Commercialised Coconut oil in São Paulo City, Brazil: Evaluation of Authenticity and Nutritional Labelling

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Abstract

Increased demand for virgin coconut oil motivated the present study, which aims to evaluate the authenticity and the compliance with nutrition labelling of oils from the São Paulo city market, Brazil. Six samples were studied. Fatty acids, Vitamin E, Vitamin A, and stigmasta 3,5-diene contents were evaluated. One sample was adulterated with the addition of soybean oil. For all samples, at least one of the above-mentioned components was not in accordance with the labelling. Two samples that were labelled as extra virgin oil presented high contents of stigmasta 3,5-diene (>4.0 mg.kg^{-1}), suggesting that the oils were submitted to refining process and were not of the quality stated. Considering the observed non-compliant commercial oils, it is essential that continuous monitoring actions be maintained to ensure the supply of safe and reliable products to the consumer.

Keywords: Specialty oil, functional food, adulteration, legislation.

1. Introduction

Worldwide there is a growing market for virgin and cold-pressed oils that are rich in biological active lipids, such as essential fatty acids, vitamins, and phenolic compounds, which can provide healthy benefit for the prevention, management or treatment of diseases (Matthäus, 2008; Azmir et al., 2013; Madawala et al., 2012). The scientific literature has presented results that suggest the beneficial effects of extra virgin coconut oil in weight and blood sugar control (Asunción et al., 2009). These effects are attributed to the high level of medium-chain triglycerides and phenolic acids (Kumar and Krishna, 2013; Marten, Pfeiffer, Schrezenmeir, 2006; Marina et al., 2009). Moreover, due to its high saturated fatty acid content, mainly lauric acid, whose excessive intake is correlated with increased levels of low density lipoprotein cholesterol (LDL-c) (Hunter et al., 2010), the recommended intake of this oil has generated controversy.

Currently in the Brazilian and international marketplace there is a large supply of these speciality oils, including coconut oil, which are products of high added value. World coconut oil production is estimated at around 3.0 million tons per year. This accounts for 2.5% of world vegetable oil production. Over 70% of global coconut oil production comes from the Philippines and Indonesia. Brazilian domestic demand for coconut oil is met by imports from these countries (UNCTAD, 2014).

The bioactive components of vegetable oils, with beneficial effects to health, are present in considerable amounts in virgin or cold-pressed oils, i.e. oils not subject to refining. Kumar and Krishna (2015) have demonstrated the differences in quality parameters and phytonutrients for virgin and refined coconut oils. Refining vegetable oils eliminates some defects of low-quality oils, such as the removal of free fatty acids, soap, organic pesticides, and other contaminants but reduces the number of components that are beneficial to people's health, such as tocopherols, phenolic compounds (Kumar and Krisma, 2015; Jorge, 2009; Verhé et al., 2006). Refining also produces steroidal alkenes (sterenes), including stigmastadiene. These compounds are formed by dehydration of desmethylsterols of the vegetable oils (Gordan and Firman, 2001).

Determination of the presence of stigmasta 3,5-diene, derived from the dehydration of beta-sitosterol, in oils declared as virgin and/or cold-pressed is a sensitive parameter for the recognition of the refining process (in particular the bleaching stage) to which the oil has been subjected (Matthäus, 2008; Ourrach et al., 2012; Aued-Pimentel et al., 2013;). However, among the edible vegetable oils, virgin olive oil is the only one that has a value set for the acceptable level of stigmasta 3,5-diene in a *Codex* standard (*Codex Alimentarius*, 2013b). Commercialised coconut oils in Brazil are regulated by the National Health Surveillance Agency (ANVISA) and are classified as "novel foods". Products are included in this category when studies on their functional properties are not conclusive. Any claim on the food's label related to these properties is prohibited (Brasil, 1999). General and nutritional information should appear on the label of such products, as they are packaged in the absence of the customer and ready to be offered to consumers. Brazilian law requires the declaration of total fat, saturated fatty acids (SFA) and *trans* fatty acids (TFA) on the labels of packaged foods and tolerates a variation of 20% between the experimental values and those reported on the label (Brasil, 2003). In the case of the declaration of the content of other nutrients such as monounsaturated fatty acids (AGM), poly-unsaturated fatty acids (PUFA), and Vitamins, the same variability is acceptable.

Considering the increased demand for coconut oil and the high commercial value, as well as the detection of similar adulterated products (Hirashima et al., 2013), the present study aimed to assess the authenticity and compliance of nutritional labelling on coconut oil that was on sale in São Paulo, Brazil. The fatty acids (including *trans*), tocopherols, and Vitamin A contents were evaluated. The content of stigmasta-3,5-diene was also determined in the samples declared as extra virgin since, depending on the content obtained, this can infer whether the product has been subjected to refining and whether it has the declared quality or not.

2. Materials and Methods

2.1. Samples

Six samples (coded as C1, C2, C3, C4, C5, and C6) of different brands were analysed, three of which were collected in the course of health and sanitation inspections in the state of São Paulo and the other three of which were acquired in trade transactions. Samples C1, C2, C3 and C4 were declared as extra virgin. A sample of vegetable oil, sent by the International Olive Oil Council, with reference values for stigmasta 3,5-diene was analysed in parallel to ensure the quality of analytical results. The reference sample consisted of 70% lampante olive oil and 30% refined grape seed oil.

2.2. Chemicals and Reagents

The following reagents were used: a mixture of 37 certified methyl esters of fatty acids ranging from C4 to C24 (Supelco, Bellefonte, PA, USA); a mixture of *cis/trans* methyl esters of fatty acid isomers of 18:2, 18:3, methyl esters of fatty acid isomers of conjugated linoleic acid (CLA) (18:2 9*c*11*t* and 18:2 10*t*12*c*), cholesta 3,5-diene, all *trans* retinol and alpha-tocopherol (> 95% purity) (Sigma Aldrich Chemical Co., St. Louis, MO, USA); silica gel 60 (70–230 mesh ASTM, Merck, Darmstadt, Germany); nanograde *n*-hexane (>95% purity), isopropanol and methanol HPLC grade (Mallinkrodt, St. Louis, MO, USA). All other reagents (96% ethanol, potassium hydroxide, sodium sulphate anhydrous, ethyl ether, and methanol) were of analytical grade.

2.3. Fatty Acid Determination by Gas Chromatography Analysis

The fatty acid composition of oils was determined as described in *Métodos Físicos e Químicos do Instituto Adolfo Lutz* (Instituto Adolfo Lutz, 2005). The fatty acid methyl esters (FAME) were obtained using a modified Hartman and Lago method (Instituto Adolfo Lutz, 2005). The sample was injected into a GC-FID from Thermo, model Focus GC, managed by the software Chrome Quest, and equipped with a fused silica column with the stationary phase of cyanopropyl siloxane (100 m x 0.25 mm x 0.25 μm) (SP 2560, Agilent, USA). The following conditions were applied: injector and detector temperature: 250°C; oven temperatures: 180°C (65 min) and rising by 15°C/min^{-1} up to 215°C for 18 min; carrier gas: hydrogen; column pressure: 170 KPa; and a split ratio of 1:100. The separated components were identified by co-injection of standards and comparison with the absolute retention times. The fatty acid profile was evaluated with area normalisation. Assessment of nutritional labelling information, as the declared fatty acids, was evaluated (in triplicate) with fatty acid methyl ester 13:0 as the internal standard.

The molecular structure of an uncommon fatty acid found in sample C6 was investigated by gas/mass spectrometry chromatography analysis on a Shimadzu GC-17A gas chromatograph, QP5000 (Kyoto, Japan). The mass spectrometer was operated in scan mode and with an impact of electrons. The mass spectrum of the component was compared to the NIST05 and NIST05s mass spectral libraries.

2.4. Tocopherol Profile, Vitamin E (Alpha-Tocopherol) and Vitamin A Contents by Liquid Chromatography

The tocopherol profile, alpha-tocopherol and Vitamin A contents were determined in one sample (C5) through high performance liquid chromatography with fluorescence detector using a system from Shimadzu (Kyoto, Japan) (LC-10AD pump, DGU-14A degasser, CBM-20A system control, RF-10A_{XL} fluorescence detector, and CTO-20A column oven), managed by the software LC Solution. The determination of tocopherols was performed according to the method AOCS Ce 8- 89 (2009) and Vitamin A by AOAC 992.06 (50.1.03) (2005). For tocopherols analysis the oils were dissolved in n-hexane (2 to 4 $mg.mL^{-1}$) and submitted directly to chromatographic analysis under the following conditions: silica column (250 mm, 5 mm, 4.6 mm) Varian, USA; λexcitation: 290 nm, λ emission: 330nm; mobile phase: n-hexano/isopropanol, 99.5:0.5 v/v; flow of 1.2 $mL.min^{-1}$ and quantification with alpha-tocopherol (Vitamin E) as external standard. Oil was submitted to a previous saponification before analysis of Vitamin A. The unsaponifiable material was analyzed in C18 Microsorb-MV column (250 mm x 4,6 mm x 5 μm) Varian, USA; λexcitation: 325 nm, λ emission: 480 nm; methanol as mobile phase; flow of 1.0 $mL.min^{-1}$; identified and quantified with a standard of all *trans* retinol as external standard. Sample was analyzed in triplicate.

2.5. Determination of Stigmastadiene

The content of stigmasta 3,5-diene was determined in samples declared as extra virgin. Four samples (C1, C2, C3, and C4) were initially analysed based on the method for oils containing more than 4.0 $mg.kg^{-1}$of stigmastadienes (IOOC, 2001b). One mL of a standard solution of cholesta 3,5-diene (25.0 $\mu g.mL^{-1}$) was added to approximately 1.0 g of oil (IOOC, 2001b). No detectable levels were obtained for samples C3 and C4. Following this, the analysis was repeated with 20.0 g of each sample. Samples C3 and C4 were saponified and the unsaponifiable material was isolated (IOOC, 2001a). Samples were analysed in triplicate. The steroidal hydrocarbon fraction of the unsaponifiable matter of samples C3 and C4, or from direct coconut oils (C1 and C2), was isolated through separation in a silica gel column and the residue was dissolved in *n*-hexane and subjected to analysis in a gas chromatograph Model 17A (Shimadzu, Japan) with a flame ionisation detector (GC-FID), managed by the software GC Solution, as described previously by Aued-Pimentel et al. (2013). Identification was performed relative to the internal standard (cholesta 3,5-diene) (IOOC, 2001a,b).

3. Results and Discussion

Table 1 shows the fatty acid contents declared on the label and those experimentally obtained from the analysed product. Table 2 shows the profile of fatty acids found in commercial samples and the reference range of authentic vegetable oils.

Table 1: Contents of Fatty Acids, Vitamin E, Vitamin A. Declared values (Label) and Experimental (Exp). Mean ±SD, n=3

	Sample	C2	C3	C4	C5	C6
Compound	Serving (g oil)	12,0	3,0 (3 softgel)	14,0	4,0 (4 softgel)	3,0 (2 softgel)
SFA	Label	11.1	2.3	13	1.3	1.0
	Exp	9.9±0.1	2.66±0.01	13.1±0.1	1.40±0.04	1.09±0.05
TFA	Label	0	0	0	NI	0
	Exp	<0.1	<0.1	<0.1	-	<0.2
MUFA	Label	0.7	0.75	0.8	0.8	NI
	Exp	1.36±0.	0.18±0.02	0.67±0.01	1.1±0.1	-
PUFA	Label	0.2	NI	0.2*	1.7	1.0
	Exp	0.23±0.	-	0.11±0.01	1.4±0.1	0.61±0.02
Lauric	Label	NI	1.50	NI	-	NI
	Exp	-	1.35±0,02	-	-	-
Miristic	Label	NI	0.75	NI	-	NI
	Exp	-	0.55±0,01	-	-	-
Oleic	Label	NI	0.75	NI	0.8	NI
	Exp	-	0.18±0,01	-	1.1±0,1	-
Linoleic	Label	NI	NI	NI	1.7	NI
	Exp	-	-	-	1.3±0,1	-
Vitamin A	Label	NI	NI	NI	600*	NI
	Exp	-	-	-	ND	-
Vitamin E	Label	NI	NI	NI	10*	NI
	Exp	-	-	-	0.68±0.06*	-

SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: poly-unsaturated fatty acid. NI: not informed. ND: not detected < 65 ng RE.g^{-1}.*mg per serving. Sample C1: no nutritional labelling.

Table 2: Composition of the Main Fatty Acids in Commercial Coconut Oils and the Reference range of Authentic oils (% w/w Methyl Esters), n=2

Sample/ Fatty acid	C1	C2	C3	C4	C5	C6	Coconut oil *	Safflower oil*	Soybean oil*
6:0	0.6	0.4	0.6	0.6	nd	nd	nd-0.7	-	-
8:0	7.9	5.8	8.0	8.7	0.9	nd	4.6-10	-	-
10:0	6.1	5.0	5.6	6.4	1.0	3.2	5.0-8.0	-	-
12:0	47.2	46.8	45.7	49.8	13.2	20.7	45.1-53.2	-	-
14:0	18.0	17.2	19.2	18.1	4.5	7.7	16.8-21.0	nd-0.2	nd-0.2
16:0	8.9	9.0	8.6	7.4	8.9	7.5	7.5-10.2	5.3-8.0	8.0-13.5
16:1	nd	nd	nd	nd	0.1	0.1	nd	nd-0.2	nd-0.2
17:0	nd	nd	nd	nd	0.1	0.1	nd	nd-0.1	nd-0.1
18:0	2.7	2.2	2.8	2.8	3.2	1.5	2-4	1.9-2.9	2.0-5.4
18:1*c*	6.9	10.5	7.2	5.0	27.8	8.2	5-10	8.4-21.3	17-30
18:1*t*	nd	nd	nd	nd	0.1	nd	-	-	-
18:2*c* (n-6)	nd	2.0	1.8	1.0	33.1	41.4	1.0-2.5	67.8-83.2	48.0-59.0
18:2*t*	nd	nd	nd	nd	0.5	0.1	-	-	-
18:3*c* (n-3)	nd	nd	nd	nd	2.1	0.4 **	nd-0.2	nd-0.1	4.5-11
18:3t	nd	nd	nd	nd	0.6	**	-	-	-
18:2*t*+ 18:3*t*	nd	nd	nd	nd	1.1	0.1	-	-	-
20:0	0.1	0.1	nd	0.1	0.3	0.2	nd-0.2	0.2-0.4	0.1-0.6
20:1	nd	0.1	0.1	nd	0.2	0.1	nd-0.2	0.1-0.3	nd-0.5
22:0	nd	nd	nd	nd	0.5	nd	nd	nd-1.0	nd-0.7
24:0	nd	nd	nd	nd	0.1	0.3	nd	nd-0.2	nd-0.5

nd: not detected < 0.05%. * Range of authentic vegetable oils (*Codex Alimentarius*, 2013a). **Isomer *cis* or *trans* 18:3 (5.4%).

Sample C1, sold in a pharmacy, had no information on its nutritional labelling. Coconut oil, despite being sold in a pharmacy, should meet the mandatory nutrition labelling legislation as it is included by ANVISA as a food product. Sample C2 had monounsaturated fatty acid (MUFA) contents above those declared on the label. For sample C3 the contents of total MUFA, myristic and oleic fatty acids were below those stated. Sample C4 presented polyunsaturated contents (PUFA) higher than those declared. There was probably a mistake from manufacturer to express unity of PUFAs on the label, i.e. in milligrams and not in gram (Table 1). The accepted tolerance variation was ± 20% (declared and experimental value) (Brasil, 2003). The results related to sample C5 showed that it was a mixture of coconut oil and soybean oil (Table 2).

The presence of soybean oil as an adulterant was evidenced by the high content of linolenic acid (about 2.0%) (Table 2) and the presence of delta-tocopherol (Figure 1), both of which are characteristic of soybean oil.

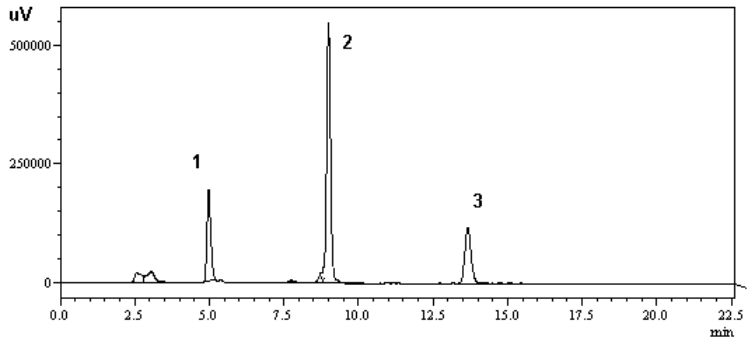

Figure 1: Tocopherol Profile of Sample C5. 1: Alpha-Tocopherol. 2: Gamma-Tocopherol. 3: Delta-Tocopherol

Sample C5, despite being presented to the consumer as only coconut oil, listed safflower oil as one of its ingredients. This oil is polyunsaturated, like soybean oil, and was not detected in sample C5 in the course of the analyses. Sample C5's label did not contain the TFA contents declared. Furthermore, the content of linoleic acid and Vitamin E (alpha-tocopherol) were below that declared and the presence of Vitamin A was not detected (detection limit: 65 ng $RE.mL^{-1}$) (Table 1). Sample C6 was reported as a mixture of coconut oil and safflower oil. The fatty acid profile of sample C6 showed the presence of saturated fatty acids characteristic of coconut oil, such as lauric acid (12:0), and polyunsaturated fatty acids, mainly C18:2, of safflower oil (Table 2). The PUFA content of sample C6 was below that declared on the label (Table 1). Considering the nutritional information, samples C2, C3, C4 and C6 had the label content of *trans* fatty acids equal to zero. These samples were in accordance with Brazilian legislation which permits up to 0.2 g of *trans* fatty acids per serving to foods that declare the value of zero on the label (Table1) (Brazil, 2003).

Considering the content of *trans* fatty acids in the samples of coconut oil investigated, values higher than the established standard for extra virgin olive oil were found only for samples C5 and C6 (Table 2). The content of *trans* fatty acids (TFA) can also be a good indicator of the presence of refined oils in virgin oils (Aparicio, 2003), and virgin olive oil is the only vegetable oil to have legal limits on the acceptable levels of *trans* fatty acid (*Codex Alimentarius*, 2013b). Sample C5 was adulterated with soybean oil and C6 was declared as mixture with safflower oil. Both polyunsaturated oils were probably refined. Sample C5 presented contents that exceeded the limit of 0.05 % (of total fatty acids), for both *trans* isomers of octadecenoic acid (18:1) and for the sum of *trans* isomers of octadecadienoic acids and octadecatrienoic acids (18:2 and 18:3); in sample C6 only the sum of isomers (18:2 and 18:3) exceeded the limit of 0.05% (Table 2). Sample C6 presented a considerable amount of a fatty acid (5.4% of total fatty acid methyl esters; Table 2) which eluted at the same retention time of a *trans* isomer of the fatty acid 18:3. The sample was also analysed in GC/MS and it was possible to conclude that the unknown compound was an isomer of the fatty acid 18:3 which can be positional or geometric. The presence of this unusual component raises suspicions that the oil contained a contaminant or by-product obtained from safflower oil, which was held in an admixture with coconut oil. The safflower oil is a raw material for the production of conjugated linoleic acid (CLA), and the presence of this component as an adulterant was verified in commercial safflower oil in Brazil (Hirashima et al., 2013). Following this finding, the presence of the main isomers of CLA were investigated in sample C6, but not detected. In Brazil, ANVISA has not authorised the sale of oils with CLA (Brasil, 2007; Hirashima et al., 2013).

Samples of coconut oil declared as extra virgin (C1, C2, C3 and C4), which were considered authentic by the fatty acid profile, were analysed in relation to stigmata 3,5-diene content, with the aim of confirming the quality. Table 3 shows the results obtained for these samples.

Table 3: Stigmasta 3,5 -Diene Contents in Commercial Coconut Oils. Mean ± SD, n=3

Sample	Stigmasta 3,5-diene ($mg.kg^{-1}$)
C1	7.3±0.3
C2	10.6±0.5
C3	< 0.15
C4	<0.15
(COI 148/2014)*	7.6±0.3

* Reference value: 7.2± 0.6 mg.kg-1

Two (C1 and C2) of the four samples declared as extra virgin showed high levels of stigmasta 3,5-diene (> 4.0 ppm, Table 3), i.e. much higher than the limit established for extra virgin olive oil (0.15 ppm) (*Codex Alimentarius*, 2013b, Brasil, 2012). The contents of stigmasta 3,5-diene in samples C1 and C2 suggesting that the oils had undergone refining processes, which promote a sensible reduction of bioactive components in oils (Jorge, 2009; Kumar and Krisma, 2015). Published data show that only traces of stigmastadienes were quantified in olive oil declared as unrefined or cold-pressed (less than 0.15 $mg.kg^{-1}$). Furthermore, it has also been found that neither the production of any conventional crude oil (virgin oil or crude vegetable oil) nor long-term storage leads to the production of measurable amounts of stigmastadiene (Grob et al., 1992; Cert et al., 1994; Schulte, 1994). Sample C2, with a high content of stigmatadiene (Table 3), presented a fatty acid profile in the limit of the range for an authentic coconut oil (18:2 and 18:3 fatty acids) (Table 2). This observation reinforces the suspected presence of refined oil in the sample C2 sold as extra virgin coconut oil. The identity of the compound stigmasta 3,5-diene in coconut oil was confirmed with the relative retention time of the internal standard (Aued-Pimentel et al., 2013). A sample of vegetable oil (sent by the International Olive Oil Council) containing reference values for the stigmasta 3,5-diene (Coi Chem 148/2014) was analysed to help identify and ensure the analytical quality of the obtained values. Figure 2 shows the peak of stigmastadiene of sample C2.

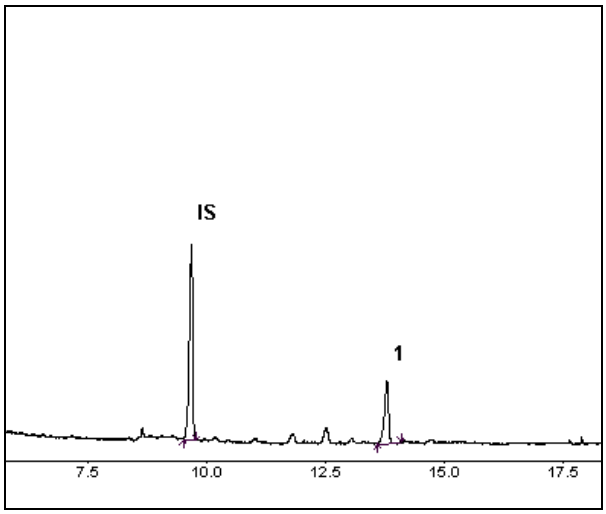

Figure 2: GC-FID Chromatogram of the Fraction of Steroidal Hydrocarbons from Sample C2. 1: Stigmasta 3, 5-Diene Peak. IS: Internal Standard: Cholesta 3,5-Diene

Samples C1 and C2 presented high levels of stigmasta 3,5-diene, but the contents of *trans* fatty acids were within the limit for extra virgin olive oil. The composition of coconut oil is predominantly made up of saturated fatty acids and even if it has been subjected to more energetic refining processes the formation of *trans* fatty acids is lower compared to a polyunsaturated oil subjected to the same procedure (Aued-Pimentel et al., 2009; Kumar and Krisma, 2015; Lutterodt et al., 2011). From the results obtained, the presence of stigmasta 3,5-diene, in the specific case of virgin coconut oil, suggest that the oil having been submitted to some sort of processing or admixing with refined oils (García-González et al.,2008; Aued-Pimentel et al., 2013; Crews et al., 2014).

4. Conclusion

Considering the large supply of specialty vegetable oils declared as virgin or cold pressed and the non-compliance observed in this study, it is evident that there is a need for more extensive control, including a larger number of samples on different types of oil, together with tougher enforcement of current standards. Such actions will collaborate to ensure the rights of consumers to purchase safer food and be provided with reliable information. More extensive studies on the content of stigmastadienes and *trans* fatty acids in virgin and cold-pressed vegetable oils should be conducted to support the proposed legal limits which may be established to assist in the legislation of quality standards for these types of oils.

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