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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