Food Research International 102 (2017) 43–50

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Rapid methodology *via* mass spectrometry to quantify addition of soybean oil in extra virgin olive oil: A comparison with traditional methods adopted by food industry to identify fraud

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ARTICLE INFO

Keywords:
Direct infusion
Mass spectrometry
Extra virgin olive oil
Soybean oil
Fraud

ABSTRACT

Fast and innovative methodology to monitors the addition of soybean oil in extra virgin olive oil was developed employing ESI-MS with ionization operating in positive mode. A certified extra virgin olive oil and refined soybean oil samples were analyzed by direct infusion, the identification of a natural lipid marker present only in soybean oil (*m/z* 886.68 $[TAG + NH_4]^+$) was possible. The certified extra virgin olive oil was purposely adulterated with soybean oil in different levels (1, 5, 10, 20, 50, 70, 90%) being possible to observe that the new methodology is able to detect even small fraud concentration, such as 1% (v/v). Additionally, commercial samples were analyzed and were observed the addition of soybean oil as a common fraud in this segment. This powerful analytical method proposed could be applied as routine analysis by control organization, as well as food industries, considering its pronounced advantages; simplicity, rapidity, elevated detectability and minor amounts of sample and solvent consumed.

1. Introduction

Nowadays consumers are particular concerned about products quality, valuing and prioritizing healthy food (INMETRO, 2015) emphasizing olive oil (*Olea europaea*) due to its combination of enjoyable flavor and nutritional benefits (Aparicio & Aparicio-Ruíz, 2000).

Olive oil is obtained through mechanical and physical processes using fresh and healthy fruits in appropriate maturation stage (Monasterio, Fernandéz, & Silva, 2013) without treatment to modify its original composition (Frankel, Bakhouche, Lozano-Sánchez, Segura-Carretero, & Fernández-Gutiérrez, 2013), therefore it is considered a high quality product (Hassen, Casabianca, & Hosni, 2015).

International Olive Council (IOC, 2016a) classifies olive oil in two main groups, primarily olive oil suitable for consumption, including extra virgin, virgin and ordinary olive oil and secondly virgin olive oil that must undergo processing prior to ingestion, including lampante, refined and olive oil composed of refined olive oil and virgin olive oils. However, extra virgin olive oil (EVOO) is recognized by its exceptional quality, being particularly desired due to its organoleptic characteristics (Aued-Pimentel, Takemoto, Minazzi-Rodrigues, & Badolato, 2002).

According to IOC (2016b), Brazil is among the major consumers of olive products, importing nearly 60% of the consumed volume. Principal producers are Spain and Italy (IOC, 2016b), nevertheless Brazil has been presenting progress in olive oil production; from January to October of 2015 an increase of 5% in its total volume comparing to the same period of 2014 was observed (INMETRO, 2015). But the production of olive oil is still reduced in comparison to other edible vegetable oils.

Owing to health appeal, desirable characteristics and limited production, EVOO has elevated commercial value, becoming focus of intentional adulteration (Aued-Pimentel et al., 2002). Among it, the most common fraud includes the addition of low cost edible vegetable oil

Abbreviations: DAG, diacylglycerol; ESI-MS, electrospray ionization mass spectrometry; EVOO, extra virgin olive oil; FA, fatty acid; FAME, fatty acid methyl ester; FFA, free fatty acid; FID, flame ionization detector; GC, gas chromatography; GLL, gadolein dillinoleo; GOL, gadolein olein linoleo; HPLC, high performance liquid chromatography; IOC, International Olive Council; L, linoleic acid; LLL/OLLn3, trilinolein/olein dilinolein; LLLn/OLnLn, dilinoleo linolein/olein dilinolein; LLPo, dilinoleo palmitolein; Ln, linolenic acid; MAG, monoglycerol; MS, mass spectrometry; MUFA, monounsaturated fatty acid; O, oleic acid; OLL/OOLn, olein dilinoleo/diolein linolein; OLMo, olein linoleo 10-heptadecenoic; OOL, diolein linoleo; OOMa, diolein heptadecanoic; OOMo/OLMa, diolein 10-heptadecenoic/olein linoleo heptadecanoic; OOO, triolein; OOO/SOL, triolein/stearo olein linoleo; P, palmitic acid; POL, palmito olein linoleo; POO, palmito diolein; PUFAs, polyunsaturated fatty acids; RSD, relative standard deviation; S, stearic acid; SO, refined soybean oil; SOO, stearo diolein; TAG, triacylglycerol; UPLC, ultra performance liquid cromatography; UV/VIS, ultraviolet and visible

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http://dx.doi.org/10.1016/j.foodres.2017.09.076
Received 14 July 2017; Received in revised form 20 September 2017; Accepted 25 September 2017
Available online 28 September 2017
0963-9969/

(Peixoto, Santana, & Abrantes, 1998) such as refined soybean oil (SO); the second most consumed vegetable oil around the world (Hartman, West, & Herman, 2011), and the main culture in Brazil. The country is the second largest producer in the world, behind only USA (EMBRAPA, 2017); besides Parana State represents 18% of the total amount of SO produced annually in Brazil (CONAB, 2017). The price difference between EVOO and SO could be a technique to industries profit from the addition of low cost oils to EVOO (Aparicio & Aparicio-Ruíz, 2000).

Food adulteration has pronounced significance from industry to regulatory agencies, but specially for final consumers, who are been deluded about the product's quality consumed (Rohman & Che Man, 2010). Aiming to prevent it, Brazilian legislation implements the criteria of quality and identity established by IOC (2016a) and Codex Alimentarius Commission (FAO/WHO, 2015).

In order to monitor oils authenticity, instrumental techniques through qualitative and quantitative parameters are adopted (Becker, Gonçalves, Grimaldi, & Fernandes, 2005). Additionally, advances in knowledge and technology have been leading to successful combat against frauds. Although, it is equally true that it has been used to tamper olive oil (Aparicio & Aparicio-Ruíz, 2000) once the complexity involved in its composition creates a problematic in fraud detection (Peixoto et al., 1998).

Hence, the present work aims to apply simple methodologies established by Codex Alimentarius claiming to ensure quality criteria, such as free acidity and absorbency in ultraviolet for later comparison with label information, plus ensures purity criteria through fatty acid composition. The results from all three analyses adopted by food industry were compared to a new developed method to identify and quantify addition of SO in EVOO, detecting a lipid marker present only in SO from triacylglycerol (TAG) composition obtained by ESI-MS direct infusion. Subsequently, all four methodologies were applied in commercial samples and compared in order to demonstrate that the current methodology adopted by industry to guarantee purity and quality of EVOO is not sufficient to detect small adulteration.

2. Materials

2.1. Experimental

Methanol, cyclohexane, chloroform and ethanol were purchased from Sigma-Aldrich (Darmstadt, Germany); ethylic ether and isooctane were purchased from Nuclear and Anidrol (São Paulo, Brazil). Analytical standard methyl tricosanoate and ammonium formate (97%) were acquired from Sigma-Aldrich (Darmstadt, Germany). For physicochemical and chromatography analysis all reagents and solvents used were analytical grade, while for MS analysis the solvents were HPLC grade.

2.2. Olive oil

Eight bottles of EVOO and two cans of commercial blends of SO and EVOO from three different lots were acquired from local market in Maringa—PR, Brazil, according to Table 1, plus one sample with international certificate identified as P was used as standard. All samples were preserved in its original container under refrigeration (6 to 10 °C) and sheltered from light.

2.3. Refined soybean oil

Three lots containing three samples each of SO, identified as S, was obtained from local market in Maringa—PR, Brazil.

2.4. Addition of soybean oil in extra virgin olive oil

The standard (P) was intentionally adulterated with addition of SO in seven different levels (1, 5, 10, 20, 50, 70, 90% (v/v)) with five replicates each, according to Table 2, to evaluate the developed method.

Table 1
Specification from oil's label.

Sample	Produced	Packaged	Label information[a]
1	Chile	Chile	EVOO[b]
2	Spain	Spain	EVOO
3	Portugal	São Paulo	EVOO
4	Portugal	Portugal	EVOO
5	European Union	Italy	EVOO
6	Paraná	Paraná	Blend of SO and EVOO (10%)
7	São Paulo	São Paulo	Blend of SO and EVOO (15%)
8	Portugal	Portugal	EVOO
9	Minas Gerais	Minas Gerais	EVOO
10	Greece	Greece	EVOO
P[d]	Italy	Italy	EVOO
S	Brazil	Brazil	SO[c]

n = 39.
[a] Classification according to the exposed in label.
[b] EVOO: Extra virgin olive oil.
[c] SO: Refined soybean oil.
[d] International certified sample.

Table 2
Intentionally adulterated EVOO with addition of SO.

% of adulteration[a]	EVOO[b]	SO[c]
0	100	0
1	99	1
5	95	5
10	90	10
20	80	20
50	50	50
70	30	70
90	10	90
100	0	100

[a] (v/v).
[b] EVOO: Extra virgin olive oil.
[c] SO: Refined soybean oil.

2.5. Quality criteria analyzes

2.5.1. Free acidity

According to COI/T.20/Doc. No. 34—"Determination of free fatty acids, cold methods" (IOC, 2015b). 2.0 g of total liquid samples were weighted in 125 mL erlenmeyer, 25.0 mL of diether:ethanol (2:1) neutral solution was added into it, few drops of phenolphthalein indicator were also added. The mixture was titrated with 0.1 mol L^{-1} sodium hydroxide solution until the consistent appearance of a light pink color. Samples were analyzed in triplicate.

2.5.2. Absorbency in ultraviolet

According to COI/T.20/Doc. No. 19/Rev. 3—"Spectrophotometric investigation in the ultraviolet" (IOC, 2015c). 0.25 g of totally liquid sample were weighted in 25 mL volumetric flask, cyclohexane was added originating solution A. 5.0 mL aliquot of solution A was transferred to 25 mL volumetric flask, cyclohexane was added originating solution B. Absorbances were read in UV/VIS calibrated with cyclohexane at wavelengths 270 nm and 232 nm, respectively. Absorbance 268 nm and 274 nm were also read in order to calculate ΔK. All samples were analyzed in triplicate.

2.6. Purity criteria analysis

2.6.1. Fatty acid (FA) composition

According to COI/T.20/Doc. No. 33—"Determination of fatty acid