Yoshime et al. Nutrire (2016) 41:12
DOI 10.1186/s41110-016-0013-y

Nutrire

RESEARCH Open Access

Bitter gourd (*Momordica charantia* L.) seed oil as a naturally rich source of bioactive compounds for nutraceutical purposes

Luciana Tedesco Yoshime, Illana Louise Pereira de Melo, José Augusto Gasparotto Sattler, Eliane Bonifácio Teixeira de Carvalho and Jorge Mancini-Filho*

Abstract

Background: Characterization of food lipids has triggered the development of applications for the food and health industries. Thus, the lipid profiles of an increasing number of fruits and their seeds have been characterized and numerous bioactive components have been isolated. The bitter gourd seed oil has aroused great interest because it contains relevant amounts of conjugated fatty acid α-eleostearic acid (C18:3 9*c*11*t*13*t*), a positional and geometric isomer of α-linolenic acid. The aim of this paper was to evaluate the phytochemical composition and quality parameters of the seed oil of bitter gourd grown in Brazil.

Methods: Bitter gourd was purchased from CEAGESP (Brazilian company of fresh food storages and warehouses). Seeds were lyophilized, and their oil was extracted using the Soxhlet and Folch extraction methods. The profiles of fatty acids and phytosterols were analyzed by gas chromatography, and the acidity and peroxide values were evaluated by methods of the American Oil Chemists' Society (AOCS).

Results: The Soxhlet extraction (40 % *w/w*) resulted in a higher yield of bitter gourd seed oil than the Folch extraction (16 % *w/w*). For both methods, α-eleostearic acid (56 and 58 %) was the major lipid in bitter gourd seed oil, followed by stearic acid (C18:0; 32 and 27 %). The oil displayed high content of phytosterols (886 mg/100 g), mainly β-sitosterol, and low acidity and peroxide values.

Conclusions: Bitter gourd seed oil from Brazil is an oil of good quality and its high contents of α-eleostearic acid and phytosterols with potential health-beneficial properties make it an attractive plant byproduct.

Keywords: α-Eleostearic acid, Phytosterols, Soxhlet, Cold-pressed oil

Background

Over the last decade, research into nutrition and dietary supplements has focused heavily on plant-derived nutraceuticals. Functional food components and sources of peculiar phytochemicals, such as new plant species, cultivars, and agro-industrial byproducts, have attracted increasing interest as they are suitable low-cost dietary supplements with potential preventive properties against numerous diseases [1].

Fats and oils are essential to human diet and are important industrial raw materials. Most oils are currently extracted from plants, and the demand for edible oils is on continuous rise. Thus, wild plants have recently been investigated as sources of unusual seed oils [2].

Momordica charantia, a member of the Cucurbitaceae family and commonly known as bitter gourd, bitter melon, or karela, thrives in humid and subtropical regions around the world [3]. Bitter gourd vines are originally from Asia but are now widely cultivated all over the world, including tropical countries such as Brazil, China, Taiwan, and India, because of the dietary value of both unripe and ripe fruits [3, 4]. They are adapted to a wide range of climates, but they grow best in warm weather. It is a revolutionary plant with versatile applications in the food industry and in therapy [5]. Numerous studies have shown that bitter gourd is a rich source of

* Correspondence: jmancini@usp.br
Department of Food and Experimental Nutrition, Lipids Laboratory, Faculty of Pharmaceutical Sciences, São Paulo University, Av. Prof. Lineu Prestes, 580-Bloco 14, CEP: 05508-900 São Paulo, SP, Brazil

conjugated α-linolenic acids (CLnA). For this reason, it has been used in traditional folk medicine (mainly in Asia) for the treatment of many diseases, such as diabetes and atherosclerosis [6].

Bitter gourd is one of a few edible fruits containing conjugated linolenic acid in its seeds, which is a byproduct from its processing. The lipid profiles of seed oils have received special attention because of its high content of polyunsaturated fatty acids (PUFAs) and other bioactive compounds [7]. Recent studies have found that bitter gourd seed oil (BSO) consists of 30–60 % of α-eleostearic acid (EA; C18:3 9*c*11*t*13*t*), a long-chain PUFA (ω-5) with conjugated double bonds [3, 4, 8]. CLnAs are a group of positional and geometric octadecadienoic fatty acid isomers with two *trans* and one *cis* double bonds [9]. The chemical and physiological properties of CLnAs along with their several potential health benefits, including antioxidant, anti-inflammatory, anti-atherosclerotic, anti-tumor, and in vitro and in vivo serum lipid-lowering activities, have increasingly fueled scientific interest [10].

In addition to the high percentage of CLnA, the presence of other bioactive compounds, such as tocopherols and polyphenolic compounds have been reported in BSO [3, 11]. However, the presence and quantification of phytosterols are very scanty. Information regarding these types of research works on varieties of this plant cultivated in Brazil is still not published. Furthermore, differences in the chemical and phytochemical composition, among cultivars, have not been well established yet [12]. According to Hussain and colleagues [13], the fat content and functional properties may be affected by ameliorated soil and climatic conditions of the production area.

This work aims to draw attention to underutilized agricultural product in Brazil and, consequently, make a contribution to avoiding waste disposal problems. Thus, to improve the economic utility of the bitter gourd seeds as a source of edible oil with nutraceutical potential. This study provides information on some bioactives in BSO grown in Brazil that can add value to bitter gourd, favoring their promotion and consumption in local and international markets. To our knowledge, no research has been reported on the phytochemical composition of BSO of Brazilian plants. Thus, conducting such analytical analysis is highly necessary.

Therefore, the aim of this study was to characterize the profiles of fatty acids and phytosterols in the seed oil of bitter gourd from Brazil and to evaluate its quality through the acidity and peroxide values using the methods of the American Oil Chemists' Society (AOCS) [14]. Identifying functional compounds in these seeds may help to increase their economic importance as a source of edible/non-edible lipids and health-beneficial phytochemicals.

Methods

Samples

Unripe bitter gourd was purchased from CEAGESP (Brazilian company of fresh food storages and warehouses) in São Paulo, Brazil, in November and December 2013. The fruit is long, dark green colored, covered with long triangular tubercles, and bitter in taste (Fig. 1). Its seeds were removed, and any flesh and mucilage was cleaned off. They were then frozen to −40 °C and dried under a vacuum of less than 4×10^{-1} Torr for 48 h in a Super Modulyo Freeze Dryer (Edwards Ltd., Crawley, UK), in accordance with the manufacturer's norms. The samples were stored in polyethylene bags under vacuum for further analysis.

A sample of bitter gourd seed oil (BSO) and a sample of linseed oil (LSO) were provided by Health & Beauty Natural Oils (Santa Barbara, CA) and Vital Âtman (Uchôa, Brazil), respectively. Both oils were obtained by cold pressing of seeds and were used for comparison.

Chemicals

Standard fatty acid methyl ester (FAME-C4:24), phytosterols (campesterol, stigmasterol, β-sitosterol), 5α-

Fig. 1 Bitter gourd (*Momordica charantia* L.) fruit

cholestane, and sodium methoxide (NaOCH3) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water, obtained using a Simplicity® Water Purification System of Millipore (Darmastadt, Germany), was used in all experiments. All other chemicals and solvents of analytical grade were purchased from Merck (Darmastadt, Germany) or from Synth (Diadema, SP, Brazil).

Oil extraction

A dried sample was ground and weighed. Two methods were employed:

1. Soxhlet—fat was extracted with petroleum ether using a Soxhlet apparatus at a maximum temperature of 60 °C for about 8 h [15].
2. Folch—fat was extracted with methanol and chloroform at room temperature according to Folch et al. [16].

Fatty acid profile

Fatty acids in BSO and LSO were converted into methyl esters using $NaOCH_3$ in methanol as described in Christie and Sédédio [17]. Samples were then injected into a Shimadzu GC-2010 chromatograph equipped with a flame ionization detector and a fused silica column (SP-2560, 100 × 0.25 mm). Samples were run under chromatographic conditions described in Baublits et al. [18]. Fatty acids were identified by comparing their retention times with those of commercial standards (C4-C24 methyl esters, Sigma 18919). Conjugated fatty acids were identified by comparing their retention times with those reported in the literature. Results were expressed as percentages of total fatty acids.

Phytosterols content

Determination of phytosterols was carried out in three steps, as recommended by Almeida [19]: (1) heat saponification using 3 % KOH in water bath at 50 °C under stirring for 3 h; (2) extraction of the unsaponifiable fraction in hexane (10 mL) under vortex mixing for 1 min; and (3) quantification of phytosterols by gas chromatography (GC). The system consisted of a Shimadzu GC-2010 chromatograph equipped with a DB-5 poly (methylphenyl) siloxane column (5 % phenyl, 60 m, 0.25 mm) and a flame ionization detector. The temperature gradient was as follows: 150 °C for 0.1 min, 150–300 °C at 10 °C/min, and 300 °C for 10 min. The temperatures of the injector and detector were 250 and 300 °C, respectively. Helium (1 mL/min) was used as the carrier gas, and the split ratio (the amount of sample entering the column) was 1:50. Identification of the peaks was performed by comparing the retention times with those of Sigma standards (campesterol-C5157, stigmasterol-S6126, and β-sitosterol-S9889), and quantification was done by internal standardization using 5α-cholestane (Sigma-C8003). Results were expressed as milligrams of phytosterols per 100 g of oil.

Quality and stability parameters

Quality and stability parameters of both oils, acidity value (Av), and peroxide value (Pv) were evaluated by classical methods described in the AOCS [14].

Statistical analysis

Quantitative data were expressed as mean values ± standard deviation ($n = 3$). Statistically significant differences were evaluated by univariate analysis of variance (ANOVA) followed by Tukey's test ($p < 0.05$). All analyses were performed using GraphPad Prism 5.0 software for Windows (San Diego, CA, USA).

Results

Soxhlet extraction resulted in a higher seed oil yield (40 %) than Folch extraction (16 %). For this reason, only samples of Soxhlet-extracted oil were assessed for phytosterols' content, quality, and stability.

Table 1 shows the profile of fatty acids in BSO, obtained using two different extraction methods, and in LSO. The levels of α-eleostearic acid ranged between 24 and 57 %. This fatty acid has been reported to be the most abundant in all previously studied bitter gourd seed oils.

Although the contents of some minor fatty acids in BSO differed significantly between the two methods of extraction (Table 1), no significant differences in the levels of the two major fatty acids, α-eleostearic acid (about 56 and 58 %) and stearic acid (about 32 and 27 %), were observed. A chromatogram of the fatty acids profile in a sample of BSO extracted by Soxhlet is shown in Fig. 2a.

Commercial cold-pressed BSO was less rich in α-eleostearic acid ($p < 0.05$) than solvent-extracted BSO. The most abundant acid in commercial BSO was linoleic acid (C18:2; 33 %) followed by α-eleostearic acid (24 %) and stearic acid (20 %).

The content of polyunsaturated fatty acids was similarly high (>60 % of total lipids) in all oils. Saturated fatty acids were significantly higher in BSO extracted by Soxhlet than in LSO but slightly higher than in other BSO samples. Monounsaturated fatty acids, on the other hand, were greatest in LSO (Table 1).

The levels of total phytosterols (Table 2) did differ among oils; they were highest in Soxhlet-extracted BSO, followed by commercial BSO and commercial LSO. Although β-sitosterol was the most abundant phytosterol in all oils, a major peak was observed immediately after the elution of β-sitosterol in chromatograms for samples of BSO extracted by Soxhlet. This peak

Table 1 Fatty acid profile in bitter gourd and linseed oil determined by GC (%)

Fatty acids	BSO (Soxhlet)	BSO (Folch)	Commercial BSO	Commercial LSO
C16:0	2.55 ± 0.25c	3.16 ± 0.02b	2.23 ± 0.03c	6.46 ± 0.02a
C18:0	32.00 ± 2.73a	27.39 ± 0.25a	20.26 ± 0.04b	5.54 ± 0.01c
C18:1 ω-9	3.77 ± 0.41c	3.46 ± 0.36c	9.29 ± 0.05b	19.20 ± 0.07a
C18:2 ω-6	4.67 ± 0.29d	7.06 ± 0.02c	33.18 ± 0.12a	14.22 ± 0.05b
C18:3 ω-3	–	–	–	54.59 ± 0.06
C18:3 9*c*11*t*13*t*	56.08 ± 3.80a	57.86 ± 0.33a	23.91 ± 0.19b	–
C18:3 9*t*11*t*13*c*	0.92 ± 0.11b	1.06 ± 0.14b	7.62 ± 0.07a	–
Saturated	34.55 ± 2.75a	27.39 ± 0.27b	25.49 ± 0.03b	12.00 ± 0.01c
Monounsaturated	3.77 ± 0.41c	3.46 ± 0.36c	9.29 ± 0.05b	19.20 ± 0.07a
Polyunsaturated	61.67 ± 4.20b	65.98 ± 0.49ab	65.23 ± 0.04ab	68.81 ± 0.07a

Results are expressed as mean ± standard deviation ($n = 3$); means in the same line followed by the same letter are not significantly different; ANOVA followed by Tukey's test ($p < 0.05$)
BSO bitter gourd seed oil, *LSO* linseed oil

suggested the presence of sitostanol and Δ-5-avenasterol in the oil sample [20]; however, later tests with a standard will confirm its presence. Figure 2b shows a chromatogram of phytosterols in a sample of BSO extracted by Soxhlet.

The acidity and peroxide values for the LSO and BSO extracted by Soxhlet met the standards of quality recommended by the Codex Alimentarius Commission Standard (CODEX STAN 210-1999) (Table 3). Commercial BSO showed significantly higher index values that were slightly above the recommended requirements.

Discussion

Solvent extraction is a common method for the measurement of lipid content. The Soxhlet method has been used as an official reference method for extracting oil from bitter gourd seeds as it is a high-yielding and rapid process. According to Kraujalis et al. [21], the yield of the oil for Soxhlet extraction can depend on a number of factors, primarily particle size and extraction time (number of cycles). Alternatively, the Folch extraction method prevents fatty acids in seeds from degrading as

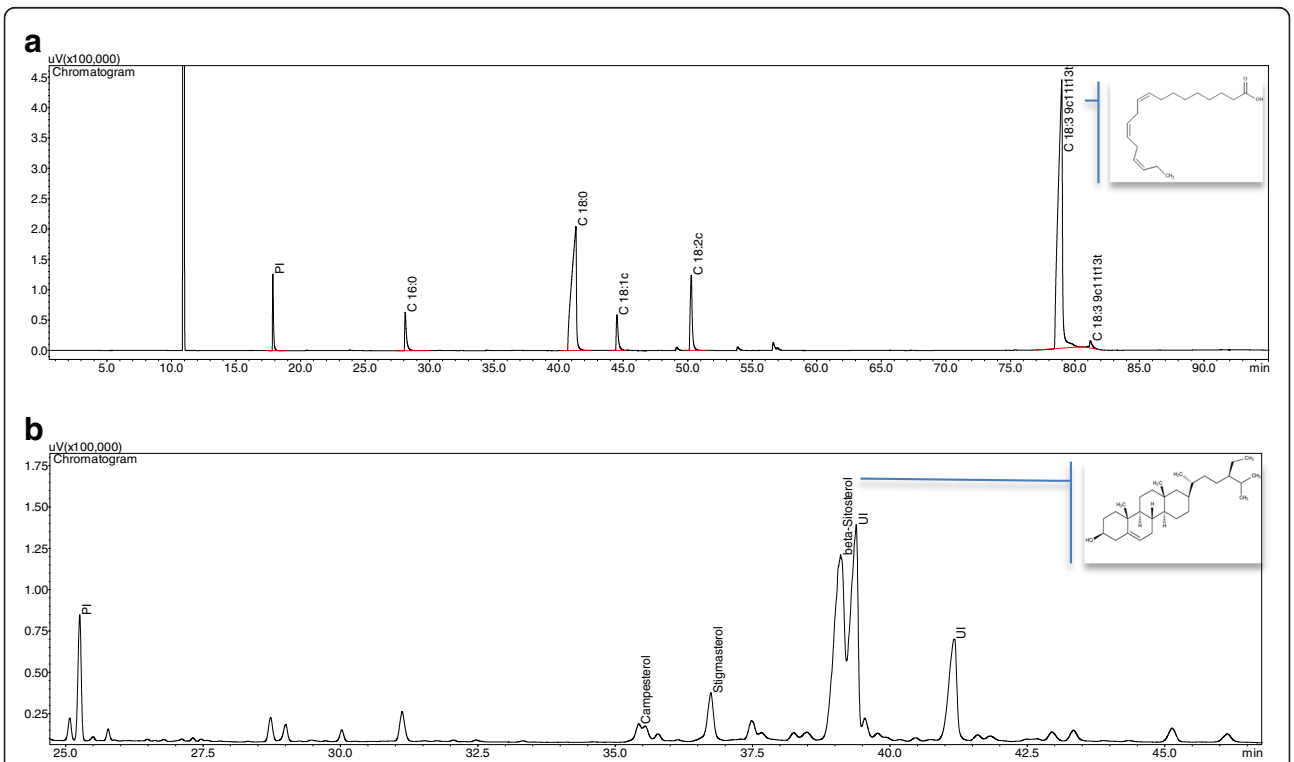

Fig. 2 Chromatograms of the profiles of fatty acids **(a)** and phytosterols **(b)** in Soxhlet-extracted bitter gourd seed oil (BSO) analyzed by GC. *PI* internal standard, *UI* unidentified peaks

Table 2 Phytosterols content (mg/100 g) in bitter gourd and linseed oil

	Phytosterols (mg/100 g)				
	Campesterol	Stigmasterol	β-sitosterol	UI	Total
BSO (Soxhlet)	60.2 ± 5.83b	57.8 ± 5.6a	265.0 ± 21.5b	531.5 ± 42.2a	886.2 ± 8.8a
Commercial BSO	29.9 ± 1.1c	0.5 ± 0.3b	440.6 ± 9.3a	124.0 ± 9.5c	595.0 ± 20.2b
Commercial LSO	111.9 ± 2.6a	8.3 ± 1.0b	150.8 ± 4.0c	210.4 ± 2.8b	481.4 ± 10.5c

Results are expressed as mean ± standard deviation ($n = 3$); means in the same column followed by different letters are significantly different ($p < 0.05$)
UI sum of unidentified peaks, *BSO* bitter gourd seed oil, *LSO* linseed oil

it is a cold process; however, the use of large volumes of toxic solvents limits its application [22].

Fatty acids significantly add up to the nutritive value of seed oils [21]. In this study, Soxhlet-extracted bitter gourd seeds, grown in Brazil, were found to be potentially richer (40 %) in oils than petroleum-ether-extracted bitter gourd seeds from India (25 %), Malaysia (19 %), and Bangladesh (26 %) (Arora and Chaudhary [8], Nyam et al. [11], and Ali et al. [5], respectively); Nyam et al. [3], on the other hand, observed similar results (40 %) for Soxhlet-extracted bitter gourd seeds grown in Pakistan.

Our findings are also in agreement with those reported by Habicht et al. [23], who investigated lipid extracts of six varieties of bitter gourd and noted that the amount of EA in seeds differed markedly among bitter gourds (from 4.75 to 65.89 % of total lipids; mean = 26.24 %). These authors suggested that the concentration of α-eleostearic acid and the fatty acid profile in bitter gourd vary according to ripeness of the fruit, which is more important than variety because of the proven biological effects of bitter gourd oil and its high concentration in seeds. Therefore, not removing that seeds during food preparation is recommended.

Overall, fatty acid profile in BSO differs among regions; for example, Ajum et al. [3] found α-eleostearic (48 and 44 %) to be the most abundant fatty acid in two BSO samples from Pakistan, followed by stearic (25 and 29 %), oleic (16 and 16 %), and linoleic (6.9 and 5.4 %). Nyam et al. [11], on the other hand, reported higher amounts of α-eleostearic (62 %) and stearic (32 %) acids but lower levels of oleic (1.5 %) and linoleic (2.6 %) acids for a BSO sample from Malaysia. Ajum et al. [3] and Ali et al. [5] suggested the fatty acid composition varies according to variety, soil type, and climatic conditions.

Table 3 Quality and stability parameters for bitter gourd and linseed oil

	Av (mg/g)	Pv (mEg/kg)
BSO (Soxhlet)	3.45 ± 0.23b	8.05 ± 0.61b
Commercial BSO	4.35 ± 0.05a	16.42 ± 1.08a
Commercial LSO	0.84 ± 0.00c	6.46 ± 0.59b

Results are expressed as mean ± standard deviation ($n = 3$); means in the same column followed by different letters are significantly different ($p < 0.05$)
Av acidity value, *Pv* peroxide value (results expressed as milliequivalents of oxygen per kilogram of oil), *BSO* bitter gourd seed oil, *LSO* linseed oil

The contents of α-eleostearic acid in BSO extracted by Soxhlet and Folch methods are in agreement with those reported in the literature (>45 %) for oils extracted from bitter gourd seeds by physical/chemical methods [3, 4, 8, 24]. The importance of α-eleostearic acid lies in its potential preventive properties against several non-transmissible diseases, such as cardiovascular disease, cancer, and diabetes [25].

The fatty acid profiles of oils extracted using conventional methods, such as Soxhlet and stirring, have been reported to be all very similar, whereas superheated hexane extraction, cold pressing, and other methods, run under various conditions, have resulted in differences in fatty acid profiles, mostly in the composition of unsaturated fatty acids [26]. These findings make BSO from Brazil very attractive to consumers wishing to increase the content of polyunsaturated fatty acid of their dietary lipid source.

Phytosterols are present in all vegetable oils and account for the largest group of compounds in their unsaponifiable fraction. The phytosterol profile is characteristic of each type of oil [27]. The phytosterol content in BSO samples from Brazil (886.2 mg/100 g) was higher than that observed in BSO from Malaysia (464 mg/100 g), reported by Nyam et al. [11]. Although the content of β-sitosterol in Soxhlet-extracted BSO (265 mg/100 g) was lower than that for commercial BSO (440 mg/100 g), it is consistent with the levels observed in the literature (405 mg/100) [11].

Several plant sterols have been added to functional food products because of their potential cholesterol-lowering effects. European legislation allows sitosterol, campesterol, and stigmasterol to be used in a higher ratio to total plant sterol content [28]. The considerable amount of sitosterol in bitter gourd seed oil encourages the consumption of BSO as a nutraceutical for modulating cholesterol absorption.

The Codex Alimentarius Commission Standard [29] recommends that the acidity and peroxide values of cold-pressed oils should not exceed 4.0 mg/g and 15 mEq/kg, respectively. Ajum et al. [3] and Ali et al. [5] reported acidity index values of 2.16 and 2.05 and peroxide index values of 5.97 and 7.40 mEg/kg for BSO from

Pakistan and Soxhlet-extracted BSO from Bangladesh, respectively. According to Ajum et al. [3], these values provide very useful information for furthering the process for refining seed oils. Sielicka et al. [30] have observed that the peroxide index test is one of the most commonly used tests to evaluate oxidative rancidity in oils and fats. It measures the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. The present experimental results show that Soxhlet-extracted BSO from Brazil is an oil of good quality.

The present results indicate that bitter gourd seed oil from Brazil could probably be used for edible purposes as it displays a high percentage of PUFAs (mainly α-eleostearic acid) and phytosterols (mainly β-sitosterol), as well as lower acidity and peroxide values.

Conclusions

Bitter gourd seed oil from Brazil contains high amounts of total lipids, mainly α-eleostearic acid, and considerable levels of phytosterols. Moreover, it is an oil of good quality. These findings increase the agricultural potential of bitter gourd and could encourage the introduction of its high-value components as potential nutraceuticals into the market. These findings also make bitter gourd very appealing as its seed oil could be used for edible purposes. Its high contents of α-eleostearic acid and phytosterols, on the other hand, encourage its medicinal use because these compounds have shown potential health-beneficial effects. Further research into these aspects is needed.

Abbreviations

AOCS, American Oil Chemists' Society; Av, acidity value; BSO, bitter gourd seed oil; CEAGESP, Brazilian company of fresh food storages and warehouses; CG, gas chromatography; CLnA, conjugated α-linolenic acids; EA, α-eleostearic acid; LSO, linseed oil; PI, internal standard; PUFAs, polyunsaturated fatty acids; Pv, peroxide value; UI, unidentified peaks

Acknowledgements

The authors would like to thank the São Paulo State Research Foundation (FAPESP, Process 13/24490-7) for funding the research and the National Council for Technological and Scientific Development (CNPq) for granting a postdoctoral scholarship.

Funding

FAPESP and CNPq.

Authors' contributions

LTY conceived the study and drafted the manuscript. ILPM conceived of the study, performed the statistical analysis, and drafted the manuscript. JAGS and EBTC participated in the carrying out of the chemical analysis. JMF participated in the design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Authors' information

Drs. Yoshime LT, Melo ILP, and Carvalho EBT are postdoctoral researchers exclude. Sattler JAG, MSc., PhD student Dr. Mancini-Filho J is a full professor exclude. The department and institution where the work was conducted was at the Food and Experimental Nutrition Department, Pharmaceutical Sciences, Lipids Laboratory, University of São Paulo.

Competing interests

The authors declare that they have no competing interests.

Received: 13 January 2016 Accepted: 6 July 2016
Published online: 07 September 2016

References

1. Caligiani A, Bonzanini F, Palla G, Cirlini M, Bruni R. Characterization of a potential nutraceutical ingredient: pomegranate (Punica granatum L.) seed oil unsaponifiable fraction. Plant Foods Hum Nutr. 2010;65:277–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20607413.
2. Sbihi HM, Nehdi IA, Al-Resayes SI. Characterization of white Mahlab (Prunus mahaleb L.) seed oil: a rich source of alpha-eleostearic acid. J Food Sci. 2014;79:795–801.
3. Anjum F, Shahid M, Bukhari SA, Anwar S, Latif S. Study of quality characteristics and efficacy of extraction solvent/technique on the antioxidant activity of bitter gourd seed. Food Process Technol. 2013;4:2–9.
4. Saha SS, Patra M, Ghosh M. In vitro antioxidant study of vegetable oils containing conjugated linolenic acid isomers. LWT - Food Sci Technol. 2012; 46:10–5. Available from: http://linkinghub.elsevier.com/retrieve/pii/S002364381100363X.
5. Ali MA, Sayeed MA, Reza MS, Yeasmin MS, Khan AM. Characteristics of seed oils and nutritional compositions of seeds from different varieties of Momordica charantia Linn. cultivated in Bangladesh. Czech J Food Sci. 2008; 26(4):275–83.
6. Bialek A, Jelinska M, Bamburowicz-klimkowska M, Tokarz A. Effect of bitter melon aqueous extract and pomegranate oil on glucose concentration and lipid profile in blood of rats—preliminary study effect of bitter melon aqueous extract and pomegranate oil on glucose concentration and lipid profile in blood of rats. Int J Cardiol Lipidol Res. 2014;1:1–7.
7. Jing P, Ye T, Shi H, Sheng Y, Slavin M, Gao B, et al. Antioxidant properties and phytochemical composition of China-grown pomegranate seeds. Food Chem. 2012;132:1457–64. Available from: http://dx.doi.org/10.1016/j.foodchem.2011.12.002.
8. Arora A, Chaudhary S. Potential source of α-eleostearic acid of Momordica charantia seed oil of arid zone plants of Rajasthan, India. Int J Basic Appl Chem Sci. 2012;2:59–62.
9. Carvalho EBT, de Melo ILP, Silva AMO, Mancini DAP, Mancini-Filho J. Effect of conjugated linoleic acid (CLA) in rats subjected to damage liver induced by carbon tetrachloride. J Mod Med Chem. 2014;2:141–8.
10. Melo ILP, Carvalho EBT, Mancini-filho J. Pomegranate seed oil (Punica granatum L.): a source of punicic acid (conjugated α-linolenic acid). J Human Nutr Food Sci. 2014;2:1–11.
11. Nyam KL, Tan CP, Lai OM, Long K, Che Man YB. Physicochemical properties and bioactive compounds of selected seed oils. LWT-Food Sci Technol. 2013;42:1396–403. Available from: http://linkinghub.elsevier.com/retrieve/pii/S002364380900070X.
12. Chirinos R, Zuloeta G, Pedreschi R, Mignolet E, Larondelle Y, Campos D. Sacha inchi (*Plukenetia volubilis*): a seed of polyunsaturated fatty acids, tocopherols, phytosterols, phenolic compounds and antioxidant capacity. Food Chem. 2013;141:1732–9.
13. Hussain J, Rehman NU, Al-Harrasi A, Ali L, Khan AL, Albroumi MA. Essential oil composition and nutrient analysis of selected medicinal plants in Sultanate of Oman. Asian Pac J Trop Dis. 2013;3(6):421–8.
14. AOCS. Official methods and recommended practices of the American Oil Chemists' Society. 5th ed. Champaign, USA: AOCS; 2004.
15. AOAC. Association of Official Analytical Chemists. Official methods of analysis of the Association of Official Analytical Chemists (method 920.39,C). Arlington: A.O.A.C. 1995, chapter 33. p. 10–12.
16. Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957; 226:497–509.
17. Christie WW, Sébédio JL, Juanéda P. A practical guide to the analysis of conjugated linoleic acid. Inform. 2001;12:147–52.
18. Baublits RT, Pohlman FW, Brown AH, Johnson ZB, Proctor A, Sawyer J, et al. Injection of conjugated linoleic acid into beef strip loins. Meat Sci. 2007;75:84–93.
19. Almeida CAS. Avaliação dos principais fitosteróis em óleos vegetais e azeite. (Dissertation) Universidade Estadual de Campinas; 2009, 89f.
20. Laakso PH. Determination of plant stanols and plant sterols in phytosterol enriched foods with a gas chromatographic-flame ionization detection method: NMKL collaborative study. J AOAC Int. 2014;97(4):1097–108.

21. Kraujalis P, Venskutonis PR, Pukalskas A, Kazernavičiūtė R. Accelerated solvent extraction of lipids from Amaranthus spp. seeds and characterization of their composition. LWT - Food Sci Technol. 2013; 54:528–34. Available from: http://www.sciencedirect.com/science/article/pii/S0023643813002314.
22. Biondo PBF, dos Santos VJ, Montanher PF, de O S Junior O, Matsushita M, Almeida VC, et al. A new method for lipid extraction using low-toxicity solvents developed for canola (Brassica napus L.) and soybean (Glycine max L. Merrill) seeds. Anal Methods. 2015;7:9773–8. Available from: http://xlink.rsc.org/?DOI=C5AY02243F.
23. Habicht SD, Kind V, Rudloff S, Borsch C, Mueller AS, Pallauf J, et al. Quantification of antidiabetic extracts and compounds in bitter gourd varieties. Food Chem. 2011;126:172–6. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0308814610013592.
24. Pal M, Ghosh M. Studies on comparative efficacy of α-linolenic acid and α-eleostearic acid on prevention of organic mercury-induced oxidative stress in kidney and liver of rat. Food Chem Toxicol. 2012;50:1066–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22269903.
25. Yuan G-F, Chen X-E, Li D. Conjugated linolenic acids and their bioactivities: a review. Food Funct. 2014;5:1360–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24760201.
26. Khoddami A, Roberts TH. Pomegranate oil as a valuable pharmaceutical and nutraceutical. Lipid Technol. 2015;27:40–2. Available from: http://doi.wiley.com/10.1002/lite.201500002.
27. Mancini-filho J, Takemoto E, Aued-Pimentel S. Parâmetros de Identidade e Qualidade de Óleos e Gorduras, Chapter 6, 63–82. In: Almeida-Muradian LB, Penteado MDVC, editors. Ciências Farmacêuticas - Vigilância Sanitária - Tópicos sobre Legislação e Análise de Alimentos. 2nd ed. Rio de Janeiro, Brazil: Guaranbara Koogan; 2015.
28. Barriuso B, Astiasarán I, Ansorena D. Unsaturated lipid matrices protect plant sterols from degradation during heating treatment. Food Chem. 2016;196: 451–8. Available from: http://linkinghub.elsevier.com/retrieve/pii/S030881461501417X.
29. CODEX. Codex Alimentarius Commission. 1999. Codex standard for named vegetable oils. Codex Stan 210–1999. 15p. http://www.fao.org/docrep/004/y2774e/y2774e04.htm#bm4.1.
30. Sielicka M, Małecka M, Purłan M. Comparison of the antioxidant capacity of lipid-soluble compounds in selected cold-pressed oils using photochemiluminescence assay (PCL) and DPPH method. Eur J Lipid Sci Technol. 2014;116:388–94. Available from: http://doi.wiley.com/10.1002/ejlt.201300356.