

Research note

The microwave-assisted process (MAP^{TM1}): Extraction and determination of fat from cocoa powder and cocoa nibs [☆]

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Abstract

This study aimed at replacing the conventional soxhlet extraction of the AOAC determination of fat by the microwave-assisted process (MAP). Fat contents were determined for cocoa powder and *Theobroma cocoa* nibs originating from the Barlovento area, Venezuela. Fats from cocoa powder and pre-treated ground nibs were extracted using different solvents that are relatively transparent to microwaves, i.e., petroleum ether, hexane, acetone, and isopropanol. The determinations were performed using a Prolabo Soxwave 3.6 MAP extraction system and the AOAC methods. The fatty acid profile of cocoa powder obtained from the different extraction methods was also characterized to establish similarities between the various conditions. Results showed that compared to conventional methods, MAP can be used as a relatively faster process of extracting fat and offering the advantages of low consumption of expensive organic solvents, short extraction time, less energy consumption, and excellent reproducibility. Crown Copyright © 2006 Published by Elsevier Ltd. All rights reserved.

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1. Introduction

The microwave-assisted process (MAP) is a relatively novel method of extracting soluble products into a fluid from a wide range of materials using microwave energy (Paré & Bélanger, 1997; Paré, Bélanger, & Stafford, 1994), and is patented by Environment Canada (Paré, 1994, 1995; Paré, Bélanger, & Punt, 2000; Paré, Sigouin, & Lapointe, 1991; Camel, 2000). It offers many advantages over currently used technologies that involve solvent extraction (AOAC, 1995; Min and Steenson, 1998); such as reduced energy consumption, smaller volumes of chemical solvents, use of less toxic solvents, and smaller quantity of waste products.

The MAP consists of heating a matrix (could be food) in a solvent that is relatively transparent to microwave. A microwave transparent medium can be defined as a medium that does not possess a significant dielectric constant with respect to the target material: hexane, (1.9), isopropanol (18.3) as opposed to large dielectric constant-possessing medium such as water (80.4). A significant fraction of these microwaves is absorbed by the target material and the absorption efficiency is largely related to the moisture content of the material at the time the extraction process is carried out.

The MAP provides a technique whereby compounds can be extracted selectively, and in a shorter period of time compared to conventional extraction methods (Paré et al., 1997). MAP is a process, which supports sustainable development as it consumes less energy than conventional extraction processes while providing in many instances for a reduction in wastes (Paré & Bélanger, 1997).

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Liquid-phase MAP extraction process is based upon the ability of a matrix to absorb microwave energy. This varies with the chemical nature of the species being exposed to the microwave irradiations. Under MAP conditions, solvents are chosen for their ability to dissolve the target compound and their relative transparency to microwaves. The chemical substances absorb microwave energy at different levels. The parameter generally used, as a measure of this physical property, is the dielectric constant. Liquid-phase extractions using MAP, is based upon the fact that it is possible to immerse the matrix to be extracted in a solvent that is characterized by a small dielectric constant and that is relatively transparent to microwaves (Paré et al., 1994; Paré et al., 1991). The application of microwave energy as a heat source causes selective heating of the matrix over the extractant. The high localized temperature and increase in pressure cause a selective migration of target compounds from the material to the solvent at a faster rate and with a similar or better recovery compared with conventional extraction methods (Paré & Bélanger, 1997).

There exists an increased consumption in low fat flavoured chocolate goods and ice creams (Zoumas & Smulden, 1992). These low fat flavoured products are made from cocoa powder prepared from cocoa nibs. Cocoa nibs are foodstuff obtained by removing the shell from cured, cleaned, dried and cracked cocoa beans (FDA, 2003). Cocoa beans are the source of chocolate cocoa powder and cocoa butter beans are processed to bring out their flavour and aroma and shelled to produce their “meat” which is called nibs (Arizona, 2004). Cocoa powder is produced by pulverizing the material remaining after part of the fat is removed from the cocoa (*Theobroma cocoa*) nibs. *T. cocoa* is the only species of major economic importance, and due to its fat-rich seeds it is the only source of cocoa solids and cocoa butter used by the confectionery industry.

As part of our continuing studies on the determination of fat in cocoa powder and cocoa nibs, we studied the extraction of fat using conventional methods but replacing the heating and extraction steps with microwave treatment of the materials immersed in different solvents that are relatively transparent to microwaves.

2. Materials and methods

2.1. Reagents and material

Petroleum ether, boiling range 35–60 °C, hexane, ethanol, isopropanol, all solvents distilled in glass were bought from Caledon Laboratory Chemicals, Canada; filter papers, qualitative 2 and 4 Whatman, Maidstone, UK; hydrochloric acid reagent ACS; cocoa powder, commercially available; Cocoa nibs *T. cocoa* commercial mixture for exportation from Barlovento, Venezuela.

Fats from cocoa powder and pre-treated ground nibs were extracted using different solvents that are relatively transparent to microwaves, i.e., petroleum ether, hexane, ethanol, and isopropanol. The determinations were per-

formed using a Prolabo Soxwave 3.6 MAP extraction system and the AOAC official methods.

2.2. Equipment

Prolabo Soxwave 3.6 MAP apparatus, operating at a frequency of 2450 MHz and variable power output ranging between 10 W and 250 W; Conventional Soxhlet equipment, Büchi Rotovapor R114.

2.3. Determination

2.3.1. Homogenization of cocoa nibs

The sample (≈5 g) was homogenized with a food processor (Waring Commercial Blender) for 10 s and the procedure was repeated five different times to give a homogeneous sample.

2.3.2. MAP extraction and fat determination from cocoa powder

The samples (5.00 ± 0.05 g; *W1*) were weighed on a weighing paper, and placed in the 250 mL quartz extraction vessel of the Soxwave 3.6 microwave-assisted extraction system. After addition of 1 mL of water, it was mixed on a vortex mixer for a few seconds. 30 mL of hexane were added and the sample was shaken again. The vessel was inserted in the microwave cavity, fitted with a condenser and irradiated in the following sequence at full power (250 W): 60 s ON 120 s OFF. Subsequently, a 10 mL aliquot of isopropanol was added and the mixture was shaken for 30 s. The solution was then irradiated for 360 s at 10% power (25 W). After irradiation, the extract was filtered through a Whatman filter paper, and collected in a tarred round-bottom flask (*W2*). The residue in the extraction vessel was washed with a 20 mL aliquot of the solvent and further washed with a 20 mL aliquot of acetone. Extract and washings were combined in the round-bottom flask and evaporated under vacuum with a rotary evaporator. Once dried the flask was cooled and reweighed (*W3*). The difference in weight (*W3* – *W2*) corresponded to the fat content of the sample:

$$\% \text{Fat} = \frac{W3 - W2}{W1} \times 100$$

Determinations were performed in triplicate, and the standard deviation (SD) was also determined. The short-term reproducibility (or repeatability) was expressed as coefficient of variation: $\text{CV}\% = \text{SD}/\text{mean} \times 100$. Experimentally determined values were compared with Cocoa standards obtained using the AOAC 991.36 (AOAC, 1995) method.

2.3.3. Control experiments for MAP extraction and fat determination from cocoa powder

Samples that were used as controls were treated exactly as per Section 2.3.2 above but the power was set a “0” (i.e., OFF at all times).

2.3.4. MAP extraction and determination of fat from cocoa Nibs

Samples of cocoa nibs previously homogenized (2.00 ± 0.05 g; *W1*) were weighed on a weighing paper and placed in a 250 mL quartz extraction vessel with a 10 mL aliquot of 50% HCL. The mixture was agitated on a vortex mixer for a few seconds. The vessel was inserted in the microwave cavity, fitted with a condenser and irradiated for 10 min at 10% power (25 W), a low power to avoid vaporization of the HCL that is required for the hydrolysis of the feedstock. Then the residue was rinsed with water onto a filter paper until the pH was neutral. The filter paper and the sample were dried overnight in an oven at 130 °C. The filter paper and sample were then placed in a quartz extraction vessel and 90 mL of petroleum ether were added. The sample was irradiated at full power (250 W) using the following sequence: 60 s ON, 30 s OFF, 60 s ON, 30 s OFF, and 60 s ON. After irradiation, the extract was filtered through a Whatman filter paper and collected in a tarred round-bottom flask (*W2*). The residue in the extraction vessel was washed with a 20 mL aliquot of the extraction solvent and further washed with a 20 mL aliquot of acetone. Then the filter paper and sample were irradiated again using the same sequence and same solvent. Extract and washings were combined in the round-bottom flask and evaporated under vacuum with a rotary evaporator. Once dried the flask was cooled and reweighed (*W3*). The fat content of the sample was calculated in the same manner as described for cocoa powder (Section 2.3.2 above).

2.3.5. Control experiments for MAP extraction and fat determination from cocoa nibs

Samples that were used as controls were treated exactly as per Section 2.3.4 above but the power was set a “0” (i.e., OFF at all times).

2.3.6. Conventional determination of fat in cocoa nibs

The fat content of cocoa nibs was determined by the AOAC official method 963.15 (AOAC, 1995). This method takes 6 h. Each sample of cocoa nibs was prepared and dehydrated following procedure described in AOAC, 1995. The thimble containing the dried samples was placed in soxhlet apparatus, supporting it with spiral beads. Rinsed digestion beaker, drying beaker, and watch glass with three 50 mL portions of petroleum ether and hexane/isopropanol, and added washings to thimble. Reflux digested sample for 4 h, adjusting heat so that extractor siphons >30 times. Each flask was removed and evaporated the solvent on a steam bath. Each flask were dried at 100 ± 1 °C to constant weight (1.5–2 h). Each flask was cooled in a dessicator to room temperature and weighted.

$$\% \text{Fat} = \frac{\text{grams of fat}}{\text{grams of sample}} \times 100$$

2.3.7. Fatty acid profile

The fatty acid profile of cocoa butter was determined following the AOAC (1995) methods 996.03 and 996.06. To avoid bias these analysis were carried out by an accredited laboratory that provided appropriate certificate of analysis.

3. Results

The data obtained demonstrated that the microwave extraction method is more effective than the conventional soxhlet extraction method. Table 1 reports that fat contents of cocoa powder samples obtained by MAP in a time of 460 s are similar to values obtained using conventional official methods (soxhlet) that take up to 6 h. Also, as noted before, the irradiation time vary with the residual moisture content of the sample. The moisture in the tissues creates localized superheating, causing a rapid and selective expulsion of the fat from their cells (Paré & Bélanger, 1997; Paré et al., 1994). In this work, materials were hydrated, or rehydrated with sufficient moisture to affect the desired microwave absorption. As expected, the extraction efficiency improved when the material contained sufficient amounts of water. Since the dipole moment of water is high, the irradiation was more effective. During this process, the irradiation causes localized heating of water in the sample causing the migration of water from the sample matrix to the surrounding solvent thus raising the temperature of the solvent. This is why the sample is left with the microwave power OFF at the end thereby allowing the temperature to decrease. Hence, the fat flows freely toward the relatively cool surrounding solvent that solubilizes it rapidly. The sample was over heated when isopropanol was added; the latter is able to keep the temperature relatively high thus creating passive thermal energy diffusion from the matrix to the surrounding solvent.

The fat contents obtained with the microwave-assisted technique for cocoa nibs ($44.71\% \pm 0.30$) are similar in terms of performance to those obtained by the soxhlet method ($44.40\% \pm 0.84$). These results not only show that the complete migration of the fat from the food matrix to the surrounding extractant (petroleum ether) is obtained after only a few minutes of irradiation, but also suggests that

Table 1
Fat content of cocoa powder and cocoa nibs

Sample Solvent	Cocoa powder Hexane/Isopropanol	Cocoa nibs Petroleum ether
Fat content obtained by control (%)	9.04 ± 0.28	35.83 ± 0.03
RSD (%)	2.54	0.37
Fat content obtained by MAP (%)	10.70 ± 0.01	44.71 ± 0.30
RSD (%)	0.74	1.01
Fat content obtained by AOAC (%)	10.55 ± 0.43	44.40 ± 0.84
RSD (%)	3.31	1.54

Samples extracted using the MAP and AOAC methods.
RSD, Relative standard deviation = (SD/mean) × 100.

Table 2
The fatty acid profile of cocoa powder extracted using MAP and soxhlet extraction methods

% Fatty acid type ^a	Soxhlet extraction		MAP extraction		
	Hexane/isopropanol	Hexane	Hexane	Hexane/isopropanol	Ethanol
C10:0	0.00221	ND	ND	0.00142	ND
C12:0	0.01310	0.00321	0.01000	0.01450	0.01170
C14:0	0.08480	0.08510	0.08240	0.07800	0.08270
C16:0	23.52000	23.84300	23.41800	23.00600	23.2680
C16:1	0.243000	0.24500	0.24000	0.20900	0.31300
C18:0	38.36700	38.35700	38.60400	39.17300	38.1020
C18:1	32.94600	33.04000	32.95300	32.98900	33.4750
C18:2	2.62900	2.50800	2.60100	2.51200	2.82100
C18:3	0.16600	0.17400	0.16400	0.15600	0.19400
C20:0	1.39200	1.33000	1.39400	1.40400	1.34300
C20:1	0.06590	0.04490	0.05680	0.04920	ND
C22:0	0.38200	0.22300	0.30000	0.24200	0.25200
C24:0	0.18800	0.14600	0.17700	0.16700	0.13700
Saturated	63.95	63.99	63.99	64.09	63.20
Monounsaturated	33.25	33.33	33.25	33.25	33.79
Polyunsaturated	2.80	2.68	2.76	2.66	3.01
Unsaturated	36.05	36.01	36.01	35.91	36.80

ND = Not detectable.

^a Expressed as mole percent and calculated from peak areas of the gas chromatograms.

a one-pot, simultaneous acid hydrolysis/extraction protocol is efficient for the determination of fat in cocoa samples.

The fatty acid composition is presented in Table 2. The results show that the saturated, mono and polyunsaturated fatty acid content were similar, for the various conditions, to fat extraction from cocoa powder, thus suggesting that the extraction with hexane/isopropanol was efficient and did not extract other constituents, such as pigments, to any significant extent. Cocoa butter contains mainly triglycerides of fatty acids that consist primarily of oleic acid (C18:1), stearic acid (C18:0), and palmitic acid (C16:0), in decreasing concentrations, with smaller amounts of linoleic acid (C18:2) (Leung & Foster, 1996). These results are similar to those reported in the literature (Anchia & Delgado, 2000; Chalseri & Dimick, 1987; Kirschenbauer, 1960; Lares, Pérez, & Álvarez, submitted for publication; Pérez, Álvarez, & Lares, 2002). The presence of oleic acid in these quantities is very important, because it influences the type of triglycerides formed. These triglycerides, when present in quantities higher than 15% interfere with monounsaturated triglyceride formation, favouring a less stable polymorphic crystalline structure that will have a low melting point (Chalseri & Dimick, 1987).

In conclusion, MAP can be used as a method of extracting fat, allowing a very rapid determination of the fat contents of cocoa with recoveries similar to or better than official methods. The MAP technology offers the added advantages of low consumption of solvent, short extraction time, low energy consumption, and excellent reproducibility.

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