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Study of the Chemical Composition of *Carica papaya* L. Seed Oils of Various Geographic Origins

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Abstract: The papaya plant (*Carica papaya* L.) is tree-like fruit plant cultivated throughout the tropics and subtropics. The aim of this study was to compare the physicochemical properties, fatty acid, sterols, and triterpenic alcohols composition of *Carica papaya* L. seed oils grown in a typical geographical location and *Carica papaya* L. seed oils grown in an untypical geographical location in greenhouse conditions (Saratov Region, Russia). The oils were extracted from the seeds of *Carica papaya* L. fruits collected in Kenya, the Dominican Republic, Angola, Ghana, and Brazil, as well as from the seeds of fruit plants grown in a similar environment (Russian Federation, Saratov). Parameters such as the oil yield, refractive index, peroxide value, iodine value, saponification value, and acid value of the extracted *Carica papaya* L. seed oils were determined. The qualitative and quantitative chemical compositions of the seed oils were determined by a combination of mass spectrometry and NMR spectroscopy. The profiles as well as the content of fatty acids, sterols, triterpenic alcohols, and benzyl isothiocyanate were established. The saponifiable fraction of the oils is mainly represented by triglycerides (98.7–99.4%), while di-(0.4–1.1%) and monoglycerides (0.1–0.3%) are also present but in smaller amounts. The content of sterols and triterpene alcohols was (537.5–918.2) mg/100 g of oil (0.54–0.92%), and up to 75% of the fraction was represented by β-sitosterol (55.9–66.7%) and its saturated analogue-sitostanol (11.0–15.7%). The physicochemical properties and the fatty acid, sterol, and triterpenic alcohol composition of seed oils from *Carica papaya* L. fruits, cultivated in Russia, is in the quantitative range of other samples, which suggests that *Carica papaya* L. can be grown in Russia for obtaining the seed oil.

Keywords: papaya; seed oil; phytochemical composition; fatty acids; unsaponifiable fraction; benzyl isothiocyanate; *Carica papaya* L.

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1. Introduction

The papaya plant (*Carica papaya* L.) is a large, tree-like fruit plant from the Caricaceae family native to Mexico and Central and South America. It is also known as the pawpaw, mummy apple, melon tree, mamão, and mamón [1,2]. Papayas are extensively cultivated throughout the tropics and subtropics, and their global fruit production exceeded 13.9 million tons in 2020 [3]. More than 40% of papayas are produced in India, followed by the Dominican Republic and Brazil. The fleshy, tasty pulp of the fruit is low in calories and contains dietary fiber up to 1.7 g per 100 g and less than 1.0 g of protein and fat. The fruit is a good source of vitamin C (61 mg per 100 g), provitamin A (950 IU per 100 g), folate (37 µg per 100 g), magnesium, and potassium [4].

The papaya fruit is widely consumed both in fresh and processed forms. Other parts of the plant are also considered edible in certain countries. The leaves and flowers are eaten steamed with rice or added to soups and stews in Southeast Asia. Young stems are cooked, while the core of the old stems can be eaten raw. The flowers are candied in Indonesia. In the Pacific islands, the leaves and skins of papaya are used as a wrapper for pork, chicken, and octopus to tenderize the meat [2,5]. The papaya fruit is "generally recognized as safe" (GRAS) as a food by the U.S. Food and Drug Administration. Fermented papaya is a product of yeast fermentation of the papaya fruit with seeds. It is sold commercially in Japan and the Philippines as a dietary supplement to support digestion and immunity, particularly in the elderly [2].

Papaya fruit production has increased by 31% from 2007 to 2020 [3]. Papaya is cultivated mostly for the pulp of the fruit, while other parts of the plant such as the stems, leaves, and seeds are often considered as waste. The "waste" has a long history of use in traditional medicine and is characterized by a high potential for extracting bioactive compounds (enzymes, polyphenols, etc.) [6,7]. A detailed review of the traditional medicinal uses of various parts of the papaya was conducted by Nguyen [8].

Compared to the pulp (8.24 mg CE/g), papaya leaves (15.54 mg CE/g), bark (15.84 mg CE/g), and roots (16.69 mg CE/g) have a higher total phenolic and flavonoid content, used as the index of the medicinal value of natural products [9]. Due to their flavonoid and enzyme content, the extracts of papaya seeds and leaves have demonstrated anti-inflammatory, immunomodulatory, free-radical scavenging, hypotensive, hypoglycemic, and hypolipidemic activity [10,11]. Additional effects include wound-healing, anti-carcinogenic, neuroprotective, and diuretic activities [8].

Both experimental and clinical studies have confirmed the antimicrobial activity of the papaya plant against bacteria, fungi, viruses, helminths, and protozoa. The antibacterial activities of different papaya parts' extracts against the multidrug-resistant bacterial strains of *S. aureus*, *B. cereus*, *E. coli*, and *P. multocida* were investigated; it was found that the ethanolic extracts of pulp and leaves were the most active [9]. The leaf extract was considered as a potential complimentary medicine in patients with fevers caused by the dengue virus [12].

Seeds make up to 15–20% of the fruit [13] and can be used as both food and medicine. Papaya seeds can substitute pepper in salad dressings, marinades, meat, and fish dishes [2,5].

To obtain oil from papaya seeds, different techniques are used in laboratory conditions such as Soxhlet extraction using an organic solvent. The oil content of papaya seeds using Soxhlet extraction was found to be in the range of 25.3–0.7% [14]. Also, maceration extraction is commonly used. In this case, oil is extracted by diffusing a solvent into oil-bearing cells via the plant's cell wall. Samaram et al. used the supercritical fluid method to extract from papaya seeds; the result shows that the yield was low along with an excessive amount of impurities detected in the oil [15].

The seeds contain 25–34% oil, 28% protein, about 19% crude fiber, and 8% ash, tocopherols, carotenoids, benzyl glucosinolates, and benzyl isothiocyanate (BITC) [16–18]. BITC is a compound also found in many types of cruciferous vegetables. It is known for its anti-carcinogenic activity [8]. The essential oil extracted from papaya seeds contains benzyl isothiocyanate (99.36% of total weight) and has shown an inhibitory effect against ten strains of *Candida* spp., including *C. albicans* [19].

In order to determine the amount of BITC in papaya peels, pulp, and seeds, an orthogonal HS-GC-MS method was employed [20]. The Head Space (HS) solvent used in this method was a 0.002% Tween 80 solution. This approach showed a low limit of detection, a broad linear range, and excellent recovery in the determination of BITC concentration in any section of the papaya fruit. The findings showed that the BITC patterns changed as the papaya fruit matured at various stages. It was shown that this approach is easy to use, quick, accurate, sensitive, and safe for the environment. The results show that the concentrations of BITC in seeds (238.0–683.0 μg/g) were higher than the concentrations found in the peel (1.4–31.0 μg/g) and pulp (0.2–2.3 μg/g).

Cold-pressed or extracted oil from papaya seeds is usually reddish-yellow and high in monounsaturated fatty acids (67–77% oleic acid of total weight), phytosterols, and benzyl isothiocyanate (0.56% of total weight) [17,18,21,22]. It has been used to treat skin conditions such as rashes, dry skin, and aged skin due to its anti-inflammatory and antioxidant activity. Papaya oil can also be used for biofuel and industrial purposes [23]. The nutritional and functional properties of papaya seed oil are similar to those of olive oil, making papaya seed oil an interesting potential source of edible oil. The only limitation is its high BITC content that can be toxic for smooth muscle cells, the reproductive system, and renal function [23]. Various methods have been elaborated to reduce the BITC levels in papaya seed oil.

Previous studies have demonstrated that papaya seed oils have a high phytosterol level (6.5–6.8 g/kg) [18,24], while most vegetable oils (palm, rapeseed, soya bean, sunflower, cottonseed, groundnut, palm kernel, coconut, and olive) contain 1–5 g/kg of sterols [25]. Phytosterols play an important role in the prevention of hypercholesterolemia and atherosclerosis.

In a recent work, Agada, Usman, and Shehu [26] reported the presence in crude extracts of *Carica papaya* L. (Nigerian) seeds of 21 compounds, including oleic acid, palmitic acid, and stearic acid, as the major components. These results were obtained using GC-MS for the analysis of methanolic and aqueous extracts of the seeds. This is in line with the work of Alfarabi and colleagues [27] who described the use of GC-MS and liquid chromatography–mass spectrometry–QTOF (LC-MS/MS-QTOF) in the study of different metabolites of two papaya seed extracts from different origins (California and Bangkok). The study showed the good potential of seed extracts as anticancer and antioxidant drugs. A total of 13 different metabolites, such as alkaloids, fatty acids, terpenoids, and flavonoids, were detected. Eight of these metabolites were identified in both extracts, with β-sitosterol and oleic acid being the largest percentage of metabolites identified in the two seed extracts. These results are quite interesting because they allow us to apply this concept to conduct a study for a larger number of samples from six different countries.

NMR spectroscopy and gas chromatography–mass spectrometry (GC-MS) have undoubted advantages and allow us to characterize different multicomponent systems (e.g., edible oils) at the component and fragment levels [24,28]. Therefore, these two methods were chosen to comparatively study the composition of papaya seed oils from different geographical regions.

2. Materials and Methods

2.1. Materials

The authentic samples of *Carica papaya* L. fruits were collected in 7 regions of the world: Kenya (1); the Dominican Republic (2); Angola (yellow pulp fruit) (3); Angola (red pulp fruit) (4); Ghana (5); Brazil (6); the Russian Federation (Saratov) (7).

Chemicals and reactives, such as Supelco® 37 component FAMEs mix, normal alcohols mix, *n*-alcanes mix, β-sitosterol, stigmasterol, campesterol, brassicasterol, cholestanol, squalene, acetyl chloride, and *bis*(trimethylsilyl)trifluoroacetamide, were purchased from Sigma (Shanghai, China), and cholesterol 95% was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Solvents, such as methanol, *n*-hexane, diethyl ether, ethanol, and acetonitrile, were purchased from Reakhim (Moscow, Russia). All the chemicals and reagents were of analytical grade. Distilled water was obtained from a Milli-Q® water purification system.

2.2. Oil Extraction

There is currently no commercial production of *Carica papaya* L. seed oil in the Russian Federation. Hence, the availability of *Carica papaya* L. seed oil on the market is very limited. The extraction of oil from the collected papaya seeds was performed at a laboratory scale using the procedure described below. The seeds were dried in a thin layer for 3 h at room temperature (22 °C) with periodic stirring (every 30 min). Then, 2.0 g of the dried seeds were ground to a fine powder (particle size less than 1 mm). The seed oil from different

papaya samples was extracted by using the Soxhlet apparatus and *n*-hexane as a solvent. The Soxhlet apparatus was approximately set to a temperature of 70 °C, and the whole process took 12 h. At the end of the process, the seed oil was separated from the hexane using a rotary vacuum evaporator (Rotavapor R-215/V, Buchi, Flawil, Switzerland), dried at 60 °C, and weighed. The oil yield was calculated based on the dry weight of the material.

2.3. Refractive Index

The sample had to be completely dry. The refractometer temperature was adjusted to 40.0 ± 0.1 °C. A few drops of the sample were placed on the lower prism of the refractometer. The prisms were closed, tightened firmly with the screwhead, and allowed to stand for 1–2 min. The instrument and light were adjusted to obtain the most distinct reading possible, and the refractive index was determined.

2.4. Acid Value

The acid value of the seed oil was determined according to the AOAC (Association of Official Agricultural Chemists) Official Method [29].

For determination of the acid value, 7.05 g of well-mixed oil was weighed into 250 mL flask and 50 mL alcohol was added. Alcohol was previously neutralized by adding 2 mL phenolphthalein solution and enough 0.1 M NaOH to produce faint, permanent pink. Then, the solution was titrated with 0.25 M NaOH with vigorous shaking until the permanent faint pink appeared.

2.5. Saponification Value

The saponification value was determined according to AOAC Official Method [30].

About 1.5 to 2.0 g of dry sample into a 250 mL Erlenmeyer flask was weighed, and 25 mL of the alcoholic potassium hydroxide solution was added into the flask. Then, a blank determination along with the sample was conducted. The sample flasks and the blank flask with air condensers were connected, kept on the water bath, and boiled for 1 h until saponification was completed. After cooling off the flask and condenser, the inside part of the condenser was washed down with about 10 mL of hot ethyl alcohol, which is neutral to phenolphthalein. The excess of potassium hydroxide was titrated with 0.5 N hydrochloric acid, using about 1.0 mL phenolphthalein indicator.

2.6. Peroxide Value

The peroxide value was determined according to AOAC Official Method [31].

For determination of the peroxide value, about 5 g (±50 mg) of sample was weighed into a 250 mL stoppered conical flask, 30 mL acetic acid chloroform solvent mixture was added and stirred until dissolution. Then, 0.5 mL saturated potassium iodide solution and about 30 mL of water was added. The iodine was titrated with 0.1 N sodium thiosulphate solution ($Na_2S_2O_3$), with vigorous shaking until the yellow color was almost gone. About 0.5 mL starch solution was added as indicator and we continued titration shaking vigorously to release all I_2 from the $CHCl_3$ layer until the blue color disappeared.

2.7. Iodine Value

Iodine value was identified according to AOAC Official Method [32].

About 0.5 g of sample was weighed into a 500 mL conical flask with a glass stopper, to which 25 mL of carbon tetrachloride was added and mixed. Then, 25 mL of Wij's solution was added. Then, 15 mL of potassium iodide solution was added, followed by 100 mL of recently boiled and cooled water. Liberated iodine was titrated with standardized sodium thiosulphate solution, using starch as an indicator until the blue color disappeared.

2.8. Unsaponifiable Matter

Unsaponifiable matter was determined according to AOAC Official Method [33].

About 5 g of well-mixed oil was weighed into a 250 mL conical flask, and 50 mL of alcoholic potassium hydroxide solution was added. Then, the contents were boiled under a reflux air condenser for one hour. The condenser was washed with about 10 mL of ethyl alcohol. The saponified mixture was transferred to a separating funnel and washed with some ethyl alcohol and then with cold water, using a total of 50 mL of water. Then it was cooled to 25 °C. A total of 50 mL of petroleum ether was added to the flask and separated. Then, the lower soap layer was transferred into another separating funnel, and the ether extraction was repeated another 3 times using 50 mL portions of petroleum ether. Then, the combined ether extract was washed three times with 25 mL portions of aqueous alcohol followed by washing with 25 mL portions of distilled water to ensure the ether extract was free of alkali. The solution was evaporated and dried at 100 °C for 30 min until constant weight was obtained. Residue was dissolved in 50 mL of warm ethanol which had been neutralized to a phenolphthalein end point and titrated with 0.02 N NaOH.

2.9. Fatty Acid Analysis

Fatty acid methyl esters (FAMEs) were obtained by the transesterification of glycerides. About 10.0 mg of the oil sample was placed in a 7.0 mL glass vial; 1.0 mL of methanol and 100.0 µL of acetyl chloride were added, and the mix was heated for 60 min at 80 °C. After cooling the reaction mixture, 3.0 mL of distilled water was added, followed by 1.0 mL of *n*-hexane; the resulting mixture was shaken on a vortex (IKA, San Diego, CA, USA). Then, 1 µL of the *n*-hexane upper layer was analyzed by GC-FID and GC-MS.

2.10. Unsaponifiable Fraction Analysis

To determine the sterol composition of the oil, 100 mg of each sample in a 5.0 mL glass vial was mixed with 1.0 mL of 2N KOH methanolic solution, and then 25 µL of internal standard solution (cholestanol, 10 mg/mL) was added. The samples were heated at 80 °C for one hour, and after cooling, 3 mL of distilled water was added to the reaction mixture. The unsaponifiable fraction was extracted with three portions (1.0 mL each) of diethyl ether; these portions were combined, passed through an anhydrous sodium sulfate cartridge, dried under a nitrogen stream, and silylated before analysis. For silylation, 300 µL of BSTFA:acetonitrile (1:2) solution was added to 1.0–3.0 mg of the isolated unsaponifiable fraction of oils, the resulting mixture was heated for 30 min at 80 °C. Then, 1 µL of the solution was injected into GC/MS.

2.11. Gas Chromatography-Flame Ionization Detection (GC-FID)

For the GC-FID analysis, an Agilent 7890A gas chromatograph (USA) was used. The operating conditions of the chromatograph were as follows: a VF-23 ms capillary column (length 30 m, internal diameter 0.32 mm, phase thickness 0.25 µm), helium as the carrier gas applied at a rate of 1.5 mL/min, the injector temperature set to 280 °C, the initial temperature of the chromatograph oven set to 50 °C, then isotherm for 2 min, then heating at a rate of 10 °C/min up to 180 °C, followed by a 5 min hold, and finally another heat-up to 240 °C at a rate of 5 °C/min. The total analysis time was 32 min. The samples were injected in split mode (1:50). FAME identification was carried out by comparing the retention times of the peaks in the experimental samples' chromatograms with the retention times of the peaks in the chromatogram of the standard sample (Supelco® 37 component FAMEs mix). Each sample was analyzed three times. The identification results were confirmed by the GC-MS analysis.

2.12. Gas Chromatography–Mass Spectrometry (GC-MS)

The GC/MS analysis was carried out on a DSQ II Thermo Fisher instrument in EI (electron ionization) mode (ionization energy 70 eV, measured mass range 40–600 Da, 2 scans/sec), equipped with a DB-5 capillary column (length 30 m, internal diameter 0.25 mm, phase thickness 0.25 µm). The carrier gas was helium, operating mode—injector temperature 290 °C, split flow mode—1:10. The oven temperature was initially pro-

grammed to 40 °C (followed by isotherm for 1 min), then came heating at 15 °C/min to 210 °C, after which came heating at 5 °C/min to 290 °C, followed by 10 min isotherm. The total analysis time was 38.3 min.

To meet the identification standards, the samples of individual compounds were used in tandem with NIST'17 mass spectral database. In the absence of mass spectra of the detected compounds in the database, the structure was established on the basis of the characteristic fragmentation processes and the data on the chromatographic properties of the studied compounds. To quantify sterols and triterpene alcohols in terms of the internal standard, their ionization coefficients were accepted to be equal.

2.13. NMR Analysis

The quantitative NMR spectra of the oils were obtained on a JNM ECA-600 spectrometer (JEOL, Tokyo, Japan) with an operating frequency of 600 MHz for protons; they were processed using the Delta 4.3 software (JEOL, Tokyo, Japan), which provides instrument control, data acquisition, and analysis. Deuterated chloroform ($CDCl_3$, 99.8 atom% D) was used as a solvent. A 30 mg portion of the oil was dissolved in 600 μL of CDCl3 and pipetted into a standard 5 mm diameter NMR tube. The acquisition parameters for the NMR spectra were as follows: WALTZ pulse sequence to eliminate satellite signals caused by spin–spin interactions ^{1}H and ^{13}C; sweep width, 19 ppm; pulse, 90°; offset, 6 ppm; the number of scans, 64; relaxation delay, 20 sec. The signal of residual solvent proton ($CHCl_3$, 7.26 ppm) was used as a secondary standard for measuring the chemical shifts of signals.

2.14. Statistical Analysis

The data are expressed as mean ± standard deviation (SD). The normality of data sets was assessed using the Shapiro–Wilk test. Statistical calculations were carried out by GraphPad Prism 8 (Version 8.0.1.). The data were subjected to a one-way ANOVA, followed by Bonferroni's post hoc test, in order to determine the statistical significance. $p < 0.05$ was considered statistically significant. All of the experiments were repeated at least three times.

3. Results and Discussion

In this work, we aimed to comparatively study the physicochemical characteristics, the fatty acid composition, and the composition of the unsaponifiable fraction of papaya seed oil samples, which vary depending on the region where the raw materials grow.

3.1. Properties of Oil

Dry papaya seeds of different origins have an oval or ovoid shape and are brown or dark brown. At the narrow end of the seed, a small residue of the yellow or orange pulp of the fruit is sometimes noticeable. The seed peel is thick. The weight of 1000 seeds ranges from 1446 to 1751 mg. The yield of the lipid complexes from seeds of various origin (Table 1) ranges from 18.3 to 27.0%. Such a high content of the hydrophobic fraction allows us to consider papaya seeds as a source of edible oil. Generally, the color of the oil was reddish yellow. The refractive indices of papaya seed oils, regardless of the place of *Carica papaya* L. plant growth, were determined to be within the range of 1.4667–1.4678, which allows us to classify papaya seed oil as a non-drying edible oil like olive or peanut oil. The obtained results are similar to the data obtained by Yanty N. A.M. et al. [34]: oil yield—27.0%, iodine value—76.9 mg/100 g oil, saponification value—193.5 mg KOH/g, unsaponifiable matter—1.5%.

Table 1. Physicochemical properties of samples 1–7.

Sample №	Oil Yield, %	Refractive Index	Acid Value, mg KOH/g	Saponification Value, mg KOH/g	Peroxide Value, meq/kg	Iodine Value, mg/100 g Oil	Unsaponifiable Matter, %
1	25.0	1.4674 ± 0.0006	0.97 ± 0.07	191.3 ± 2.1	0.87 ± 0.07	67.4 ± 0.9	1.99 ± 0.09
2	27.0	1.4678 ± 0.0005	1.11 ± 0.09	189.1 ± 1.9	0.94 ± 0.06	70.4 ± 0.9	2.16 ± 0.03
3	26.0	1.4689 ± 0.0009	0.89 ± 0.05	189.9 ± 2.4	1.22 ± 0.08	69.5 ± 0.7	2.19 ± 0.12
4	27.0	1.4676 ± 0.0006	1.29 ± 0.09	196.4 ± 2.2	0.82 ± 0.05	66.1 ± 1.1	1.65 ± 0.06
5	18.3	1.4667 ± 0.0004	1.04 ± 0.06	192.4 ± 1.8	1.04 ± 0.08	72.3 ± 0.3	1.82 ± 0.07
6	21.1	1.4666 ± 0.0007	1.02 ± 0.08	193.4 ± 1.6	0.91 ± 0.04	71.5 ± 0.6	1.76 ± 0.09
7	23.0	1.4673 ± 0.0006	0.88 ± 0.03	190.8 ± 2.2	0.88 ± 0.06	69.8 ± 0.4	2.07 ± 0.14

Values are expressed as mean ± SD of three (n = 3) measurements.

3.2. GC-FID Analysis

The results of the chromatographic analysis of the fatty acid composition of the papaya oil samples are presented in Table 2. The identified components were further confirmed by GC-MS. The main fatty acids in papaya oil are oleic (68.7–74.9%), palmitic (14.2–16.9%), stearic (4.8–5.9%), and linoleic (3.4–6.8%) acid. In terms of fatty acid composition, papaya seed oil belongs to a relatively small group of non-drying oils such as olive, almond, peanut, and peach oils. Comparing the obtained results with the published data [13], the average content of each FA is the same.

Table 2. Fatty acids of samples 1–7 (%).

Fatty Acid (in Methyl Ester Form)	C:D	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Capric	C10:0	0.08 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.13 ± 0.02	0.09 ± 0.01	0.06 ± 0.02	0.08 ± 0.01
Lauric	C12:0	0.02 ± 0.01	0.08 ± 0.01	0.01 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.06 ± 0.01	0.02 ± 0.01
Myristic	C14:0	0.42 ± 0.04	0.56 ± 0.09	0.33 ± 0.07	0.29 ± 0.01	0.28 ± 0.02	0.53 ± 0.04	0.46 ± 0.06
Palmitic	C16:0	15.16 ± 0.14	14.22 ± 0.16	16.67 ± 0.18	16.93 ± 0.09	16.62 ± 0.23	16.13 ± 0.11	15.33 ± 0.21
Palmitoleic	C16:1	0.63 ± 0.06	0.51 ± 0.05	0.47 ± 0.07	0.54 ± 0.02	0.52 ± 0.04	0.34 ± 0.08	0.59 ± 0.08
Stearic	C18:0	5.81 ± 0.11	4.77 ± 0.14	5.29 ± 0.07	5.83 ± 0.07	5.87 ± 0.09	5.08 ± 0.10	5.63 ± 0.12
Elaidic	C18:1trans	0.09 ± 0.01	0.13 ± 0.02	0.12 ± 0.01	0.15 ± 0.02	0.09 ± 0.01	0.09 ± 0.02	0.14 ± 0.02
Oleic	C18:1cis	72.60 ± 0.36	74.91 ± 0.28	72.79 ± 0.41	70.44 ± 0.51	68.71 ± 0.35	70.54 ± 0.52	71.88 ± 0.33
Linoleic	C18:2cis	4.36 ± 0.16	3.56 ± 0.08	3.37 ± 0.09	4.62 ± 0.17	6.77 ± 0.11	6.33 ± 0.04	4.71 ± 0.06
α-Linolenic	C18:3	n/f	n/f	n/f	n/f	n/f	n/f	n/f
Arachidic	C20:0	0.26 ± 0.03	0.44 ± 0.03	0.41 ± 0.05	0.53 ± 0.05	0.36 ± 0.02	0.28 ± 0.03	0.48 ± 0.06
Gondoic	C20:1	0.10 ± 0.01	0.11 ± 0.01	0.08 ± 0.02	0.11 ± 0.02	0.14 ± 0.03	0.09 ± 0.01	0.17 ± 0.02
Behenic	C22:0	0.13 ± 0.03	0.38 ± 0.04	0.21 ± 0.03	0.16 ± 0.03	0.33 ± 0.02	0.21 ± 0.06	0.19 ± 0.02
Erucic	C22:1	0.12 ± 0.01	0.11 ± 0.01	0.08 ± 0.02	0.08 ± 0.01	0.06 ± 0.01	0.11 ± 0.01	0.09 ± 0.01
Lignoceric	C24:0	0.22 ± 0.03	0.19 ± 0.02	0.12 ± 0.02	0.16 ± 0.01	0.13 ± 0.01	0.15 ± 0.04	0.23 ± 0.02
Total		100.0	100.0	100.0	100.0	100.0	100.0	100.0

Values are expressed as mean ± SD of three (n = 3) measurements, C:D—Chain length:number of double bonds; n/f—not found.

The data of the study carried out by Alfarabi [27] agree with our determination of oleic acid (~70%) and palmitic acid (~15%) as the major components of the fatty acid fraction of papaya seeds from different geographical origins. However, in sample 5, there was less oleic acid than in samples 1–3,6. In sample 2, less palmitic acid was found than in samples 3,4,5,6. The composition of other minor fatty acids in the samples also varied depending on the region. The p-value can be found in Supplementary Materials Table S1.

3.3. GC-MS Analysis

The GC-MS analysis was performed to confirm the fatty acid composition of the extracted seed oil by reanalyzing the FAMEs mixtures of papaya seed oil samples. The components of the unsaponifiable fraction were identified by matching their recorded mass spectra with the standard mass spectra from the National Institute of Standards and Technology (NIST'17) libraries, provided by the software of the GC-MS system (Xcalibur ver

1.4 SR2), as well as the literature data and the results of our previous research [28,35]. The results were confirmed by analyzing the mass spectra of the produced compounds and also by comparing their retention indices with those indices reported in the literature [29,35]. In the EI mass spectra of all the studied trimethylsilyl (TMS) derivatives of sterols and triterpene alcohols, a molecular cation radical $M^{+\bullet}$ and a fragment ion with the composition $[M\text{-}CH_3]^+$, which are characteristic of TMS derivatives and suitable for determining the molecular weight of compounds, are presented. In addition, in the case of EI mass spectrometry, the ionized molecules are given a sufficient amount of energy (70 eV) to form a significant number of fragment ions (ionized atoms). The presence of characteristic fragment ions with the composition $[M\text{-}(CH_3)_3SiOH]^+$, $[M\text{-}(CH_3)_3SiOH\text{-}CH_3]^+$, and the products of the elimination of the hydrocarbon tail from the radical cation are useful in determining the chemical structure of the analytes. A typical total ion current (TIC) mass chromatogram of the unsaponifiable fraction of papaya seed oil is shown in Figure 1.

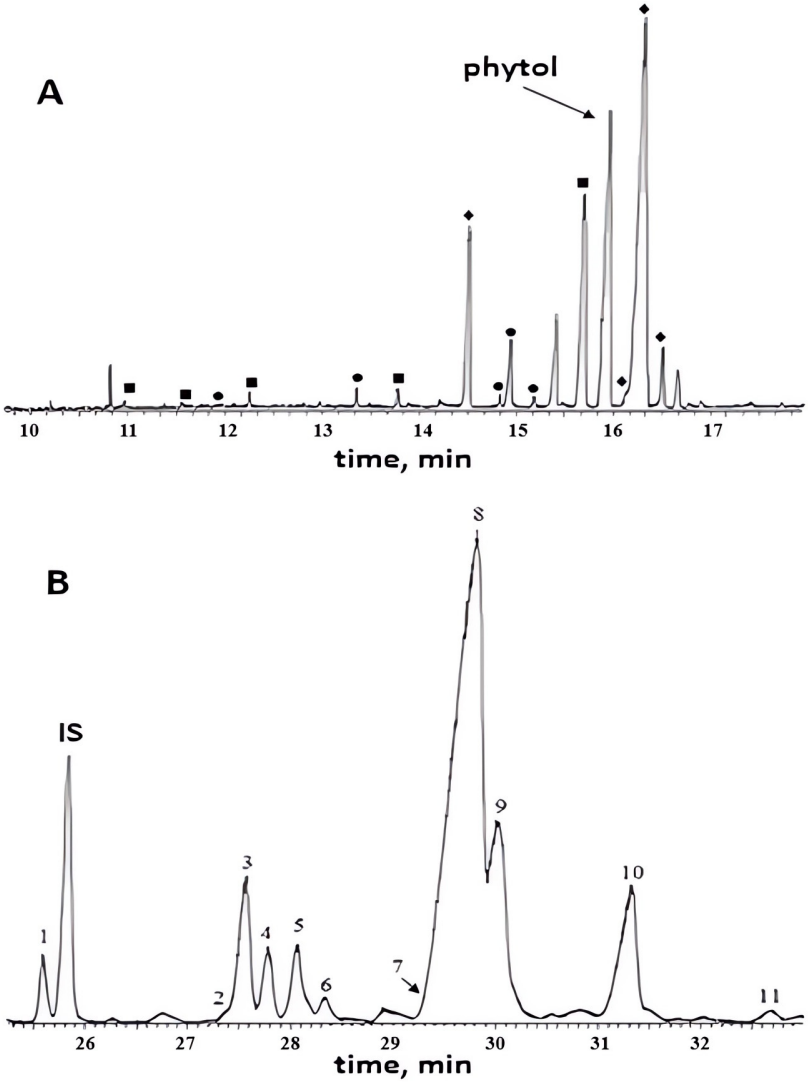

Figure 1. Typical TIC chromatogram of unsaponifiable fraction of papaya seed oil, (**A**): ■—free fatty acids (in TMS esters form), •—free fatty alcohols (in TMS esters form), ♦—fatty acids methyl esters; (**B**): sterols and triterpenic alcohols (1—cholesterol; 2—24-methylene cholesterol; 3—campesterol; 4—campestanol; 5—stigmasterol; 6—unidentified compound (428 Da); 7—lanosterol; 8—β-sitosterol; 9—sitostanol; 10—cycloartenol; 11—24-methylene cycloartanol; IS—internal standard (cholestanol)).

Apart from sterols and triterpene alcohols, the unsaponifiable fraction of all samples contained free fatty acids and free fatty alcohols in the form of TMS esters, methyl esters of

fatty acids, phytol, and benzyl isothiocyanate (BITC). Apparently, the presence of benzyl isothiocyanate (BITC) in the unsaponifiable fraction of papaya oil is associated with the presence of glucotropaeolin (benzylglucosinolate), which is converted into BITC upon enzymatic activity of myrosinase. In fact, the hydrolysis of benzylglucosinolate by myrosinase can yield a variety of products, but the primary product is BITC.

As mentioned above, the fatty acid composition of papaya seed oil was found to be similar to that of other oleic acid type oils such as olive, almond, and peach oil (ranges from 7.5 to 20.0% in palmitic acid, 0.5 to 5.0% in stearic acid, 0.3 to 3.5% in palmitoleic acid, 55.0 to 83.0% in oleic acid, 3.5 to 21.0% in linoleic acid, 0.0 to 1.5% in linolenic acid, 0.0 to 0.8% in arachidic acid, 0.0 to 0.2% in behenic acid, and 0.0 to 1.0% in lignoceric acid). Nevertheless, significant differences were observed in the composition of the unsaponifiable fraction. The main feature is a relatively high content of fatty alcohols and FAMEs. The profile of sterols and triterpene alcohols found in papaya seed oil samples (Table 3) allows us to distinguish them from other non-drying oils. The quantitative content of sterols in the oil samples was 537.5–918.2 mg/100 g of oil (0.54–0.92%). The content of the main sterols, i.e., β-sitosterol, campesterol, and stigmasterol, ranged from 55.9–65.9%, 7.1–8.8%, and 2.9–4.0%, respectively. Cholesterol and 24-methylene cholesterol were also found (up to 4.0%). The relative amount of saturated sterols (campestanol and sitostanol) ranged from 12.5 to 18.8%. Mass chromatograms with m/z values corresponding to the molecular ions of trimethylsilyl derivatives of β-sitosterol and lanosterol (m/z 486 and m/z 498) revealed the presence of trace amounts of the latter in all samples. As for the component presented in smaller amounts (about 1%), its chemical structure has not been established. The analysis of its unsaponifiable fraction in the native form (without BSTFA derivatization) showed that the mass of this compound is 428 Da, while the mass of the TMS derivative is 500 Da, which corresponds to the introduction of one TMS functional group (72 Da) into the analyte molecule, similarly to other sterols. This, together with a similar type of fragmentation, allowed this compound to be assigned to sterols and triterpene alcohols.

Table 3. Sterols and triterpenic alcohols of samples 1–7 (mg/100 g).

Sterol/Triterpene Alcohol (in Trimethylsilyl Ester Form)	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Cholesterol	10.6 ± 0.9	12.5 ± 0.6	28.4 ± 1.8	17.1 ± 1.3	26.6 ± 1.8	9.9 ± 0.7	11.2 ± 0.9
24-Methylene cholesterol	1.9 ± 0.3	2.4 ± 0.2	2.6 ± 0.3	1.8 ± 0.3	2.2 ± 0.1	1.2 ± 0.2	1.5 ± 0.2
Campesterol	43.9 ± 3.4	62.1 ± 2.6	73.1 ± 4.1	38.4 ± 1.9	57.0 ± 3.5	39.3 ± 3.2	66.8 ± 1.7
Campestanol	11.8 ± 0.7	7.1 ± 0.3	13.3 ± 0.5	10.9 ± 0.5	11.4 ± 0.7	16.9 ± 0.9	12.6 ± 0.7
Stigmasterol	27.5 ± 1.0	28.3 ± 0.8	27.0 ± 0.9	20.5 ± 1.3	29.0 ± 1.4	20.9 ± 1.3	30.6 ± 1.5
Unidentified compound (428 Da)	6.9 ± 0.5	8.7 ± 0.4	9.1 ± 0.4	7.8 ± 0.6	6.5 ± 0.6	5.2 ± 0.4	6.8 ± 0.4
β-Sitosterol + Lanosterol	451.9 ± 13.9	464.8 ± 8.6	513.0 ± 22.1	314.1 ± 11.2	479.7 ± 11.8	309.8 ± 9.9	493.8 ± 10.5
Sitostanol	76.9 ± 4.9	83.8 ± 8.1	140.0 ± 9.1	66.7 ± 5.5	83.2 ± 5.9	84.6 ± 7.0	92.5 ± 7.7
Cycloartenol	43.5 ± 3.3	33.3 ± 0.9	108.3 ± 6.2	55.3 ± 4.8	54.4 ± 4.4	45.2 ± 2.7	77.3 ± 5.0
24-Methylene cycloartanol	3.1 ± 0.2	2.3 ± 0.2	3.4 ± 0.1	5.2 ± 0.3	7.1 ± 0.4	4.5 ± 0.3	2.9 ± 0.2
Total	678.0 ± 29.1	705.3 ± 22.7	918.2 ± 45.5	537.8 ± 27.7	757.1 ± 30.6	537.5 ± 26.6	796.0 ± 28.8

Values are expressed as mean ± SD of three (n = 3) measurements.

β-sitosterol and stigmasterol have been identified as the two main constituents of the fatty acid fraction of papaya seeds from various geographical origins based on a comparison of our results with the data from the study conducted by Alfarabi [27]. The p-value can be found in Supplementary Materials Table S2.

As it is shown in the histograms (Figure 2A), sample 3 and sample 5 contained more cholesterol than the other samples. The content of 24-methylene cholesterol (Figure 2A) was similar in all samples. The content of campesterol was highest in sample 3. Sample 6 contained more campestanol (Figure 2B). The content of the total amount of β-sitosterol and lanosterol was similar in all samples, except for sample 4 and sample 6. The content of sitostanol and cycloartenol was higher in sample 3 than in other samples. The content of

24-methylene cycloartanol was similar in all samples except in sample 5, where its content was highest.

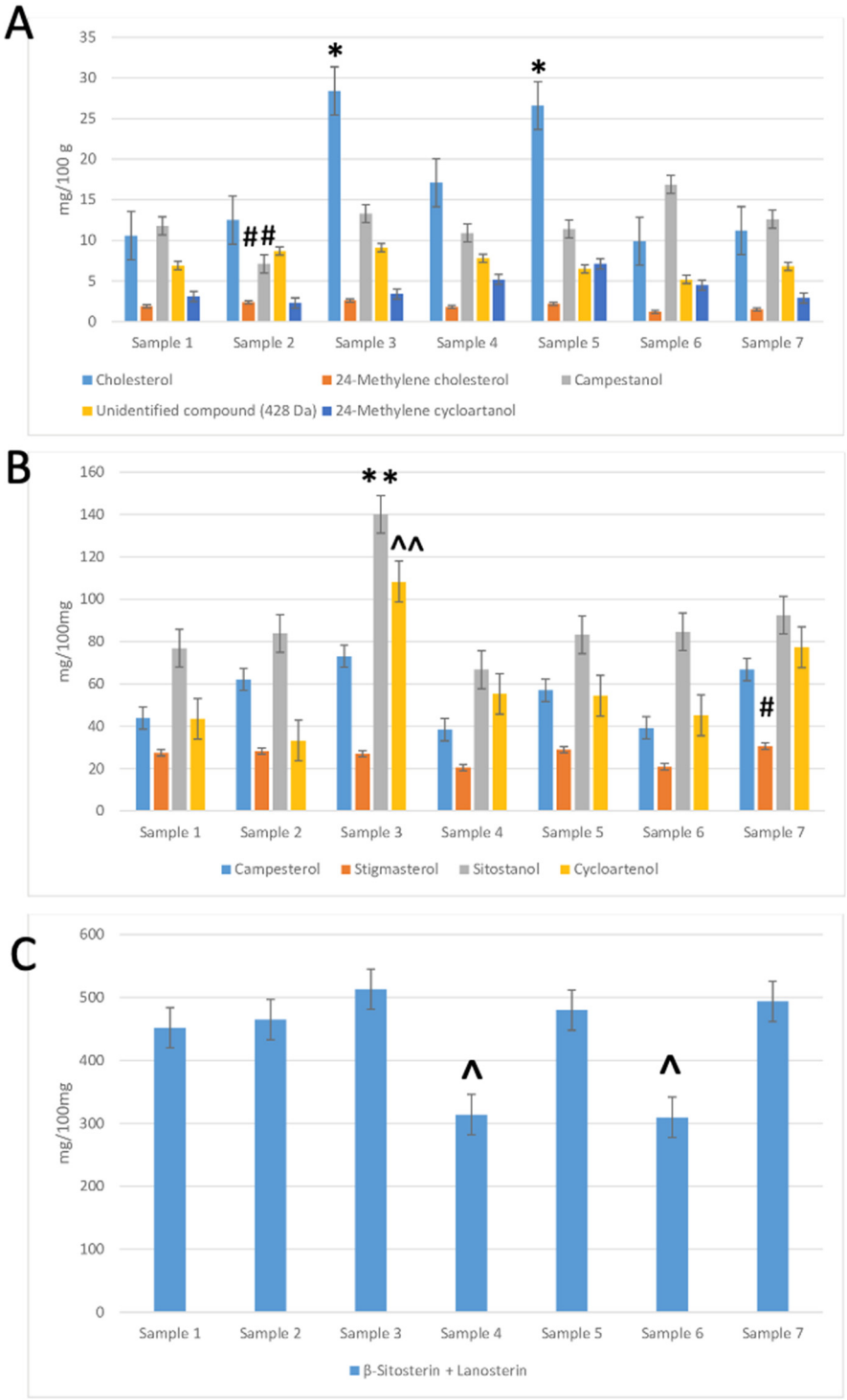

Figure 2. Histograms (**A**–**C**) of sterols and triterpenic alcohols content of samples 1–7. * $p < 0.05$ compared with samples 1,2,4,6,7; # $p < 0.05$ compared with samples 4,6; ^ $p < 0.05$ compared with samples 1,2,3,5,7; ** $p < 0.05$ compared with samples 1,2,4,5,7; ^^ $p < 0.05$ compared with samples 1,2,4,5,6; ## $p < 0.05$ compared with samples 1,3,4,5,6,7.

3.4. NMR Studies

A typical ^{1}H NMR spectrum of papaya oil is shown in Figure 3. The NMR spectra of edible oils usually contain nine main signals corresponding to the functional groups of the fatty acids that make up their composition: -CH_3 (0.82–0.94 ppm), -(CH_2)n-(1.20–1.43 ppm), -OCO-CH_2-CH_2 (1.55–1.69 ppm), -CH_2-CH=CH-(1.93–2.13 ppm), -OCO-CH_2-(2.25–2.36 ppm), =CH-CH_2-CH= (2.73–2.87 ppm), -CH=CH-(5.29–5.43 ppm), as well as the methylene and methine protons of the glycerol fragment, -CH_2OCOR (4.10–4.35 ppm) and -CHOCOR (5.23–5.29 ppm). The absence of an additional signal within the range of 0.94–1.03 ppm corresponding to the protons of the terminal –CH_3 group of linolenic acid indicates its absence in papaya seed oil. The intensity of the methylene proton triplet located between the double bonds within the range of 2.7–2.9 ppm in all ^{1}H NMR spectra of the samples is very small. This indicates the predominance of glycerol esters with monounsaturated acid in triglyceride (TAG) in all papaya seed samples of different origins.

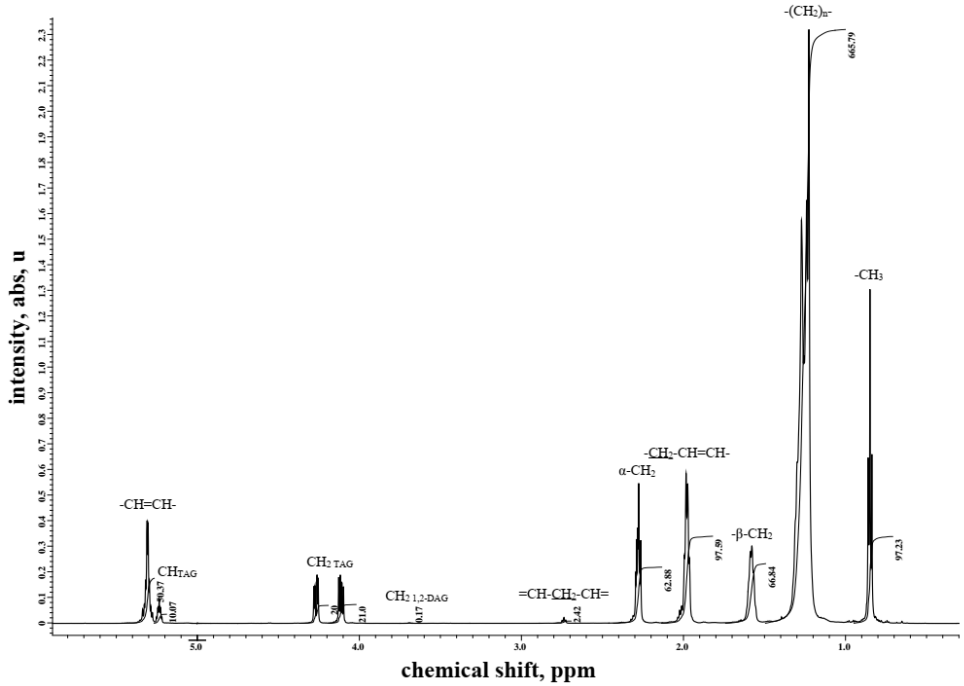

Figure 3. Typical NMR ^{1}H spectra of papaya seed oil (sample 7).

This is also evidenced by the low iodine value of the analogue of the iodine number of TAG in papaya seed oil samples, from 66.1 to 72.3 (see Table 1). They also contained about 20–25% of saturated fatty acids and a significant amount, i.e., about 75–80%, of unsaturated fatty acids. The signals in the ^{1}H NMR spectra of the samples corresponding to mono- and diacylglycerides, respectively, are of a low intensity; almost the entire saponified portion of papaya seed oil is represented by triacylglycerides. Based on the physical principle of NMR spectroscopy, a strict linear relationship between the signal areas and the content in the analyte of molecule fragments responsible for these signals is observed. The ratios between the signal areas allow us to calculate the component composition of the samples (Table 4). The *p*-value can be found in Supplementary Table S3. Analysis of the glycerol components content showed that in samples 2 and 4, the content of DAG was lower than in other samples. The results also show that in samples 5 and 6, there is a significant increase in the amount of diunsaturated fatty acids compared to that in the other samples.

Table 4. Chemical composition of samples 1–7 obtained by NMR analysis (TAG—triacylglyceride, DAG—diacylglyceride, MAG—monoacylglyceride).

Sample №	Glycerol Components Content, %				Fatty Acids Components Content, %			
	TAG	DAG	MAG	Total	Saturated	Monoun-Saturated	Diun-Saturated	Total
1	99.2 ± 0.1	0.7 ± 0.1	0.1 ± 0.05	100.0	21.3 ± 0.2	74.1 ± 0.1	4.6 ± 0.1	100.0
2	99.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.04	100.0	19.8 ± 0.1	76.6 ± 0.2	3.6 ± 0.2	100.0
3	98.7 ± 0.2	1.1 ± 0.1	0.2 ± 0.03	100.0	23.9 ± 0.1	72.3 ± 0.2	3.8 ± 0.1	100.0
4	99.2 ± 0.1	0.6 ± 0.1	0.2 ± 0.03	100.0	24.8 ± 0.1	70.9 ± 0.1	4.3 ± 0.1	100.0
5	98.9 ± 0.2	0.8 ± 0.1	0.3 ± 0.04	100.0	25.3 ± 0.2	67.7 ± 0.3	7.0 ± 0.2	100.0
6	99.0 ± 0.1	0.9 ± 0.1	0.1 ± 0.05	100.0	22.6 ± 0.1	71.1 ± 0.2	6.3 ± 0.2	100.0
7	98.9 ± 0.1	0.9 ± 0.1	0.2 ± 0.02	100.0	22.9 ± 0.1	72.3 ± 0.3	4.8 ± 0.2	100.0

Values are expressed as mean ± SD of three (n = 3) measurements.

4. Conclusions

This paper provides information on the physicochemical properties and the chemical composition of papaya seed oil of different geographic origins. *Carica papaya* L. can be a valuable source of oil due to the high content of oleic acid in its seeds. The obtained data on the physicochemical properties of *Carica papaya* L. seed oil, such as the refractive index, acid value, iodine value, peroxide value, and saponification value, were found to be similar with the data obtained by many other researchers [17] and thus confirm their results. The same applies to the data on the fatty acid composition of papaya oil. However, prior to our investigation, the available data on the composition of the unsaponifiable fraction was limited and indicated the presence of only five compounds in the oil: β-sitosterol, campesterol, stigmasterol, and trace amounts of stigmastanol and stigmast-4-en-3-one. We found that the unsaponifiable fraction also contains cholesterol, 24-methylene cholesterol, sitostanol, cycloartenol, and 24-methylene cycloartanol. We estimated the difference in the composition of the unsaponifiable fraction, which can be used for the authentication purposes of papaya seed oil among other vegetable oils. The content of fatty acids, sterols, and triterpenic alcohols in papaya seed oil from Russia is in the same quantitative range as that in other samples. This means that *Carica papaya* L. can be grown in more northern latitudes without losing its nutritional properties because *Carica papaya* L. is considered to be a promising source of valuable seed oil.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9111227/s1, Table S1: One-way ANOVA analysis results of fatty acids of samples 1–7; Table S2: One-way ANOVA analysis results of sterols and triterpenic alcohols of samples 1–7; Table S3: One-way ANOVA analysis results of glycerol (TAG, DAG, MAG) and fatty acids of samples 1–7.

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