Camellia Seed Oils

Subjects: Plant Sciences Contributor: Efrén Pérez-Santín

Camellia genus (Theaceae) is comprised of world famous ornamental flowering plants. C. japonica L. and C. sasanqua Thunb are the most cultivated species due to their good adaptation. The commercial interest in this plant linked to its seed oil increased in the last few years due to its health attributes, which significantly depend on different aspects such as species and environmental conditions. Therefore, it is essential to develop fast and reliable methods to distinguish between different varieties and ensure the quality of Camellia seed oils. The present work explores the study of Camellia seed oils by species and location. Two standardized gas chromatography methods were applied and compared with that of data obtained from proton nuclear magnetic resonance spectroscopy (1H-NMR) for fatty acids profiling. The principal component analysis indicated that the proposed 1H-NMR methodology can be quickly and reliably applied to separate specific Camellia species, which could be extended to other species in future works.

Camellia oil authentication quality chromatogra

chromatographic techniques

nuclear magnetic resonance chemometrics

1. Introduction

Camellia is a genus of flowering plants in the family *Theaceae*, native to East Asia and widely distributed in China, India, Japan, and South-East Asian countries, whose seeds and leaves present high nutritional and medicinal values. This subtropical evergreen shrub or small tree arrived in Europe around the 16th century ^[1], and was introduced into the gardens of the highest social classes of Galicia (NW of Spain) at the beginning of the 19th.

Nowadays, cultivars of *Camellia* species are found worldwide in public and private gardens thanks to their excellent adaptation to climatic and edaphic conditions, easy spread, and resistance to pests and diseases. Particularly, *Camellia japonica* L. is the best known internationally as a cultivated species for ornamental value. In the last decade, commercial interest was remarkable, and consequently, production in Spain reached about 2.5 million *Camellia* plants per year, which are exported throughout Europe as ornamentals ^{[2][3][4]}.

Camellia oil is obtained from the seeds, known as one of the most popular edible vegetable oils that was utilized for more than 1000 years in China, and also abundantly used in southeast Asian countries (Japan, Korea, India, Sri Lanka, Indonesia, and Vietnam), where *Camellias* are abundantly available ^[5].

Camellia oil is also known as "Eastern Olive Oil" because it shares a similar chemical composition with olive oil ^[6]. It contains several natural antioxidants, such as squalene, phytosterol, polyphenols, fat-soluble vitamins (vitamins

A, B, E), sasanqua saponin, and other functional substances. It was recommended by the Food and Agriculture Organization of the United Nations as a high-quality, healthy vegetable oil because of its nutritional value and excellent storage qualities ^[Z]. For these reasons, it is commonly used as cooking oil (edible oil) ^{[8][9]}. In China, the main species used for oil production is *Camellia oleifera* C. Abel ^[10], while in Japan this is *C. japonica* ^[11], and *C. sasangua* in Vietnam ^[12].

Camellia oil is an expensive product with a particular and characteristic aroma and taste, good storage stability, and high nutritional and medicinal values, with high value interest for trade ^[13]. Thus, the economic interest in this crop increased exponentially in recent years for a variety of purposes ^[14]. Specifically, *Camellia* oil extracted from seeds of different species, including *C. reticulata Lindl., C. sinensis* L., *C. oleifera*, and *C. japonica*, was long processed as an industrial oil used for oligosaccharide production ^[15], as a surfactant, in soaps, as a hair oil, and now it is generating interest as a biofuel source, lubricant, and biopolymer ^{[16][17][18][19][20]}. Although, in cosmetics *C. japonica* oil has a long history of traditional cosmetic usage in Japan as a protectant to maintain skin and hair health, where other species are nowadays commonly used for this purpose (e.g., *C. oleifera, C. grijsii* Hance, and *C. sasanqua*) ^{[11][21]}. *Camellia* oil has fat-soluble natural compounds with health benefits, reducing cholesterol and triglycerides in the blood, lowering blood pressure, and promoting effects such as antioxidation, antipermeability, anti-inflammation, as an analgesic, and anticancer properties ^{[22][23][24]}, as well as antimicrobial and antiviral activities ^[25]. In addition to this, they are used in traditional treatments in China to prevent cardiovascular diseases, arteriosclerosis, and burn injuries ^{[26][27][28]}.

Triacylglycerols are the principal components of Camellia oils, with a high proportion of oleic and linoleic acids and low saturated acids. This general lipidic profile is associated with well-known health properties. The oil yield of seeds from this plant is high, being on average 30% oil per seed. However, the seed oil content varies according to species, cultivar, and environmental conditions ^{[29][30]}. The profile of fatty acids (FAs) allows correlation to be made with their botanical origin, which is a very important aspect from a commercial point of view, since the traceability of these oils is mandatory to avoid fraud by adulteration. The properties of the oils are also dependent on the FAs' composition. The degree of unsaturation and chain length, and the presence of polyunsaturated FAs, appear to increase the potential beneficial properties of these oils ^[31]. The unsaturated FAs content in *Camellia* oil can reach as much as 90%, which is the highest amount so far reported for unsaturated FAs in edible oils [22][32][33]. In recent years, Camellia oil became one of the most popular and expensive edible vegetable oils on the market in China, being more susceptible to adulteration with other cheaper oils by unscrupulous traders for high profits. Another aspect of fraud, the mislabeling of oil extraction methods, and geographical or origin, also destabilize the local Camellia oil market economies [34]. The method for Camellia oil authentication currently used officially, employing gas chromatography (GC) techniques, includes the FAs' composition. The increased demand for Camellia oil made the development of rapid and reliable methods for the unequivocal chemical plant species oil characterization associated with the quality of the edible oil a priority objective to avoid commercialization of adulterated *Camellia* oils [35][36][37][38][39].

To determine the FA composition, a wide variety of analytical methods are available. In this context, traditional methods are gas chromatography with flame ionization detectors (GC-FID) ^[40] or gas chromatography-mass

spectrometry (GC-MS) ^[41]. In these methods, a pretreatment of the sample is necessary to convert the FA into the corresponding methyl esters (FAMEs). So, these methodologies are tedious, time-consuming, require the use of FAs standards, and involve complicated pretreatment of the samples prior to analysis, such as the triacylglycerol hydrolysis and esterification that could face problems of oxidation during the derivatization process ^{[42][43][44]}.

Currently, new, rapid, and nondestructive methods such as Near-InfraRed (NIR), Raman Spectroscopy, and Nuclear Magnetic Resonance (NMR) techniques were recognized as alternative analytical tools in combination with appropriate chemometrics in oil quality control ^[45]. Specifically, recent studies confirmed that NMR is a powerful tool for qualitative and quantitative analysis of FAs composition in edible vegetable oils ^{[32][40][46][47][48][49][50]}.

2. Results and Discussion on Camellia Seed Oils

2.1. Oil Content

Seeds of all *Camellia* species contain oil. However, oil content and quality may vary with species ^[51]. High seed oil variability is likely the result of several factors, including environmental variables such as soil, altitude, light, rainfall, humidity, and temperature, all playing a key role, as previously demonstrated for a variety of plants ^[30]. Thus, seed oil content (SOC) of traditional *Camellia* varieties can range between 24% and 50%, with an average about 30% ^[29]. *C. oleifera*, which is the earliest species exploited for edible oil, accounting for 98% of the *Camellia* cultivated area in China, was previously reported to provide an SOC between 21% and 34% ^[52]. Moreover, some of the new *C. oleifera* cultivars can reach as much as 53% oil per dry seed ^[53].

In this study, seeds from different *Camellia* species (*C. japonica*, *C. sasanqua*, *C. reticulata*, and *C. hiemalis* Nakai) were harvested in various locations in the province of Pontevedra (Galicia, NW Spain, **Figure 1**) during the last four months of 2019. The percentage of seed oil extracted from *Camellias* varied from 16.1% to 31.9% for *C. japonica*, and from 22% to 30.1% for *C. sasanqua*, providing mean values of 23.1% and 25.8%, respectively (**Table 1**). Thus, both species are appropriate candidates for use in *Camellia* oil production. *C. reticulata* and *C. hiemalis* showed slightly lower values of 16.6% and 22.6%, respectively.



Figure 1. Camellia locations.

	- ·			Extraction Yield	Acid Value	lodine Value
Sample	Species	Origin-Code	Harvest	(w/w, %)	(mg KOH/g Oil)	(g l ₂ /100 g Oil)
1	C. japonica	Cuntis	Sep.	26.0	5.61 ± 0.02 jk	79.1 ± 0.5 de
2	C. japonica	EFA-826	Sep.	31.9	0.39 ± 0.00 b	82.2 ± 0.0 g
3	C. japonica	EFA-942	Sep.	21.6	1.81 ± 0.02 e	82.2 ± 0.2 g
4	C. japonica	Quiñones de León/O Castro- 876	Aug.	24.0	5.55 ± 0.04 j	83.2 ± 0.1 gh
5	C. japonica	Quiñones de León/O Castro- 877	Aug.	24.0	5.66 ± 0.00 k	85.6 ± 0.0 i
6	C. japonica	Pazo de Lourizán	Sep.	28.4	5.60 ± 0.01 jk	78.7 ± 0.4 cd
7	C. japonica	Pazo de Gandarón	Aug.	23.2	4.52 ± 0.04 i	76.5 ± 0.1 b
8	C. japonica	Castelo de Soutomaior	Sep.	19.7	5.61 ± 0.00 jk	80.9 ± 0.2 f
9	C. japonica	Pazo de Rubianes–Hob Hope	Nov.	16.1	5.62 ± 0.00 jk	79.4 ± 0.1 de
10	C. japonica	Pazo de Rubianes–Augusto Leal	Nov.	17.5	5.63 ± 0.00 jk	78.8 ± 0.5 cd
11	C. japonica	Pazo de Rubianes–Momoiro– Bokuhan	Nov.	27.3	5.62 ± 0.00 jk	80.1 ± 0.2 ef
12	C. japonica	Pazo de Rubianes–Bento de Amorim	Nov.	16.1	5.62 ± 0.02 jk	70.3 ± 0.4 a
13	C. japonica	Pazo de Rubianes–Royal Velvet	Nov.	24.1	5.61 ± 0.00 jk	83.1 ± 0.3 gh
14	C. sasanqua	EFA-826	Sep.	30.1	0.52 ± 0.00 c	89.8 ± 0.1 j
15	C. sasanqua	EFA-942	Sep.	25.0	1.07 ± 0.00 d	82.3 ± 0.0 g

Table 1. Origin and quality parameters of Camellia seed oils.

Comula	Creasian	Origin Code	lloweet	Extraction Yield	Acid Value	lodine Value
Sample	Species	Origin-Code	Harvest	(w/w, %)	(mg KOH/g Oil)	(g l ₂ /100 g Oil)
16	C. sasanqua	Pazo de A Saleta	Oct.	22.1	2.17 ± 0.01 f	92.0 ± 0.5 k
17	C. sasanqua	Pazo de Rubianes	Nov.	26.1 [<u>54</u>]	3.41 ± 0.06 g	83.9 ± 0.4 h
18	C. reticulata	San Vicente do Mar	Oct.	[<mark>51</mark>] 16.6	3.68 ± 0.01 h	77.2 ± 0.3 b
19	C. hiemalis	Pazo de Rubianes	Nov.	22.6	5.64 ± 0.00 ik	83.0 ± 0.4 ah

this parameter was determined for all *Camellia* oils in this study to compare with the standard values legislated by the official olive oil method, according to the Spanish and International regulation [55]. Thus, Extra Virgin Olive Oil FFA; Fatación Fitopatolóxica Argeiro cresults are expressed as mean \pm standard deviation ($n_{\rm e}$, $n_{\rm e}$). Different letters frust have an action value indicate international values in this study to compare with the standard values legislated by the official olive oil method, according to the Spanish and International regulation [55]. Thus, Extra Virgin Olive Oil FFA; Fatación Fitopatolóxica Argeiro cresults are expressed as mean \pm standard deviation ($n_{\rm e}$, $n_{\rm e}$), bifferent letters ($a_{\rm e}$) in same column indicate international values, below the maximum authorized in olive oil for food/industrial purposes. Among species, *C. japonica*, with a greater number of samples analyzed, presented great variability in its composition (**Table 1**), with the oils from EFA being the ones that presented the lowest values (0.39 and 1.81 mg KOH/g oil). These results were also similar to the one (1.7 mg/g) found in the literature for the same species [56].

lodine value is also an oil quality index representative of the number of unsaturated C-C bonds from FAs. Results obtained for the iodine index of *Camellia* oils were compared with those set by the official method for olive oil, ranging from 70.3 to 92.0 g $I_2/100$ g oil (**Table 1**). There is no regulation for *Camellia* oil in Spain, but values between 75 and 90 g $I_2/100$ g oil are set as healthy by Spanish legislation, and therefore they were used as a reference ^[55]. Thus, iodine values obtained for the different species of *Camellia* oils were, in general, similar to those referred to as healthy by Spanish legislation, with only two samples (S12 and S16) out of this range, since they showed iodine values slightly out of this range (Sample 12, Pazo de Rubiáns–Bento de Amorim, with 70.3 ± 0.4, and Sample 16, *C. sasanqua* from Pazo de A Saleta, with a value of 92.0 ± 0.5). Furthermore, the values obtained in *C. japonica* were really close to that of 79.9 g/100 g obtained by Zeng and Endo, (2019) ^[56] for the same species.

2.3. GC-FID Analysis

FAs composition is one of the most important indexes in edible oils, closely related to their price ^[57]. The proportion of saturated and unsaturated FAs varies in edible oils. This FAs profile of edible oils is closely related to lipid oxidation, product quality, and function of vegetable oils. Thus, highly unsaturated FAs' (UFAs) oil content is more expensive because consumers assume that they are healthier. Furthermore, the price of edible oils is different in any place depending on factors such as the local availability of the vegetable source needed to extract the oils, the mechanization of agriculture, and the economy of the oil production area, among others ^[51]. For example, the price of olive oil with a fairly mechanized production and cultivated in large areas of the south of Europe is relatively

higher than that of soybean oil produced mainly in China, US, Argentina, and Brazil, with the latter more expensive than palm oil, which is the most widely consumed vegetable oil. Indonesia and Malaysia are the top palm oil producers, followed by Thailand, Nigeria, and Colombia.

Camellia oil has a very similar FAs profile and physicochemical properties to olive oil, being given with the designation of "oriental olive oil". It is rich in UFAs (>90%), especially oleic acid (74–87%), as well as in other type of compounds such as polyphenols, fat-soluble vitamins (Vitamins A, B, E), and minor unsaponifiable matters (2–5%), including squalene and phytosterol, etc., ^{[51][58]}.

In this work, the FAs composition of *Camellia* oils from different species were analyzed by GC-FID as methylated derivatives (FAMEs) and the results expressed as mean values ± standard deviations as shown in **Table 2**. All tested samples contained similar FAs composition, showing nine common FAs compounds. Among them, oleic (C18:1), palmitic (C16:0), linoleic (C18:2), and stearic (C18:0) acids were the predominant FAs, which accounted for 98.5–99.5% of the total, similarly to the results found for total FAs composition of extra virgin olive oil (97.5%) used as a control. Oleic acid (C18:1) was the major component in *Camellia* samples, ranging from 77.9% to 83.6%, followed by palmitic acid (C16:0, 8.2% to 10.8%), linoleic acid (C18:2, 3.9% to 8.0%), stearic acid (C18:0, 1.7% to 3.9%), and linolenic acid (C18:3, 0.23% to 0.45%). Other fatty acids, such as myristic (C14:0), palmitoleic (C16:1), arachidic (20:0), and eicosenoic (C20:1) acids, were found in concentrations lower than 0.2%. Due to the *Camellia* oil characteristics based on a high oleic acid content and the presence of essential fatty acids (C18:2 and C18:3), which cannot be synthesized by the human body and need to be solely supplied through diet, *Camellia* oils may provide health functions, such as the lowering of blood pressure, cholesterol, and triglycerides, and thus prevent cardiovascular diseases, cancer, hypertension, and autoimmune disorders. It is also of value in protecting the liver against peroxidative damage, as was stated by the carbon tetrachloride-induced hepatotoxicity model ^[59].

Table 2. FAs composition by GC/FID, expressed as % total fatty acids.

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1	0.06 ± 0.01 bc	8.24 ± 0.26 a	0.10 ± 0.02 a-c	2.05 ± 0.05 b–d	82.20 ± 0.66 f-h	5.56 ± 0.12 c-e	0.29 ± 0.02 a-c	0.05 ± 0.01 ab	0.29 ± 0.02 ab	10.40	82.59	5.85	88.45
2	0.06 ± 0.01 bc	9.17 ± 0.05 d–f	0.10 ± 0.01 a-c	2.43 ± 0.09 g—i	81.59 ± 0.48 e-h	5.12 ± 0.09 b-d	0.23 ± 0.02 a	0.05 ± 0.01 ab	0.57 ± 0.07 f	11.70	82.26	5.35	87.61
3	0.04 ± 0.01 a	9.46 ± 0.23 e-g	0.12 ± 0.01 a-c	2.36 ± 0.08 f–h	80.96 ± 0.47 c-g	5.65 ± 0.06 e	0.31 ± 0.03 a-d	0.04 ± 0.01 a	0.36 ± 0.03 b-d	11.90	81.44	5.96	87.40

Sample C14:0 C16:0 C16:1 C18:0 C18:1 C18:2 C18:3 C20:0 C20:1 SFA MUFA PUFA SUFA

Samp	le C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	∑SFA	MUFA	PUFA	∑UFA
4	0.07 ± 0.01 c	9.80 ± 0.11 gh	0.09 ± 0.01 ab	2.14 ± 0.09 c-f	81.07 ± 0.56 d–g	6.41 ± 0.07 f	0.30 ± 0.04 a–d	0.08 ± 0.01 bc	0.37 ± 0.03 b–d	12.09	81.53	6.71	88.24
5	0.07 ± 0.01 c	9.53 ± 0.08 fg	0.12 ± 0.01 a-c	2.11 ± 0.06 c-e	81.12 ± 0.47 d-g	6.37 ± 0.07 f	0.25 ± 0.03 ab	0.07 ± 0.01 a-c	0.24 ± 0.03 a	11.78	81.48	6.62	88.10
6	0.06 ± 0.01 bc	9.26 ± 0.05 d-f	0.13 ± 0.01 bc	2.29 ± 0.07 e-g	81.06 ± 0.56 d-g	5.61 ± 0.05 de	0.32 ± 0.02 a-d	0.07 ± 0.01 a-c	0.33 ± 0.02 a-c	11.67	81.51	5.93	87.44
7	0.05 ± 0.01 ab	10.41 ± 0.22 ij	0.18 ± 0.02 d	2.28 ± 0.07 defg	78.88 ± 0.33 b–d	7.12 ± 0.09 gh	0.26 ± 0.04 abc	0.05 ± 0.01 ab	0.28 ± 0.02 ab	12.78	79.33	7.38	86.72
8	0.05 ± 0.01 ab	9.05 ± 0.05 c-e	0.10 ± 0.01 a-c	2.46 ± 0.05 g–i	79.18 ± 0.59 b–d	7.43 ± 0.06 h	0.32 ± 0.03 a-d	0.09 ± 0.01 c	0.53 ± 0.03 ef	11.64	79.81	7.75	87.56
9	0.05 ± 0.01 ab	8.21 ± 0.11 a	0.12 ± 0.01 a-c	1.85 ± 0.05 ab	83.04 ± 0.54 gh	5.08 ± 0.08 bc	0.35 ± 0.03 b-e	0.05 ± 0.01 ab	0.44 ± 0.03 c-e	10.15	83.59	5.43	89.02
10	0.05 ± 0.01 ab	9.43 ± 0.11 e-g	0.12 ± 0.01 a-c	2.61 ± 0.04 ij	81.65 ± 0.64 e-h	5.83 ± 0.08 e	0.33 ± 0.02 a-d	0.06 ± 0.01 a-c	0.44 ± 0.04 c-e	12.14	82.20	6.16	88.36
11	0.07 ± 0.01 c	9.13 ± 0.07 def	0.14 ± 0.01 cd	1.72 ± 0.06 a	82.58 ± 0.80 f-h	5.05 ± 0.14 b	0.25 ± 0.04 ab	0.06 ± 0.01 a-c	0.53 ± 0.03 ef	10.98	83.25	5.30	88.55
12	0.06 ± 0.01 bc	8.67 ± 0.08 bc	0.10 ± 0.01 a-c	3.88 ± 0.09 m	83.62 ± 1.26 h	3.91 ± 0.06 a	0.32 ± 0.03 a-d	0.08 ± 0.01 c	0.44 ± 0.04 c-e	12.69	84.16	4.23	88.39
13	0.06 ± 0.01 bc	8.99 ± 0.10 b-d	0.12 ± 0.01 a-c	2.73 ± 0.03 j	82.86 ± 1.16 gh	5.06 ± 0.07 bc	0.28 ± 0.06 a-c	0.06 ± 0.01 a-c	0.44 ± 0.04 c-e	11.83	83.41	5.34	88.76

Sample	eC14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	∑SFA	MUFA	PUFA	∑UFA
14	0.05 ± 0.01 abc	8.59 ± 0.16 ab	0.07 ± 0.01 a	2.12 ± 0.07 c-e	80.54 ± 0.46 c-f	6.82 ± 0.12 fg	0.30 ± 0.01 a-d	0.06 ± 0.01 a-c	0.57 ± 0.05 f	10.82	81.18	7.12	88.30
15	0.05 ± 0.01 abc	8.86 ± 0.10 b–d	0.10 ± 0.01 a-c	2.57 ± 0.05 h–j	79.00 ± 0.48 b–d	7.44 ± 0.09 h	0.45 ± 0.04 ef	0.05 ± 0.01 ab	0.82 ± 0.05 g	11.53	79.93	7.89	87.81
16	0.06 ± 0.01 bc	9.05 ± 0.08 c-e	0.13 ± 0.02 bc	2.48 ± 0.07 g–i	78.68 ± 0.53 bc	8.00 ± 0.09 i	0.31 ± 0.03 a-d	0.08 ± 0.01 bc	0.52 ± 0.03 ef	11.66	79.33	8.31	87.64
17	0.07 ± 0.01 c	10.77 ± 0.09 j	0.11 ± 0.02 a-c	1.95 ± 0.12 bc	79.36 ± 1.20 b-e	6.95 ± 0.13 gh	0.36 ± 0.05 c-e	0.06 ± 0.01 abc	0.43 ± 0.03 c-e	12.84	79.90	7.31	87.22
18	0.05 ± 0.01 ab	10.32 ± 0.11 i	0.11 ± 0.01 a-c	3.17 ± 0.07 k	77.97 ± 0.76 b	7.18 ± 0.07 gh	0.41 ± 0.04 d-f	0.04 ± 0.01 a	0.35 ± 0.04 a–c	13.58	78.43	7.59 [<mark>5</mark>	86.01 6
19	0.06 ± 0.01 bc	10.20 ± 0.11 hi	0.13 ± 0.01 bc	1.85 ± 0.06 ab	79.23 ± 0.51 b-d	7.12 ± 0.10 gh	0.36 ± 0.01 c-e	0.07 ± 0.01 abc	0.44 ± 0.04 c-e	12.17	79.79	7.49	87.28

which showed values between 54.1 and 75.5% [60].

Also, slight differences between total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and solve the status of the species of the species of the species of the species studied fatty acids (FA) with a minor contribution from other monounsaturated acids, with *C. japonica* being the species with the highest percentage in reference to the other species studied, 79.3% to 84.2% and 78.4% to 81.2%, respectively. However, this trend is the opposite in the case of PUFA, showing values from 4.2% to 7.7% in *C. Japonica*, while the values were higher in the other species, ranging between 7.1% and 8.3%. In general, oleic acid (C18:1) is usually considered to be more stable than linoleic (C18:2) and linolenic acid (C18:3). The results showed that *Camellia* oils contained high levels of MUFA and low PUFA, favoring the nonappearance of unpleasant odors due to oxidation. Therefore, this may be a justification of the suitability of this oils for cosmetic applications and for cooking at high temperatures [56].

2.4. GC-MS Analysis

Gas chromatography-mass spectrometry is a practical and powerful analytical technique used for the quantification of fatty acids, and also commonly used as a separating criterion for *Camellia* oil authentication ^[61]. The results

obtained using the method based on GC-MS (**Table 3**) were analogous to those using the GC-FID methodology previously described. However, some differences were found. Although the values for the main compounds, namely oleic (C18:1), palmitic (C16:0), linoleic (C18:2), and stearic (C18:0) acids showed similar ranges in both techniques, the minor fatty acids myristic (C14:0), palmitic (C16:1), linolenic (C18:3), and arachidic (C20:0) acids presented values lower than 0.2%, and therefore, they were not quantified. The limits of quantification from GC-MS are usually higher than those from GC-FID. For example, Dodds et al., (2005) ^[62] found for standard FAMES that the limit of quantification (LOQ) of myristic acid (C14:0) is five times higher for GC-MS than that of GC-FID, e.g., 2.52 pmol and 0.50 pmol, respectively. Also, higher LOQs were found by GC-MS for the compounds palmitic (C16:1), linolenic (C18:3), and arachidic (C20:0) acids, which, due to the low concentrations found in the samples, did not allow for their quantification.

Scheme 16.	C16:0	C18:0	C18:1 ω-9 <i>ci</i> s	C18:1 ω-9 <i>trans</i>	C18:2 ω-6,-9	C20:1 ω-9	∑SFA	MUFA	PUFA	∑UFA
1	6.69 ± 0.00 c	1.66 ± 0.02 ef	87.1 ± 0.1 gh	0.72 ± 0.03 d–f	3.59 ± 0.02 d	0.25 ± 0.01 ab	8.35	88.07	3.59	91.65
2	7.44 ± 0.07 fg	1.94 ± 0.04 ij	86.5 ± 0.1 fg	0.76 ± 0.04 d–g	3.08 ± 0.03 bc	0.24 ± 0.02 bc	9.38	87.53	3.08	90.62
3	6.84 ± 0.03 cd	1.64 ± 0.02 d– f	87.7 ± 0.1 hi	0.66 ± 0.03 c–e	3.12 ± 0.05 bc	ND	8.48	88.43	3.12	91.52
4	7.80 ± 0.06 hi	1.68 ± 0.01 e– g	85.3 ± 0.2 с–е	0.92 ± 0.06 h	4.06 ± 0.09 ef	0.22 ± 0.00 a	9.48	86.43	4.06	90.52
5	7.51 ± 0.03 gh	1.58 ± 0.01 de	86.1 ± 0.1 ef	0.90 ± 0.07 gh	3.75 ± 0.04 de	0.20 ± 0.01 a	9.09	87.17	3.75	90.91
6	8.04 ± 0.17 i	1.49 ± 0.02 cd	85.5 ± 0.3 de	0.74 ± 0.01 d–f	4.27 ± 0.09 f– h	ND	9.53	86.20	4.27	90.47
7	8.46 ± 0.01 j	0.89 ± 0.02 a	84.6 ± 0.1 c	0.89 ± 0.02 gh	4.35 ± 0.06 f– h	ND	9.35	85.53	4.35	89.86
8	6.92 ± 0.19 c– e	1.86 ± 0.03 g– i	87.6 ± 0.4 hi	0.66 ± 0.03 c–e	3.01 ± 0.14 b	ND	8.78	88.23	3.01	91.22
9	6.07 ± 0.02 a	1.27 ± 0.01 b	89.2 ± 0.1 j	0.62 ± 0.04 c–e	2.79 ± 0.06 b	ND	7.34	89.87	2.79	92.66

 Table 3. FAs composition by GC/MS, expressed as % total fatty acids.

Scheme 16.	C16:0	C18:0	C18:1 ω-9 cis	C18:1 ω-9 <i>trans</i>	C18:2 ω-6,-9	C20:1 ω-9	∑SFA	MUFA	PUFA	∑UFA
10	7.45 ± 0.11 fg	2.05 ± 0.08 j	85.5 ± 0.6 de	1.18 ± 0.06 i	3.49 ± 0.39 cd	0.29 ± 0.01 c	9.50	87.03	3.49	90.50
11	7.17 ± 0.08 ef	1.39 ± 0.05 bc	87.4 ± 0.3 hi	0.80 ± 0.06 f–h	2.92 ± 0.11 b	0.34 ± 0.01 c	8.56	88.50	2.92	91.44
12	6.38 ± 0.05 b	2.76 ± 0.05 l	87.9 ± 0.2 i	0.92 ± 0.01 gh	2.08 ± 0.09 a	ND	9.14	88.77	2.08	90.86
13	7.06 ± 0.11 de	2.02 ± 0.03 j	87.1 ± 0.3 g—i	0.77 ± 0.02 e–h	3.02 ± 0.14 b	ND	9.08	87.90	3.02	90.92
14	7.11 ± 0.10 de	1.78 ± 0.03 f– h	85.6 ± 0.2 de	0.53 ± 0.01 a–c	4.54 ± 0.05 gh	0.41 ± 0.00 d	8.89	86.57	4.54	91.11
15	7.17 ± 0.03 e	1.97 ± 0.01 j	85.2 ± 0.1 cd	0.57 ± 0.00 b–d	4.70 ± 0.03 h	0.38 ± 0.00 d	9.14	86.13	4.70	90.86
16	7.50 ± 0.11 gh	1.84 ± 0.04 g– i	83.4 ± 0.3 b	0.42 ± 0.04 a	6.53 ± 0.15 i	0.33 ± 0.01 c	9.34	84.13	6.53	90.66
17	8.39 ± 0.06 j	1.46 ± 0.05 bc	85.2 ± 0.3 cd	0.75 ± 0.05 d–h	4.21 ± 0.20 fg	ND	9.85	85.93	4.21	90.15
18	9.32 ± 0.07 k	2.64 ± 0.02 kl	83.3 ± 0.2 b	0.46 ± 0.02 ab	4.03 ± 0.15 ef	0.21 ± 0.01 a	[<u>62</u>].96	84.00	4.03	88.04
19	7.84 ± 0.07 i	1.28 ± 0.03 b	86.0 ± 0.1 ef	0.65 ± 0.04 c–f	4.23 ± 0.03 fg	ND	9.12	86.67	4.23	90.88

The oils found in nature are in the form of triglycerides, fatty acids generally found with saturated and unsaturated bonds, and the FAs containing double bonds are usually stable as cis isomers. A small percentage of these acids

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MS-chromatographic techniques were widely employed in oil quality and safety assessments, with a high specificity and sensitivity to quantify those targeted analytes (FAs) to have a rigorous control (authentication and classification) of samples. However, as in the case of the GC-FID technique, it involves tedious, destructive, and extensive sample preparation. So, these conventional chromatographic techniques have a number of limitations for further quality control oil applications.

2.5. H-NMR Analysis

The NMR spectroscopy was extensively used for oil analysis, and it was established as a valuable tool for the assessment of the quality and authenticity of olive oil ^{[64][65]}. NMR was used to develop accurate analytical fingerprinting methods for the authentication or certification of the geographical origin of olive oils aided by suitable chemometric analysis ^{[66][67]}. Studies of time, thermal, and oxidative stability of olive oils by NMR analysis were also powered by multiway chemometric methodologies ^{[68][69]}. Also, ¹H-NMR combined with chemometrics were employed for the prediction of fatty acid composition ^[50], to detect the adulteration of *Camellia* oil ^[49], and to determine oxidative stability in *Camellia* oils ^[70].

In previous work, Feás et al., (2013) ^[32] determined the FA profile of three species of Galician *Camellia* oils (*C. oleifera, C. reticulata* and *C. sasanqua*, see **Table 4** samples 21–23) collected at the *Estación Fitopatolóxica do Areeiro* in 2011, with values ranging between 82.3% and 84.5%, 5.69% and 7.78%, 0.26% and 0.41%, and 8.04% and 11.2%, for oleic, linoleic, linolenic, and saturated acids, respectively. These values demonstrate that the FAs composition remained fairly stable over time for these species in the region. In this methodology, Feás et al. used the tertiary hydrogen of the glyceryl group (δ 5.25 ppm) as the key indicator to estimate the FAs composition. The magnetic field for providing good results was established as 17.6 T (750 MHz) to avoid signal overlapping of protons of the acyl and glyceryl groups (5.32 and 5.25 ppm, respectively, see **Table 5**). However, the NMR equipment at 750 MHz is of high cost, which would make the technique not easily available and therefore not applicable. To improve the applicability of the ¹H-NMR technique for the determination of the FA composition in *Camellia* oils, an adaptation of the Barison method was carried out in the present work taking as reference a more common NMR instrument of 400 MHz ^[71] (**Table 6**).

Sample	Species	C18:1 (MUFA)	C18:2	C18:3	∑SFA	PUFA	∑UFA	
1	C. japonica	89.9 ± 0.4 f	5.78 ± 0.19 b–e	ND	12.36	5.78	95.63	
2	C. japonica	86.3 ± 0.4 e	4.33 ± 0.00 a	ND	12.92	4.33	90.63	
3	C. japonica	86.0 ± 0.2 de	5.33 ± 0.00 bc	ND	12.64	5.33	91.35	
4	C. japonica	94.3 ± 0.3 g	7.33 ± 0.00 hi	ND	15.25	7.33	101.63	
5	C. japonica	96.4 ± 0.6 hi	7.33 ± 0.00 hi	ND	14.75	7.33	103.69	
6	C. japonica	86.4 ± 0.2 e	7.11 ± 0.19 g–i	ND	13.75	7.11	93.46	
7	C. japonica	85.6 ± 0.9 de	6.67 ± 0.33 e–h	ND	14.25	6.67	92.30	
8	C. japonica	89.6 ± 0.3 f	5.33 ± 0.00 bc	ND	13.36	5.33	94.96	
9	C. japonica	90.4 ± 0.7 f	5.22 ± 0.19 b	ND	11.64	5.22	95.58	

Table 4. FAs composition by ¹H-NMR, expressed as % total fatty acids.

Sampl	le Sp	ecies	C18:1 (MUFA)	C18:2	C18:3	∑SFA	PUFA	∑UFA	
10	C. ja	aponica	98.1 ± 0.8 i	6.33 ± 0.33 d–g	ND	14.69	6.33	104.41	
11	C. ja	aponica	97.8 ± 0.3 i	5.11 ± 0.19 ab	ND	13.63	5.11	102.91	
12	C. ja	aponica	94.5 ± 1.0 gh	5.11 ± 0.19 ab	ND	16.02	5.11	99.63	
13	C. ja	aponica	93.3 ± 0.4 g	5.56 ± 0.19 b–d	ND	13.91	5.56	98.85	
14	C. sa	asanqua	84.7 ± 0.1 c–e	6.67 ± 0.00 f–h	ND	12.25	6.67	91.41	
15	C. sa	asanqua	83.6 ± 0.1 c	7.67 ± 0.00 i	ND	12.86	7.67	91.24	
16	C. sa	asanqua	85.7 ± 0.2 de	10.33 ± 0.00 j	ND	13.80	10.3	96.08	
17	C. sa	asanqua	84.1 ± 0.3 cd	7.33 ± 0.00 hi	ND	14.36	7.33	91.41	
18	C. re	eticulata	81.0 ± 0.5 b	7.11 ± 0.19 g–i	ND	17.25	7.11	88.07	
19	C. h	niemalis	91.1 ± 1.7 f	7.89 ± 0.77 i	ND	14.58	7.89	98.96	
20 *	C. ja	aponica	80.7	6.65	0.29	12.4	6.94	87.64	
21 **	C. sa	asanqua	82.3	6.20	0.30	11.2	6.50	88.80	
22 **	C. re	eticulata	1 84.5	5.69	0.26	9.58	5.95	90.42	
Peak a	δ (ppm)	Multiplic	ity Functional	Group	(Compou	nd		
1	5.32	m	-CH=Cł	H–		acyl grou	ıp		
2	5.25	m	-CH-O-C	COR	Q	lyceryl gro	oup		
3	4.27	dd	CH ₂ O-(COR	Q	lyceryl gro	oup		d. ati
4	2.74	t	=CH–CH ₂ -	-CH= a	icyl group (lir	noleic and	linolenic g	roup)	b
5	2.29	dt	-0C0-C	H ₂ -		acyl grou	ib		
6	2.01	m	-CH ₂ -CH=	=CH-		acyl grou	ıp		
7	1.61	m	-0C0-CH ₂	CH ₂		acyl grou	ıр		
8	1.29	m	-(CH ₂)r	1—		acyl grou	ib		
9	0.98	t	-CH=CH-CF	H ₂ CH ₃	linc	oleic acyl (group		
9	0.88	t	-CH ₂ -CH ₂ -C	CH ₂ CH ₃ sa	aturated oleic	c except lii	noleic acyl	group	

Table 6. Signal identification and quantification according to Barison's method.

Fatty Acid	Label	¹ H NMR Signal	Reference Area (Signal)	Subtration
Linolenic	Е	0.98 ppm	22.2	
Linoleic	А	2.74 ppm	33.3	2 × linoleic

Fatty Acid	Label	¹ H NMR Signal	Reference Area (Signal)	Subtration
Oleic	С	2.01 ppm	16.7	linolenic and linoleic
Saturated	В	2.29 ppm	33.3	linolenic + linoleic + oleic

from 81.0% to 98.1%, 4.33% to 10.4%, and 11.6% to 17.3% for oleic acid (C18:1), linoleic acid (C18:2), and saturated acids, respectively. In most cases, the fatty acid contents found were close to the levels showed in chromatographic analysis and comparable with data from the literature based on NMR analysis of Galician *Camellia* oils ^{[3][32]}. In general, the content of oleic acid (C18:1) in *C. Japonica* (91.4%) and *C. hiemalis* (91.1%) showed average values higher than in *C. sasanqua* (84.5%) and *C. reticulata* (81.0%), although *C. japonica* showed a wide variability, including that of linoleic acid in the range 4.3–7.3%. No significant amounts of linolenic acid (C18:3) were detected. The slight differences in the FA profile between chromatographic and NMR samples may be due to the approximations implied in Barison's method based on two approaches: (1) All fatty acid acyl chains were esterified on the glycerol moiety, and (2) there were no free fatty acids in the samples in the samples in the spectrum at 4.12 and 2.27 ppm, respectively. Also, the acid value in all *Camellia* oil samples is lower than 6 mg KOH/g of oil, and therefore *Camellia* oils are optimal candidates for the application of this methodology.

The application of the ¹H-NMR methodology developed to determine FA content in *Camellia* oils is simpler and faster than conventional methods due to the absence of sample pretreatment, low-reagent consumption, short analysis (approx. 3–4 min), excellent repeatability, and fully automatic routine protocol in the NMR software ^{[20][50]}. Although currently the costs per sample are affordable, however, professional operating personnel are necessary. Moreover, this technique avoids problems such as lipid oxidation present in the traditional GC analysis, it does not require the use of standards, it is a nondestructive technique, and it provides information about distribution of FAs (**Figure 2**) ^{[72][73][74]}.



Figure 2. Structure of FAs and ¹H-NMR spectrum at 400 MHz of camelia seed oil.

2.6. Principal Component Analysis (PCA)

Principal component analysis (PCA) was used to identify the parameters, mainly fatty acids, that better separate 19 seed oils from four species of *Camellia*, namely the most widespread *C. japonica* and *C. sasanqua*, and the less common species *C. reticulata* and *C. hiemalis*. **Figure 3**A–C show the biplot of the two main principal components (PC1 and PC2) characterized by the common parameters studied in samples including iodine and acid values, extraction efficiency, and the FAs profile studied with the gas chromatography techniques (GC-FID and GC-MS) and the proton nuclear magnetic resonance technique (¹H-NMR). This FA profile presented saturated FAs (C14:0, C16:0, C18:0 and C20:0), total saturated FA (Σ SFA), total unsaturated FAs (C16:1, C18:1, C18:2, C18:3, and C20:1), total monounsaturated FA (MUFA), total polyunsaturated FA (PUFA), and total unsaturated FA (Σ UFA). The cumulative explained total variance ranged from 54.31% (GC-FID) and 67.76% (GC-MS) for the chromatographic techniques to 67.84% for ¹H-NMR technique.



Figure 3. Principal component analysis plot of *Camellia* oils from different species. FAs were determined by (**A**) GC-FID, (**B**) GC-MS, (**C**) ¹H-NMR.

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