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Fraud in olive oils of Brazilian market: evaluation by fatty acid profile, difference of ECN 42 and quality parameters

Fraude em azeites de oliva do comércio brasileiro: avaliação pelo perfil de ácidos graxos, diferença do ECN 42 e parâmetros de qualidade

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ABSTRACT

Introduction: Considering the high incidence of adulterations in olive oil marketed in Brazil, a continuous monitoring of this product is fundamental. **Objective:** In the present study, 41 samples from 18 brands were evaluated, of which 26 were declared as extra virgin olive oil (AOEV) and 15 as olive oil (AO). **Method:** The samples were analyzed at Adolfo Lutz Institute, São Paulo, Brazil, between 2014 and 2016. Fatty acids profile, acidity and peroxides indexes, specific extinction at 270 nm, ECN 42 difference, tocopherols profile and adequacy of nutritional information were determined. **Results:** Nineteen samples (46%), of 12 brands, did not present a characteristic fatty acid profile. The ECN 42 difference was sensitive to indicate the adulteration of two other samples whose fatty acid profile was of authentic olive oil. Of the 26 samples declared as AOEV, only 9 were in this category. Twenty-two samples had a monounsaturated fatty acid content (AGM) and / or polyunsaturated fatty acid content (AGP), varying more than 20% of that declared on the label. **Conclusions:** The adulterated samples were bottled in Brazil, evidencing the need for a more rigorous control in the production and commercialization of the product aiming at the nutritional safety of this food.

KEYWORDS: Olive Oil; Adulteration; Legislation; Health Surveillance

RESUMO

Introdução: Considerando a alta incidência de adulterações no azeite de oliva comercializado no Brasil, é fundamental o monitoramento contínuo deste produto. **Objetivo:** No presente estudo foram avaliadas 41 amostras de 18 marcas, sendo 26 declaradas como azeite de oliva extra virgem (AOEV) e 15 como azeite de oliva (AO). **Método:** As amostras foram analisadas no Instituto Adolfo Lutz, São Paulo, Brasil, entre os anos de 2014 e 2016. Foram determinados: o perfil de ácidos graxos, os índices de acidez e peróxidos a extinção específica a 270 nm, a diferença do ECN 42, o perfil de tocoferóis e a adequação da informação nutricional. **Resultados:** Dezenove amostras (46%), de 12 marcas, não apresentaram perfil de ácidos graxos característico. A diferença do ECN 42 mostrou-se sensível para indicar a adulteração de outras duas amostras cujo perfil de ácidos graxos era de azeite autêntico. Das 26 amostras declaradas como AOEV, somente 9 enquadraram-se nesta categoria. Vinte e duas amostras apresentaram teor de ácidos graxos monoinsaturados (AGM) e/ou poli-insaturados (AGP) variando mais que 20% do declarado no rótulo. **Conclusões:** As amostras adulteradas foram envasadas no Brasil, evidenciando a necessidade de um controle mais rigoroso na produção e na comercialização do produto com vistas à segurança nutricional deste alimento.

PALAVRAS-CHAVE: Azeite de Oliva; Adulteração; Legislação; Vigilância Sanitária

INTRODUCTION

Olive oil is highly appreciated in Brazilian cuisine due to the influence of European colonizers and immigrants. This is one of the reasons that led Brazil to be one of the main importers of olive oil in the world. Brazilian domestic demand is met by imported oil, with about 90% being supplied by European countries like Spain and Portugal and 10% by Argentina[1,2]. Brazil imports 70,000 thousand tons of olive oil a year[1] and in the last decade there has been a substantial increase of about 500%[2]. The expansion of the market and the prospect of domestic commercial production have intensified the efforts of the Brazilian government to improve the legal requirements for the control of this product. Despite these initiatives, control remains a challenge in view of evidence of persistent fraud in oil bottled in Brazil[3,4,5,6,7]. Worldwide, due to the economic importance of this product, there is much research and discussion in order to establish increasingly sensitive analytical parameters that ensure the quality of the marketed product[8,9,10].

The unique sensory and nutritional attributes of oil extracted from olives and its limited production are some aspects that explain the high market value of this product. However, oils of different categories and quality levels can be obtained from olives[11,12,13].

Extra virgin olive oil obtained from the first press of fresh olives and in a suitable state of ripeness is the one with best quality. This oil has a maximum acid content of 0.8% (expressed as oleic acid). Other olive oils of good quality flavor and aroma, but with higher values of acidity, are classified as virgin olive oil. Lower quality categories include refined olive oil and olive oil, i.e. a blend of virgin and refined olive oil. *Lampante* is a virgin olive oil made from poor quality olives. Olive pomace oil is obtained by the solvent extraction of the residual pressing cake of the olives. Both *lampante* olive oil and olive pomace oil must be refined to be fit for human consumption[11,12,13]. Defining each type of oil is a difficult task that often requires a wide variety of analytical tests[11,12,13].

The physico-chemical properties that define the identity of olive oil and other vegetable oils are mainly related to the structure of the predominant molecules. Each vegetable oil has a characteristic profile of triacylglycerol (TAG) and fatty acids (FA)[11,12].

Concerning quality, the analytical determinations that differentiate the categories of olive oil and olive pomace oil are based on the identification or dosage of certain compounds formed, for example, in olive maturation, oil extraction, storage and deterioration of the oil or other technological process that the oil might have undergone[11,12]. Some of the parameters that may indicate the quality of those oils are: acid value, peroxides, specific extinction in ultraviolet (270 and 232 nm). The trans fatty acid content also indicates the quality of olive oils[11,12].

Codex Stan 33 establishes the quality and identity standards of olive oil and olive pomace oil[14].

In Brazil, Normative Instruction n. 1 of the Ministry of Agriculture, Livestock and Food Supply (MAPA) regulates tolerance thresholds for various parameters of identity and quality of olive oil and olive pomace oil, based on what is established in the standards of the *Codex Alimentarius* and in the commercial standard of the International Olive Oil Council (IOOC) [14,15,16]. The classification standard for olive oil and olive pomace oil marketed in Brazil was also defined according to those legislations[16]. In the area of Health, RDC n. 270, of September 22, 2005, of the Brazilian Sanitary Surveillance Agency, is the technical regulation for vegetable oils, vegetable fats and vegetable cream, which establishes the identity and minimum characteristics of quality, such as the acid and peroxide values of these products[17]. Nowadays, the control and inspection of the product obtained from olives sold in Brazil must be done by the MAPA and the Ministry of Health, supported by compatible and complementary legislation[5].

Considering the evidence of continuous fraudulent practices in oils marketed in Brazil[3,4,5,6,7,18], the present work had the objective of evaluating the quality and identity of commercial oils marketed as olive oils and submitted to the Adolfo Lutz Institute for analysis by inspection services between the years 2014 and 2016.

METHOD

Samples

Forty-one samples from different batches and 18 brands were analyzed (Table). The brands were coded with letters A to R. Twenty-six samples were declared as extra virgin olive oil (EVOO) (15 brands) and 15 as olive oil (OO) (5 brands). Twenty-six samples were sent by the Sanitary Surveillance agency of the state of São Paulo, 12 by the Institute of Forensic Medicine of São Paulo and 3 by the Sanitary Surveillance agencies of other Brazilian states (Bahia, Minas Gerais and Espírito Santo). The F brand samples were bottled in Portugal and the J and G brands were bottled in Spain. The other samples were bottled in Brazil.

Acid, peroxide and specific extinction values at 270 nm

The acid and peroxide values and specific extinction at 270 nm were determined according to physical and chemical methods of the Adolfo Lutz Institute (2005)[19].

Table. Characteristics of commercial oil samples

Sample	Brand	Type	Sample	Brand	Type
1-6	A	OO	28	I	EVOO
7	B	EVOO	29	J	EVOO
8	C	EVOO	30-34	K	EVOO
9	D	OO	35	L	EVOO
10-12	E	EVOO	36	M	OO
13,15,18,21,22	F	OO	37	N	EVOO
14,16,17,19,20	F	EVOO	38	O	EVOO
23,25	G	EVOO	39	P	EVOO
24,26	G	OO	40	Q	EVOO
27	H	EVOO	41	R	EVOO

EVOO: extra virgin olive oil; OO: olive oil

The acidity, expressed as a percentage of oleic acid, was determined by titration with 0.1 M sodium hydroxide. The peroxide value, expressed in milliequivalents per 1,000 g of sample, was determined by solubilizing the sample in acetic acid-chloroform (3:2) and adding saturated potassium iodide solution. The liberated iodine, after reacting with the peroxides, was titrated with 0.01 M sodium thiosulphate solution.

The specific extinction measure at 270 nm was done on a single-beam spectrophotometer, model Specord S 600 (Analytikjena, Germany), with 1 cm quartz cuvettes in the 1% solution of the oil in spectroscopic grade cyclohexane.

Composition of fatty acids

Fatty acids were analyzed by gas chromatography (GC) with flame ionization detector (FID).

Fat was converted to fatty acid methyl esters (FAME) by reaction with methanol solution of 2M KOH. The FAME were separated on a 100 m fused silica capillary column (SP 2560), installed on gas chromatograph, Focus model (Thermo, USA), with the following chromatographic conditions: column oven temperature: isothermal at 180° C , injector and detector temperature: 250° C, carrier gas H2; column pressure: 170 kPa[19]. The components were identified by co-injection of standards and comparison with absolute retention times. Fatty acids were quantified by area normalization and expressed as area percentage. The profiles were evaluated in comparison with the reference values of the *Codex Alimentarius*[14] and the Brazilian legislation[16].

The values stated on the label were compared with those obtained experimentally for saturated (SFA), trans (TFA), mono (MUFA) and polyunsaturated fatty acids (PUFA).

ECN 42 difference

The ECN 42 difference was evaluated in two samples (1 and 11), which presented alpha-linolenic acid content (18:3 n-3) very close to 1%, which is the maximum limit of this parameter, according to NI n. 1, of January 30, 2012, of the MAPA. The triacylglycerols were separated and quantified by high performance liquid chromatography (HPLC) with refractive index detector, and the difference between the experimental value (HPLC) and theoretical value from the fatty acid composition was obtained according to the official method of the International Olive Oil Council (2001)[20]. The high performance liquid chromatograph with refractive index detector (Japan, Shimadzu) was composed of the: LC-10AD pump, DGU-14A degasser, CBM-20A interface, RID refractive index detector, and of the CTO-20A column oven. The following conditions were used: C-18 (250 mm x 4.6 mm x 5 µm) reverse phase column (Varian, USA); mobile phase: acetone: acetonitrile (1:1), flow: 1.15 mL.min^{-1}.

Tocopherol profile

The tocopherol profile was determined by HPLC with fluorescence detector. The chromatograph was composed of the following modules: LC-10AD pump, DGU-14A degasser, CBM-20A interface, RF-10AXL fluorescence detector and CTO-20A column oven (Shimadzu, Japan). The determination of the tocopherol profile followed method AOCS Ce 8-89[21]. The profile was determined in samples 1 and 11, which were also submitted to analysis of the ECN 42 difference.

RESULTS AND DISCUSSION

Figure 1 shows the identity parameters (composition of the main fatty acids) and Figure 2 shows the quality parameters (acidity, peroxide and E 270 nm values) obtained for the commercial oils samples, in relation to the values of reference according to NI n. 1/2012 of MAPA[16].

Of the 26 samples declared as EVOO, only nine fit into this category according to the parameters evaluated (acidity, peroxides, E 270 nm and trans fatty acids) (Figures 1D, 1E and Figure 2). Sixteen of these samples were adulterated with another vegetable oil, as indicated by the fatty acid profile (Figure 1). These also presented very high values of E 270 nm (Figure 2C), exceeding the limits of the category, which reinforces the presence of refined oil in the samples[5,11]. Two samples, with fatty acid profile characteristic of olive oil, presented E 270 nm equal to 0.82 and 0.44, (samples 11 and 38), that is, above the limit for EVOO (Figure 2C).

In relation to the profile of fatty acids, 19 samples (46%) of 12 brands did not present a characteristic profile of olive oil. Nine had a soybean oil profile (samples 6, 7, 9, 10, 12, 31, 32, 34 and 35). In 18 samples, the alpha-linolenic acid content (18:3 n-3) was higher than 1%, the threshold established in the Brazilian legislation for this parameter in olive oil (Figure 1C)[16]. Commercial samples with values close to or higher than 1% may be adulterated with oils with a considerable content of alpha-linolenic acid, as is the case of soybean, with 4.5 to 11%[22]. It should be noted that soybean oil has a low commercial value when compared to olive oil and is the most common adulterant in Brazil[5, 6, 7]. Figure 3 shows the fatty acid profile of a sample of authentic olive oil and soybean oil.

The samples, probably adulterated with soybean oil, had a high content of trans fatty acids (TFA), mainly the sum of 18:2t and 18:3t, which are formed in the refining process of poly-unsaturated vegetable oils like soybean oil[23]. That sum exceeded the threshold for both EVOO and OO (Figure 1E).

Two samples, one marketed as OO (Sample 1) and another as EVOO (Sample 11), presented alpha-linolenic acid content between 0.9 and 1.0%, very close to the maximum threshold of the parameter according to IN n. 1/2012 of the MAPA (Figure 1C). These samples were also analyzed for the ECN 42 difference and the tocopherol profile. The values obtained for the ECN 42 difference in samples 1 and 11 were 0.60 and 0.85, respectively, that is, higher than 0.50, indicating the presence of vegetable oils rich in linoleic acid in the sample, like soybean oil[12,14,15,16]. Figure 4 presents the chromatogram of the triacylglycerols from a sample marketed as extra virgin olive oil, highlighting the region of triacylglycerols with ECN 42.

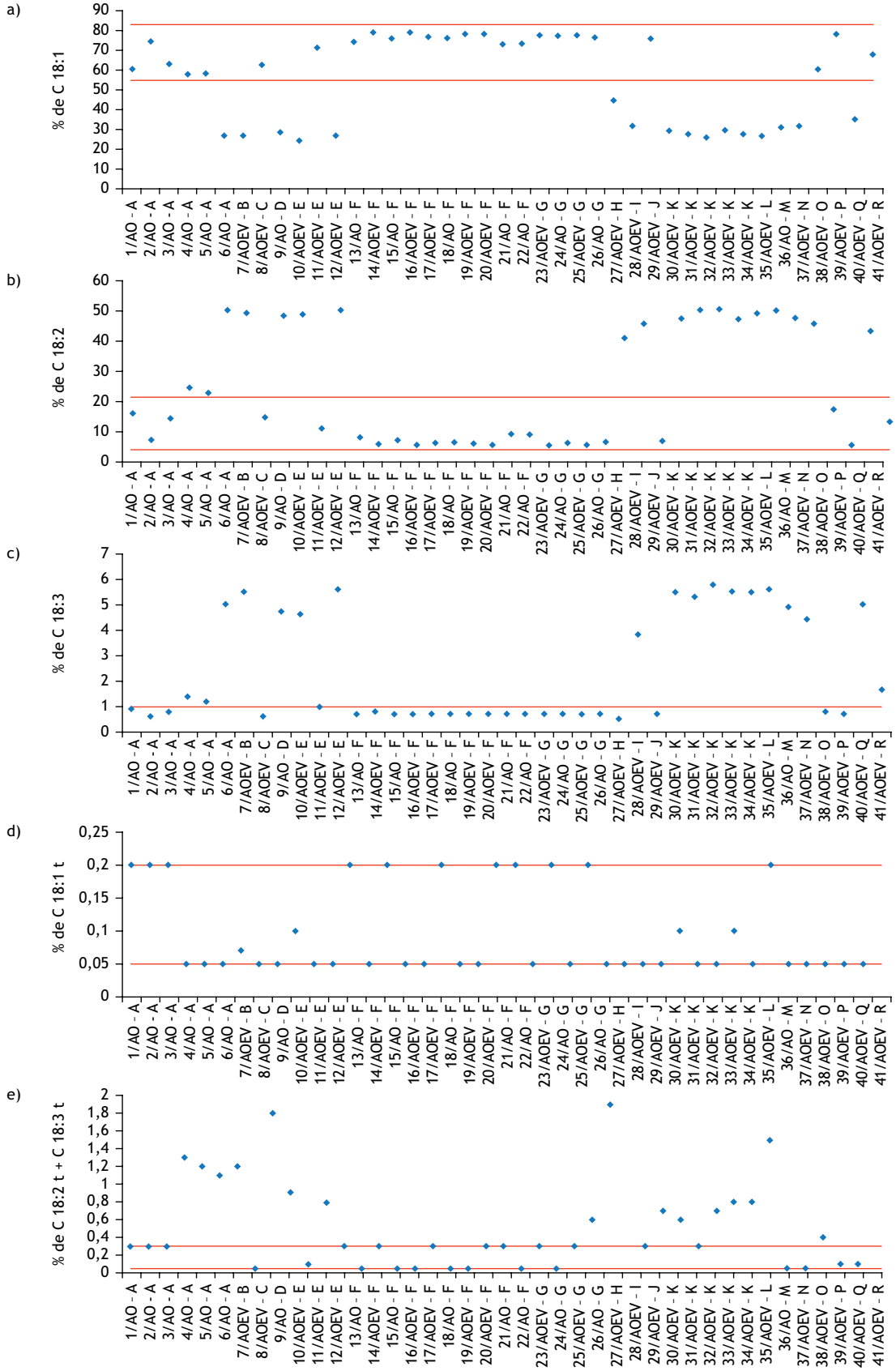

Figure 1. Fatty acid values for EVOO and OO samples, compared to the thresholds of NI n. 1/2012. 1A - fatty acid C 18:1 n-9; 1B - fatty acid C 18:2 n-6; 1C - fatty acid C 18:3 n-3; 1D - fatty acid C 18:1 t; 1E - fatty acid C 18:2 t + C 18:3 t;— thresholds of NI n. 1/2012 for each fatty acid (Figures 1A, 1B and 1C)— thresholds of NI n. 1/2012 for Figure 1 D (0.05% for OO)— thresholds of NI n. 1/2012 for Figure 1 E (0.05% for EVOO and 0.3% for OO). Different letters for samples mean different brands.

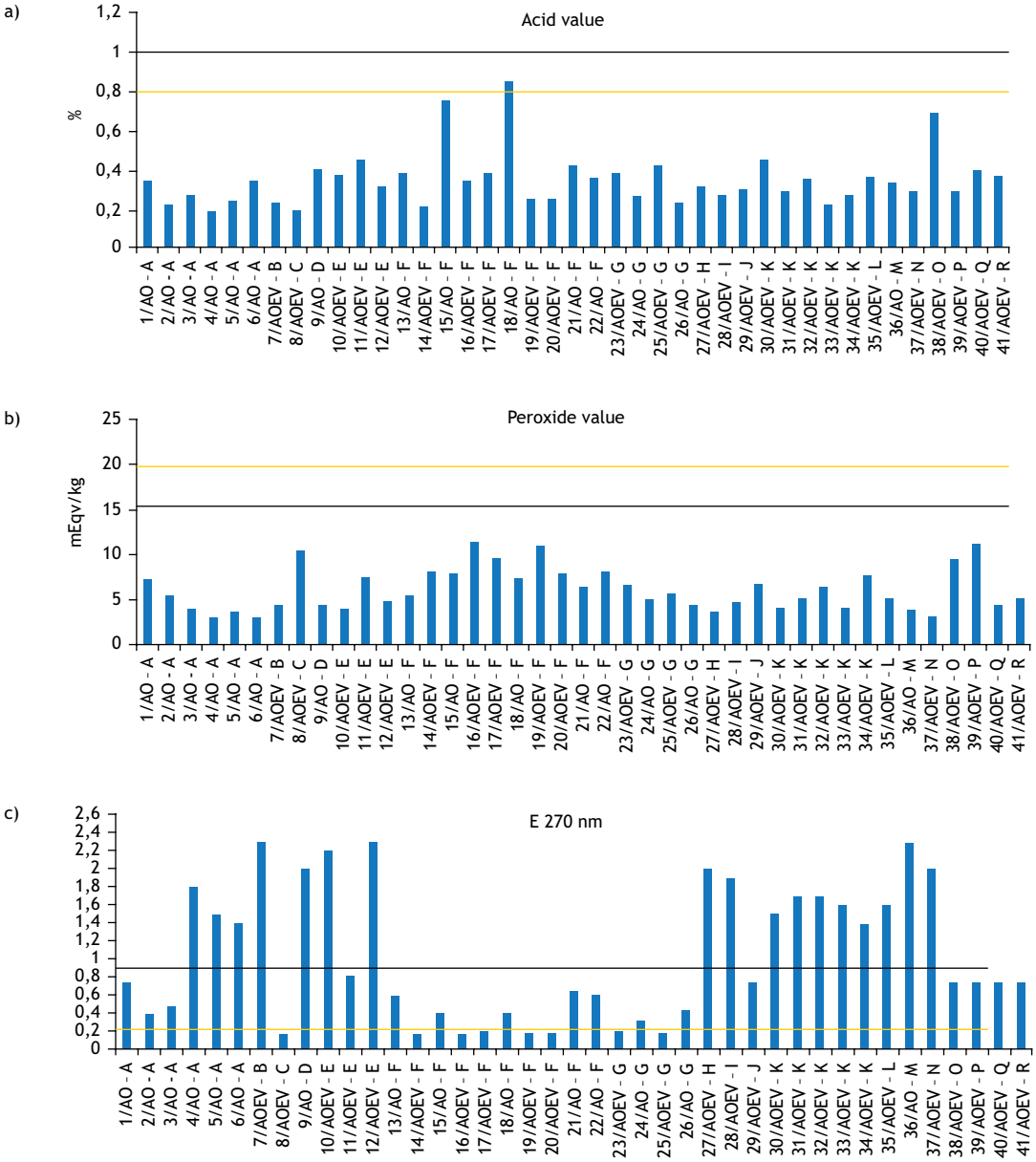

Figure 2. Parameters of quality and identity of oils marketed as olive oil. 2A - Acid value; 2B - Peroxide value; 2C - Extinction at 270 nm. - thresholds for EVOO according to NI n. 1/2012; - limits for OO according to NI n. 1/2012. Different letters for samples mean different brands.

The triacylglycerols with ECN 42, especially trilinolein (LLL), which in Figure 4 is the major component in the ECN 42 group, are only present as traces in authentic olive oil[7,12]. The ECN 42 difference is a very important parameter in the evaluation of the oils marketed in Brazil, since it is sensitive to indicate adulteration by the addition of up to 2.5% of oils rich in linoleic acid, like soybean, corn and sunflower oil[7,11,12].

Samples 1 and 11 were also evaluated for the tocopherol profile. Both presented delta-tocopherol in the profile, which is characteristic of soybean oil and not detected in olive oil[14,22,24] (Figure 5).

Although the tocopherol profile is not included in the parameters of the *Codex Alimentarius* standard for olive oil, the observed result in this specific case reinforces the presence of soybean oil in the two samples. The E 270 nm measure for Sample 11, declared with EVOO, was 0.82, which is well above the category threshold (0.22), reinforcing the presence of a refined oil blend (Figure 2C).

Considering the nutrition facts on the labels of the samples, 22 samples presented MUFA and/ or PUFA content varying more than 20% of the declared in the 13 mL portion, 18 of which were adulterated samples. For 20 samples the levels of monounsaturated fatty acids were below and the levels of polyunsaturated fatty acids were much higher than those reported (Figure 6).

Since 2003 Brazil has adopted mandatory nutritional information on the label of processed food as a strategy for the prevention

of chronic diseases[25]. It is mandatory to declare the content of saturated and trans fatty acids, but most part of vegetable oil manufactures include information on MUFA and PUFA fatty acids. In this study, 25 samples declared, besides the obligatory nutrients, the monounsaturated and polyunsaturated fatty acids in the serving (13 mL). However, the results show that the information on MUFA and PUFA, for the most part, did not correspond to the information declared (Figure 6).

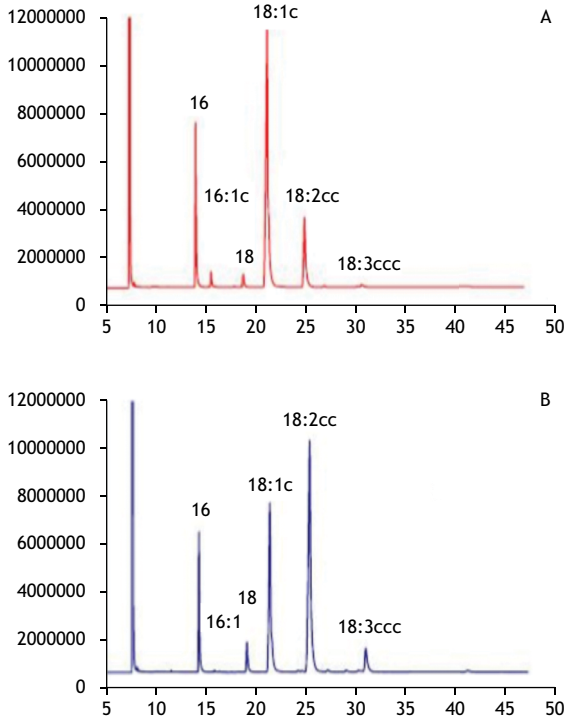

Figure 3. Profile of fatty acids: A) authentic olive oil; B) soybean oil

CONCLUSIONS

Twenty-one samples of 12 brands (51%) collected in the Brazilian market were adulterated with vegetable oil of lower commercial value, mainly soybean oil. Most of the observed adulteration was detected by the fatty acid profile. However, for two samples, whose fatty acid profile was that of authentic olive oil, the adulteration could only be verified using the determination of the ECN 42 difference. The tocopherol profile corroborated the observed adulteration.

Regarding nutritional information, 54% of the samples had levels that were not in agreement with the information declared on the label for MUFA and PUFA.

The adulterated samples were bottled in Brazil, evidencing the need for more strict control in the manufacturing and marketing of this product, with a view to food safety assurance.

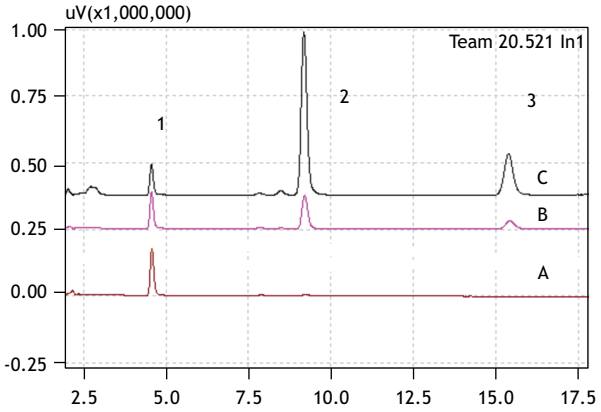

Figure 5. Tocopherol profile of samples of vegetable oils. A) Authentic olive oil, B) Commercial oil labeled as olive oil and C) Soybean oil. 1. Alpha-tocopherol. 2. Gamma-tocopherol. 3. Delta-tocopherol.

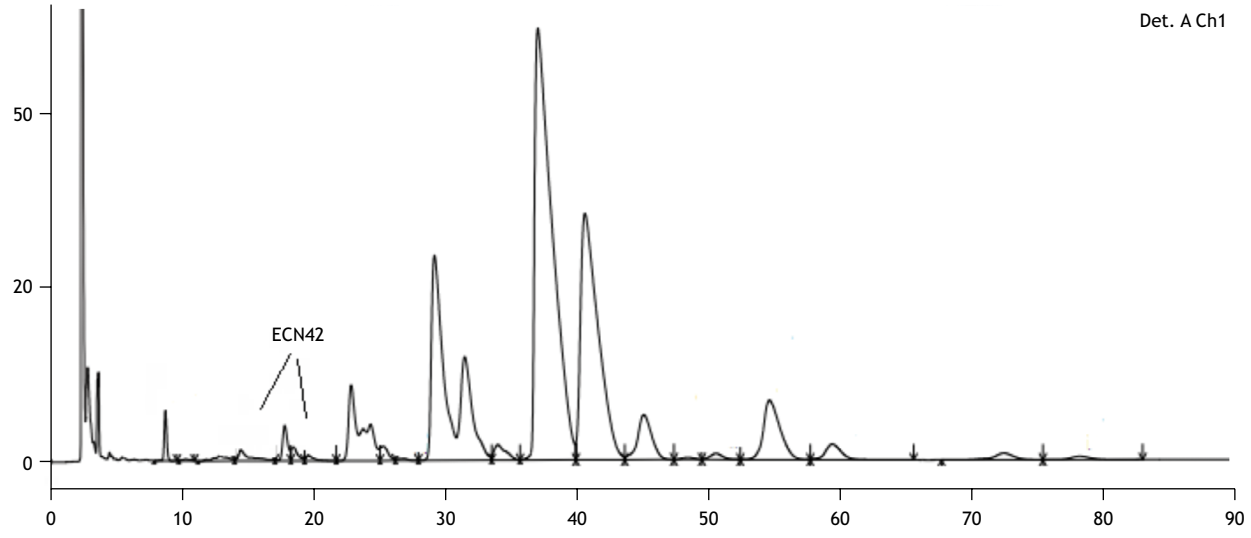

Figure 4. Profile of triacylglycerols (TAG) of commercial sample labeled as olive oil. TAGs with ECN 42: LLL, OLnL, PLnL. L: linoleic acid; O: oleic acid; P: palmitic acid; Ln: linolenic acid.

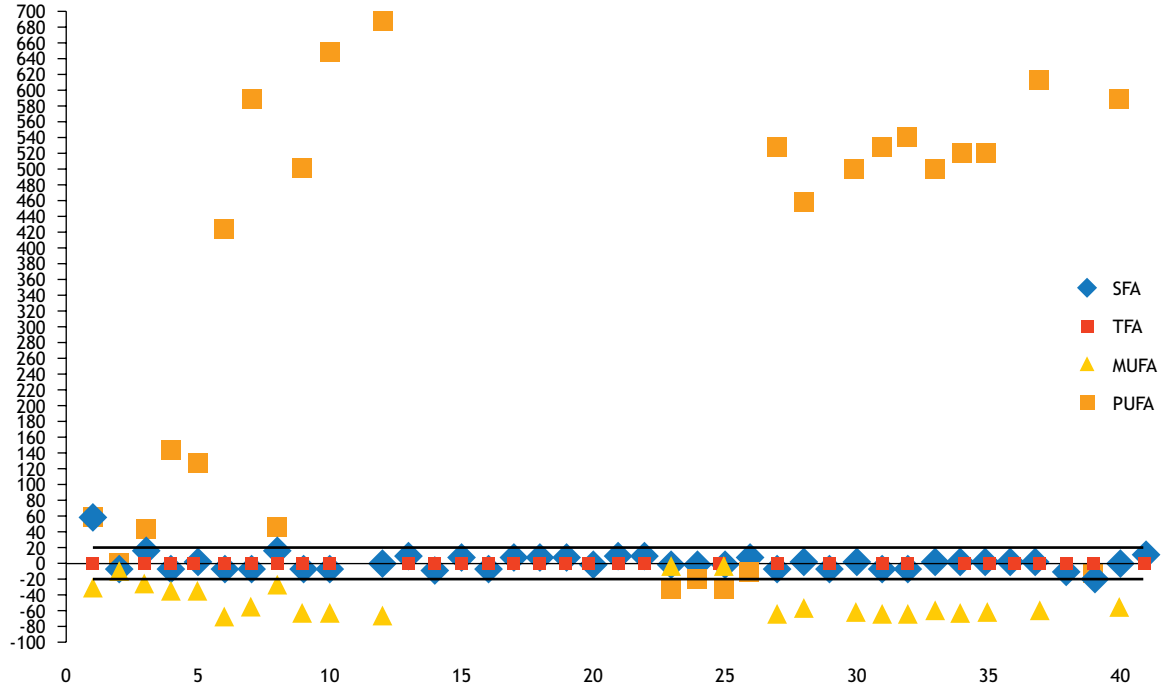

Figure 6. Percentage of variation of the components analyzed in the samples (SFA, TFA, MUFA and PUFA) in relation to the values declared on the label. Tolerance of 20%.

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Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.