammonium thiocyanate were obtained from Synth (Diadema, SP, Brazil).

2.3. Extraction solvent

In an attempt to substitute the chloroform:methanol (2:1 v/v) with a solvent less harmful to human health and to environment, hexane, ethyl acetate, isopropanol, iso-octane, ethyl acetate:isopropanol (7:3, v/v), hexane:isopropanol (3:1, v/v), ethyl acetate:methanol (2:1, 4:1 and 9:1, v/v) and hexane:methanol (3:1, v/v) were evaluated as extraction solvent. MAE conditions were established in preliminary assays using chloroform:methanol (2:1, v/v) as extraction solvent. MAE conditions were 400 mg of sample, 5 mL of solvent, microwave extraction during 10 min at 50 °C and 400 W.

2.4. Dielectric permittivity parameters

The dielectric properties (dielectric constant and dielectric loss) of the solvents and solvent mixtures used to extract the lipids were determined in a HP 85070B measurement system (Agilent Technologies, Palo Alto, CA, USA) connected to a HP 8752C network analyzer (Agilent Technologies, Palo Alto, CA, USA). Readings were made in 201 point frequency scan from 300 kHz to 6 GHz. Calibration was carried out using the 3 point method (short-circuit, air and water at 25 °C).

2.5. Experimental statistical design

Optimization of lipid extraction using MAE was carried out by way of an experimental design considering the following variables: sample mass (230 to 670 mg), irradiation time (2 to 18 min) and temperature (30 to 60 °C). The independent variable levels were selected based on preliminary experiments. A 2^3 CCD with 3 central points and 6 axial points was carried out randomly according to the analysis order arranged by the software Statistica 7.0 (StatSoft, Inc., Tulsa, OK, USA), giving a total of 17 trials.

2.6. Lipid extraction by conventional method

Lipids were extracted according to Folch et al. (1957) and lipid content was gravimetrically determined.

2.7. Microwave assisted extraction

Microwave assisted extraction was carried out using the Start-E microwave extraction system (Milestone, Sorisole, Italy). Extraction parameters (sample weight, irradiation time and temperature) were set according to the experimental design and maximum power was programmed to be 400 W. Sample was weighed in a Teflon tube and 5 mL of extraction solvent was added. A temperature program was set in the equipment to reach the desired temperature within 1 min, and after that to maintain a constant temperature during the process. After irradiation, the tubes were automatically cooled for 10 min and the samples were filtered through a qualitative filter paper. Water (3.5 mL) was added to the filtered extract and the mixture was centrifuged at 3000 *g* (Alegra 64R Centrifuge, Beckman Coulter, Fullerton, CA, USA) for 5 min at 10 °C. The lipid phase was separated, transferred to a previously weighed test tube and the solvent was removed under nitrogen flow. The extracted lipid content was determined gravimetrically.

2.8. Fatty acid determination

An aliquot of the lipids (25 mg) obtained by MAE was saponified and methylated according to Joseph and Ackman (1992). The fatty acid methyl esters were separated in a CP-SIL 88 column (Chromopack, 100 m × 0.25 mm × 0.20 μm) in a gas chromatograph (GC-2010, Shimadzu) equipped with a flame ionization detector and split injector (1/50). The chromatographic conditions were according to Sancho, De Lima, Costa, Mariutti, and Bragagnolo (2011).

The fatty acids were identified by comparison of the retention times of the fatty acid methyl ester standards with those of the fatty acid methyl ester peaks in the samples. Quantification was carried out by internal standardization, using undecanoic and tricosanoic acid methyl esters as internal standards. Fatty acid

content was calculated in mg/100 g of meat according to the AOCS (1997).

2.9. Lipid oxidation products

2.9.1. Conjugated dienes

Conjugated dienes (CD) were measured according to the IUPAC official method number 2.505 with the modifications described by Dimakou, Kiokias, Tsaprouni, and Oreopoulou (2008). Absorbance was measured at 232 nm in a UV–Vis spectrophotometer (Agilent, Santa Clara, CA, USA). The limit of detection (LOD) was 0.2454 g/kg lipid and limit of quantification (LOQ) was 0.3589 g/kg lipid, calculated by visual method (Ribani, Bottoli, Collins, Jardim, & Melo, 2004).

2.9.2. Peroxide content

Method 74 A:1991 of International IDF Standards (1991) was used to determine the peroxide content, using a UV–Vis spectrophotometer (Agilent, Santa Clara, CA, USA). The LOD was 0.0729 meq O_2/kg lipid and LOQ was 0.220 meq O_2/kg lipid, calculated seven-point using analytical curves with ferrous chloride as standard (linear range 1 to 40 μg Fe) (Ribani et al., 2004).

2.9.3. Volatile compounds

Sample preparation for the analysis of the volatile compounds hexanal, pentanal and propanal was carried out according to Ulberth and Roubicek (1993), with modifications. A 25 mg lipid aliquot was added to a 10 mL vial together with the internal standard (2-heptanone, 0.1 μg/mL). The vials were sealed with magnetized aluminum caps and Teflon covered silicone septa and heated in the incubator of the auto-injector at 60 °C and 250 rpm for 1 h before injection.

The chromatographic analyses were carried out based on the method described by Shahidi and Pegg (1994) with modifications on the initial and final temperatures of the chromatographic run. A gas chromatograph (GC) coupled to a mass spectrometer detector (GCMS-QP2010 Ultra Shimadzu, Kyoto, Japan) with an ion source operating in the electron ionization (EI) mode, quadrupole *m/z* analyzer and AOC-5000 injection system was used. The compounds were separated on a RTX-Wax capillary column (Restek, 30 m × 0.25 mm i.d. × 0.25 μm) using the following temperature program: 30 °C for 8 min, increasing to 115 °C at 10 °C per min, maintaining this temperature for 4 min, then increasing to 200 °C at 30 °C per min and maintaining this temperature for 2 min, giving a total run time of 23 min. The stripping gas was helium at a constant flow of 1.2 mL/min. Injection volume was 1 mL of the volatile compounds present in the headspace of the vials with the injector operating in the split mode (1:7) at 180 °C. Interface temperature was maintained at 210 °C. Ionization energy was 70 eV. The target ions (28, 29, 44, 56, 57, 58 and 72) were monitored in the selected ion monitoring (SIM) mode.

The volatile compounds were identified by comparison of the mass spectra obtained for the samples with the mass spectra of analytical standards analyzed under the same experimental conditions and with those in the Wiley Registry of Mass Spectral Data (2011), and by comparison of the retention times of the standards with those of the peaks in the samples. Quantification was carried out by comparison of the area of the sample peak with that of the internal standard 2-heptanone. The detection limits for hexanal, pentanal and propanal were, respectively, 0.04, 0.02 and 0.01 ng/g of lipids.

2.10. Validation of lipid MAE

Validation of the lipid MAE method for chicken breast was carried out by evaluation of precision and accuracy by comparing the results obtained with the optimized method with those obtained by Folch extraction, and by the use of standard homogenized meat reference material (SRM 1546, NIST). The Z score, which represents the performance of the laboratory, was also determined.

If $|Z| \leq 2$, the results are satisfactory; $2 < |Z| \leq 3$, the results are questionable; and $|Z| > 3$, the results are unsatisfactory.

Method robustness of the validated method was evaluated by analyzing different meats (chicken leg, chicken thigh, fresh ham, pork loin, eye round and beef hump).

2.11. Statistical analysis

The statistical experimental design and the statistical analysis of the data were carried out using the software Statistica 7.0 (StatSoft Inc., Oklahoma, USA). The results obtained and the means were compared using Tukey's test (confidence level of 90%).

3. Results and discussion

3.1. Moisture content

Moisture contents varied from 70.1 to 76.9 g/100 g (Supplementary information, table S1), indicating that most of the microwave energy could be absorbed during MAE.

Microwave effect is strongly dependent on the dielectric properties; therefore, to facilitate matrix heating and hence increasing the mass transference of the desired compound to the extraction solvent, the extraction of the compounds is carried out in solvents containing small amounts of water (Pan, Niu, & Liu, 2001; Talebi, Ghassempour, Talebpour, Rassouli, & Dolatyari, 2004), or the sample is immersed in water prior to extraction (Pan, Liu, Jia, & Shu, 2000; Pan, Niu, & Liu, 2003). In principle, this step is not necessary in lipid extraction from meat because of its high moisture content.

3.2. Extraction solvent and dielectric permittivity parameters

Distinct behaviors were noticed for the different solvents or solvent mixtures in MAE and in Folch extraction. In Folch extraction, extraction depends mainly on the compound solubility in the solvent, mass transfer and force of the solute/matrix interactions. However, under the influence of microwave, heating has an important role in extraction efficiency and hence MAE success is also determined by the dielectric properties of the extraction solvent.

The dielectric properties of a material are defined as function of the dielectric constant (ε') and the dielectric loss (ε"). Table 1 shows the dielectric constants and dielectric losses of the solvents used at 2450 MHz frequency, and also the recoveries of the extracted lipids when compared to Folch extraction using chloroform:methanol. Chloroform:methanol (2:1, v/v), ethyl acetate:methanol (2:1, v/v) and ethyl acetate:methanol (4:1, v/v) showed high ε' values, indicating that these solvent mixtures are good media for microwave energy absorption, and ε" values indicated efficient conversion of the absorbed microwave energy into heat. The use of these tree solvent mixtures for lipid microwave assisted extraction resulted in lipid contents similar to the values obtained by using the conventional method.

Hexane and iso-octane have low ε' and ε" values and were not efficient in extracting the lipids, possibly due to these characteristics and the extraction conditions used in this study, such as temperature below the boiling point of these solvents and relatively short extraction time. On the contrary, some studies indicate these solvents for the extraction of thermolabile compounds at low temperatures (Paré et al., 1994; Camel, 2000).

The mixture ethyl acetate:methanol (2:1, v/v) was chosen as the lipid extraction solvent because it is less toxic and the results of lipid extract obtained with this mixture was similar to the results obtained with chloroform:methanol (2:1, v/v).

Table 1
Dielectric permittivity parameters at 25 °C and recovery of the extracted lipids.

Solvent	Dielectric constant (ε')	Dielectric loss (ε")	Lipid recovery (%)[a]
Chloroform:methanol (2:1, v/v)	10.6	5.3	100
Hexane	2.3	0.6	10
Isopropanol	4.8	4.5	0
Isooctane	2.2	0.5	6.2
Ethyl acetate	6.8	1.4	50
Hexane:isopropanol (3:1, v/v)	2.8	1.0	8.7
Hexane:chloroform (3:1, v/v)	2.8	0.5	3.7
Ethyl acetate:isopropanol (7:3, v/v)	8.4	2.9	0
Ethyl acetate:methanol (2:1, v/v)	13.0	3.6	100
Ethyl acetate:methanol (4:1, v/v)	10.3	1.7	100
Ethyl acetate:methanol (9:1, v/v)	8.2	0.9	75

[a] Relative to Folch extraction.

3.3. Optimization of the microwave assisted extraction of lipids

Lipid content of chicken breast assayed by Folch extraction was 2.18 ± 0,11 g/100 g. Table 2 shows the CCD matrix for the trials and results for lipid MAE. The central points showed little variation (RSD = 1.9%), indicating good repeatability of the extraction process.

The regression coefficients for the microwave assisted extraction of lipids (supplementary information, table S2) showed that the linear parameters sample mass (m) and extraction time (t), and the interaction parameter between sample mass and extraction time (m t) were significant ($p < 0.1$). Eq. (2) corresponds to the model equation.

$$y = 1.72 - 0.15\,m + 0.16\,t + 0.09\,m\,t \quad (2)$$

Considering only the significant terms, ANOVA table (Supplementary information, table S3) shows the analysis of variance for MAE. The coefficient of determination for MAE was 80%. The model was significant and adequate to describe the results by way of a surface response, as evidenced by the calculated F value.

Sample mass and extraction time were statistically significant; thus, only one surface response was generated (Fig. 1). Lipid contents ranged from 1.35 to 2.15 g/100 g, and the surface response indicated that the highest lipid content was extracted when sample mass was below 300 mg and extraction time was above 15 min.

Lipid content increased with the increase in extraction time. Lipid extraction was always efficient with longer extraction times, even with variations in sample mass (Fig. 1) or at lower temperatures (Table 2). Thus, the optimum extraction conditions were achieved at 16 min (1 min to reach 54 °C and this temperature was maintained for 15 min).

Lipid extraction was not efficient in the trials with larger sample masses. The maximum temperature and extraction time used in this study possibly were not sufficient for the exhaustive extraction of the lipids from larger sample masses. Under the studied conditions, the optimum mass to guarantee an efficient lipid extraction was 300 mg.

An increase in extraction temperature normally favors the dissolution of compounds because high temperatures decrease the intermolecular interactions with the solvent, increasing cell mobility consequently increasing solubility. An increase in temperature can also cause cell rupture, leaving the cell compounds more available for solvent extraction. In addition, solvent viscosity decreases at high temperatures and diffusivity increases; thus, also increasing extraction efficiency (Hao, Han, Huang, Xue, & Deng, 2002). On the other hand, lipid oxidation can occur at high temperatures. Although the temperature was not statistically significant in this study, the best extraction was obtained at higher temperatures (Table 2). Thus, 54 °C was chosen as the optimum extraction temperature.

The optimized conditions for lipid MAE (300 mg sample, 5 ml ethyl acetate:methanol (2:1, v/v), 54 °C, 400 W and 16 min; 1 min to reach 54 °C and this temperature was maintained for 15 min) were validated in order to compare the predicted value with the experimental one. A triplicate analysis gave a value of 2.11 ± 0.05 g/100 g of total lipids, which was very close to that of 2.05 g/100 g predicted by the model equation. The good correlation between these results confirmed the

Table 2
CCD matrix for the microwave assisted extraction of lipids from chicken breast.

Trials	Variables			Lipids (g/100 g)
	Temperature (°C)	Sample mass (mg)	Time (min)	
1	36	300	5	1.95
2	54	300	5	1.55
3	36	500	5	1.38
4	54	500	5	1.35
5	36	300	15	2.07
6	54	300	15	2.15
7	36	500	15	1.74
8	54	500	15	1.97
9	30	400	10	1.58
10	60	400	10	1.86
11	45	230	10	1.92
12	45	670	10	1.45
13	45	400	2	1.40
14	45	400	18	1.75
15	45	400	10	1.80
16	45	400	10	1.66
17	45	400	10	1.75

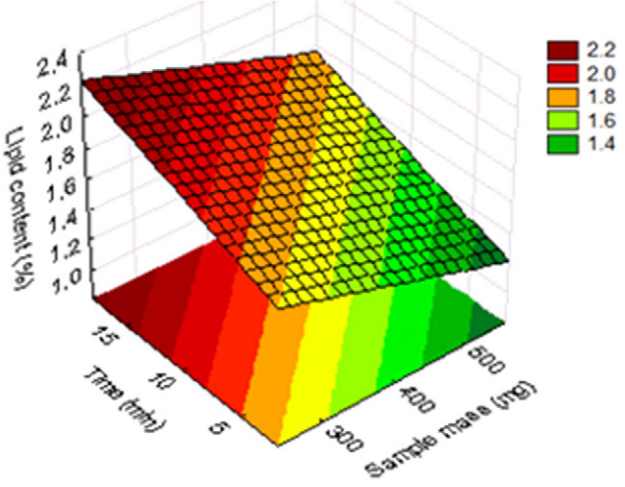

Fig. 1. Surface response for microwave assisted extraction of lipids from chicken breast (g/100 g) as a function of extraction time and sample mass.

Table 3
Fatty acid composition of chicken breast lipids (mg/100 g) obtained by Folch extraction and MAE method.

Fatty acids	Folch extraction[a]	MAE[a]
10:0	0.29 ± 0.02	0.23 ± 0.00
12:0	2.21 ± 0.10	2.34 ± 0.23
14:0	10.98 ± 0.55	10.44 ± 1.17
15:0	2.05 ± 0.11	1.94 ± 0.19
16:0	408.03 ± 15.82	415.02 ± 33.47
17:0	3.33 ± 0.07	3.31 ± 0.31
18:0	133.19 ± 8.53	113.34 ± 7.16
20:0	1.60 ± 0.06	1.78 ± 0.17
22:0	0.94 ± 0.05	0.90 ± 0.10
14:1n-5	2.49 ± 0.15	2.42 ± 0.25
16:1n-6	81.80 ± 5.83	80.14 ± 7.48
17:1n-7	7.54 ± 0.25	8.89 ± 1.04
18:1(9*t*)	2.88 ± 0.16	3.35 ± 0.06
18:1n-9	628.05 ± 30.10	626.57 ± 32.94
18:2n-6	471.43 ± 20.91	459.18 ± 24.31
18:3n-6	3.31 ± 0.11	3.55 ± 0.42
20:1n-9	4.32 ± 0.11	4.49 ± 0.42
18:3n-3	25.02 ± 0.69	25.19 ± 2.86
20:2n-6	6.42 ± 0.20	6.87 ± 0.59
20:3n-6	7.30 ± 0.26	8.50 ± 0.31
22:1n-9	0.30 ± 0.01	0.38 ± 0.04
20:3n-3	0.45 ± 0.03	0.47 ± 0.03
20:4n-6	45.31 ± 3.06	47.99 ± 1.78
20:5n-3	1.54 ± 0.04	1.72 ± 0.15
24:1n-9	0.80 ± 0.05	0.81 ± 0.06
22:6n-3	3.58 ± 0.07	3.35 ± 0.39

Fatty acid composition did not present statistical difference between the extraction methods ($p < 0.1$).
[a] Mean and standard deviation ($n = 3$).

validity of the model and demonstrated that the chosen conditions were adequate for lipid extraction.

The fatty acid composition of the lipids obtained by the MAE method was compared with that obtained by Folch extraction to verify if MAE caused any changes in the fatty acid composition (Table 3). The major fatty acids were oleic (18:1n-9), linoleic (18:2n-6), palmitic (16:0) and stearic (18:0) acids. No fatty acid showed a significant statistical difference. In general, MAE did not alter the fatty acid composition of the analyzed samples.

There were no statistical differences between the fatty acid contents using the lipids obtained by the two extraction methods ($P < 0.1$). The fatty acids 8:0, 13:0, 15:1n-5, 18:2 (9*t*, 12*t*), 19:0, 21:0, 22:2n-6 and 24:0 were not detected.

3.4. Lipid oxidation products

Lipid oxidation is a degradation process that decreases the sensory and nutritive quality of food products, also forming undesirable toxic compounds, making the product less acceptable or unacceptable for consumption. The main components involved in lipid oxidation are the unsaturated fatty acids and oxygen. This reaction is also affected by several factors such as type, content and activity of pro- and antioxidants, irradiation, temperature, natural sensitizers, contact surface area with oxygen and water activity. Radicals are formed during the initial steps of oxidation generating unstable intermediates, such as conjugated dienes and hydroperoxides, which degrade to produce low molecular weight compounds responsible for off-flavor development during the propagation step of lipid oxidation (Pan et al., 2001; Talebi et al., 2004). Chicken meat is especially sensitive to lipid oxidation due to its high unsaturated lipid content. Fig. 2 shows the contents of conjugated dienes, peroxides and hexanal found in the lipids extracted from chicken breast by the MAE and Folch extraction methods. Pentanal and propanal were not detected in both methods. According to Kiokias, Dimakou, and Oreopoulou (2007) the initial stage of lipid oxidation starts when the values obtained for conjugated dienes are above 5 g/kg. With respect to peroxides, low lipid oxidation of a food

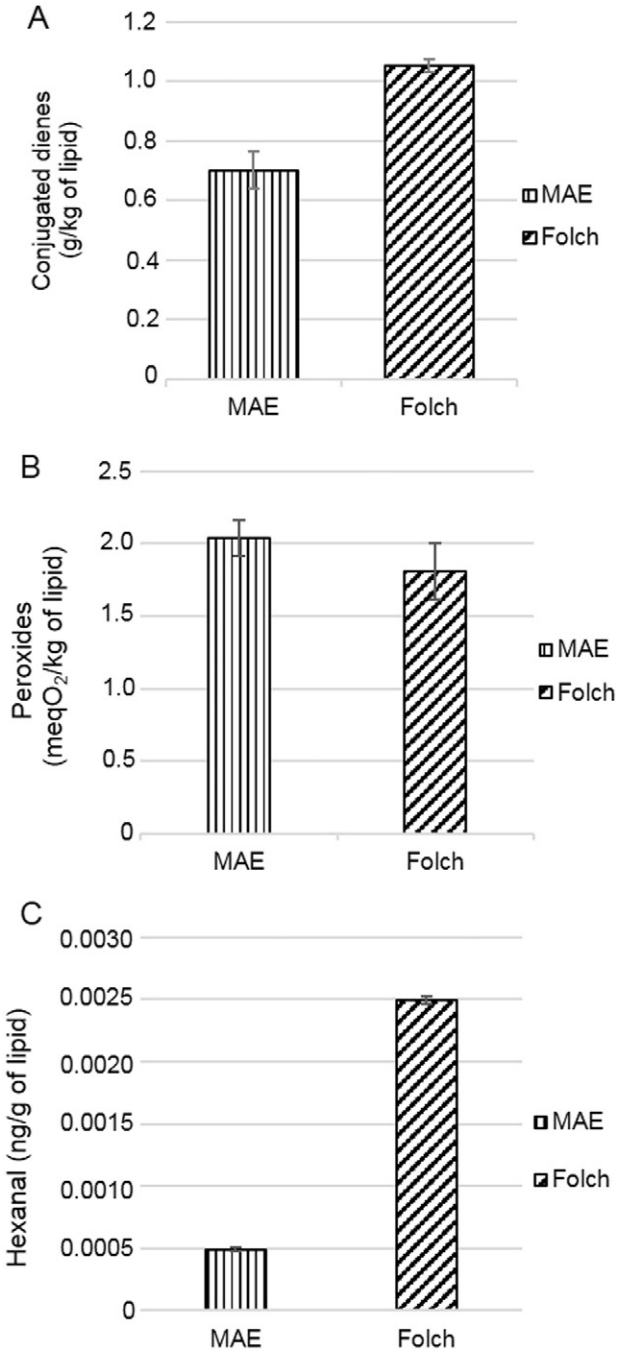

Fig. 2. Content of conjugated dienes (g/kg of lipid) (A), peroxides (meqO_2/kg of lipid) (B) and hexanal (ng/g of lipid) in the lipid extracted from chicken breast by Folch method and MAE method.

product is considered when peroxide values are below 10 meq O_2/kg (Codex Alimentarius Commission, 1999). The conjugated diene and peroxide contents found in the samples extracted by the two extraction methods were below the indices suggested in the literature.

For both extraction methods, hexanal was the only aldehyde detected among the analyzed compounds. In general, low levels of hexanal were found after using the two extraction methods when compared with other studies (Elmore, Mottram, Enser, & Wood, 1999; Kiokias, Dimakou, & Oreopoulou, 2009). Propanal is the main marker of n-3 PUFA oxidation, whereas pentanal and hexanal are markers of n-6 PUFA oxidation (Laguerre, Lecomte, & Villeneuve, 2007). Linoleic acid was the most abundant polyunsaturated acid present in the analyzed sample, which explains the formation of hexanal in the extracted lipids.

These results show that under the conditions applied in this study, MAE did not promote lipid oxidation when compared with the Folch method; therefore it can be used for the extraction and study of meat lipids.

3.5. Validation of MAE

Lipid MAE method was validated by comparison of the results obtained by the developed MAE method with Folch extraction using chicken breast and standard homogenized reference material (SRM 1546, NIST). The replicates (n = 10) of each extraction presented a relative standard deviation (RSD) below 8%.

No statistical differences were found between both extraction methods for lipid and individual fatty acid contents (Table 3). Table 4 shows the results obtained for the extraction of lipids and fatty acid composition from the SRM 1546. The results obtained in this study are in agreement with the certificate values. Z-score values were $|Z| \leq 2$ for total lipid and for all fatty acid contents; thus, the method is capable of producing satisfactory results. Lipid extraction by MAE was shown to be a precise and accurate method. Thus, the lipids obtained by MAE can be used in the determination of the fatty acid composition of meat samples.

In addition to its precision and accuracy, the validated MAE method shows other advantages when compared with Folch extraction, such as fast simultaneous lipid extraction from multiple samples (reduction of 75% in extraction time), reduced costs, less harmful to the environment (95% reduction in solvent consumption and the substitution of chloroform by ethyl acetate), smaller sample size and less laborious, since most of the process is carried out automatically by the equipment.

3.6. Application

The optimized and validated MAE method was applied to meat samples containing different lipid contents. Table 5 shows the lipid contents of meat samples extracted by Folch extraction and by MAE. The lipid content varied between 1 and 5 g/100 g and no differences were observed between the two extraction methods, which indicate that MAE is capable of extracting variable lipid contents, as also demonstrated for the standard reference material containing 20 g/100 g of lipids.

The fatty acids obtained by Folch extraction and by MAE are presented in tables S4, S5 and S6 (Supplementary information). Most of the fatty acids did not show statistical differences between the two extraction methods, except 24:1n-9 for chicken leg, 20:0 for chicken thigh, 15:0 and 22:6n-3 for pork loin and fresh ham, 20:1n-9 and 20:4n-6 for eye round. These few differences between methods were found only for minor fatty acids, where a small variation in the fatty acid content can result in a statistical difference. However, the total fatty acid contents of the evaluated samples were not altered.

Table 4
Lipid and fatty acid contents extracted by MAE from the standard reference material (SRM 1546, NIST) and values declared on the SRM.

	MAE[a]	SRM 1546	z-score
Lipids (g/100 g)	21.61 ± 0.53	21.00 ± 1.40	0.435
Fatty acids (g/kg)			
10:0	0.193 ± 0.014	0.170 ± 0.032	0.718
12:0	0.123 ± 0.013	0.133 ± 0.028	0.357
14:0	2.479 ± 0.130	2.530 ± 0.190	0.268
16:0	51.170 ± 4.048	45.600 ± 3.900	1.428
18:0	21.945 ± 1.952	21.700 ± 2.900	0.084
20:0	0.375 ± 0.042	0.315 ± 0.063	0.952
16:1n-7	6.681 ± 0.484	6.830 ± 0.660	0.225
18:1n-9	88.950 ± 7.157	82.000 ± 9.600	0.723
18:2n-6	19.820 ± 0.859	19.600 ± 2.000	0.110
18:3n-3	1.246 ± 0.132	1.410 ± 0.350	0.468
20:1n-9	1.687 ± 0.102	1.560 ± 0.230	0.552
20:4n-6	0.561 ± 0.059	0.560 ± 0.250	0.004

[a] Mean ± standard deviation (n = 3).

Table 5
Lipid contents (g/100 g)* extracted by Folch extraction and by MAE from chicken, pork and beef meats.

Sample	Folch extraction[a]	MAE[a]
Chicken		
Leg	2.06 ± 0.22	2.10 ± 0.15
Thigh	2.48 ± 0.19	2.48 ± 0.22
Pork		
Fresh ham	3.56 ± 0.26	3.57 ± 0.27
Loin	3.36 ± 0.20	3.21 ± 0.31
Beef		
Eye round	1.15 ± 0.08	1.15 ± 0.08
Hump	5.41 ± 0.15	5.35 ± 0.43

Lipid contents did not present statistical difference between the extraction methods ($p < 0.1$).

[a] Mean ± standard deviation (n = 10).

4. Conclusions

The new microwave assisted extraction method of meat lipids was developed and validated becoming the method of choice because of its precision, accuracy and fastness. This is the first time that a study assessing compounds from lipid degradation after microwave assisted extraction is reported. Under optimal conditions, extraction time is reduced by 75%. Other advantages of the lipid MAE method are the reduction of manual manipulation leading to less labor costs, reduction of sample size and reduced consumption of organic solvents in sample preparation step (95% reduction). In addition, the present method requires less solvent, less time and lower temperature than any other MAE method found in the literature.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.foodres.2015.10.028.

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