Seed Oil Biochemical Composition of Cultivated
Cucurbita L. Species from the VIR Collections Grown
in the Astrakhan Province of the Russian Federation

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Abstract: Cucurbita crops are among the most valuable and widely cultivated vegetable crops in
global agriculture. Cucurbita seed oil meets the requirements of functional nutrition; it is material for
the food industry and medicines based on natural ingredients. The present research was aimed at
studying features of the biochemical composition of oil in seeds of the main cultivated
Cucurbita L. species from the collection of the N.I. Vavilov Institute (VIR) grown in the conditions of the Astrakhan
Experiment Station (AES) of VIR. The oil content in seeds of Cucurbita L. species varied from 40
to 49.7%. Over 80% of the fatty acids (FA) composition (FAC) was represented by oleic (13.6–49.6)
and linoleic (33.5–69.3%) acids. The accessions of Cucurbita mixta Pang. were noted for high values
of saturated FAs (palmitic and stearic), those of Cucurbita pepo var. melopepo (L.) Harz. of linoleic
and linolenic, and those of Cucurbita pepo L. of oleic acid. The multiple factor analysis showed
significant differences in FAC in accessions of C. pepo var. melopepo, Cucurbita maxima Duch. and
C. pepo. The performed study allowed us to reveal FAC features in seeds of each Cucurbita species
from the VIR collection and to identify the accessions with the best economically important indicators.

Keywords: functional nutrition; oleic; linoleic; linolenic fatty acids; hull content

1. Introduction

At the present time, the healthy diet concept is becoming increasingly popular and relevant. In
this regard, vegetables occupy one of the main places in a person’s healthy diet [1,2]. Cucurbita L.
species are among the most nutritionally valuable and widely cultivated vegetable crops in global
agriculture. In terms of gross output of Cucurbita crops, Russia ranks third in the world after China
and India [1,2]. In Russia alone, 152 squash and 138 pumpkin varieties are cultivated [3–5].

Cucurbita crops are among the most common food products and raw materials for manufacturing
a wide range of dietetic, therapeutic and prophylactic products, as well as baby foods [5]. Their
biochemical composition includes such valuable food components as different groups of
vitamins, dietary fibers, as well as low calorie content [5]. Not only the flesh of Cucurbita, but also their
seeds have nutritional benefits, being used in the national cuisine of Austria, Slovenia, Hungary and
Africa [5–7]. The cucurbit seed oil is one of the most famous products in the modern healthy nutrition
market, especially in Austria [5,7]. This oil is also becoming increasingly popular in Russia and the
countries of the former USSR [5]. The use of the cucurbit seed oil as a healthy diet component, as well
as for medicinal purposes, is receiving great attention in China and Japan [8–10]. The cucurbit seed
oil is considered a unique remedy for preventing cardiovascular diseases, hypertension, urogenital
system disorders (prostatitis therapy), oncological and dermatological diseases [11]. Previous studies have shown that treatment of skin lesions with cucurbit seed oil with a high content (more than 50% of the total fatty acids) of polyunsaturated fatty acids (PUFAs) promotes wound healing and removal of inflammation [12,13]. In addition, the oil has antimicrobial properties [12,13]. At present, there is a growing demand for medicines produced based on natural ingredients, including cucurbit seed oil. Such medicines are often more effective, safer and cheaper, in comparison with synthetic and semi-synthetic analogs [5,12,13]. The cucurbit seed oil was found to contain over 30% of protein and oil, and more than 40% of α- and γ-tocopherols [6,7]. The cucurbit seed oil is rich in phytosterols, of which β-sitosterol (over 39%) and the carotenoids β-carotene and lutein are the main ones [5–8].

Among the phenolic compounds, cucurbit seeds contain protocatechuic, caffeic, syringic, vanillic, paracumaric and ferulic acids, among which syringic acid predominates (up to 7.96 mg/100 g) [14]. The cucurbit seed oil is a source of essential ω-3, ω-6 and ω-9 FAs [1,5,6]. The main FAs are palmitic, oleic and linoleic acids, with a predominance of oleic acid (about 16, 44 and 35%, respectively) [11,12]. The presence of all the above-described components in cucurbit seeds ensures their useful nutritional qualities, including antioxidant activity. A higher content of oleic acid compared to that of the polyunsaturated linoleic acid ensures the stability of the oil in storage and makes it a ‘convenient’ raw material for the food industry [14]. In addition, the cucurbit seed oil, especially the cold-pressed one, is actively used in the pharmacological and cosmetic industries [11,12,14,15].

The collection of Cucurbita crops at VIR contains all five cultivated species of the genus Cucurbita L.: C. maxima Duch., C. pepo L., Cucurbita moschata Duch., Cucurbita ficifolia Bouche, and C. mixta Pang. The collection numbers about three thousand accessions from 99 countries. Squash and pattypan squash are varieties of Cucurbita pepo (L. var. giromontia and var. melopepo (L.) Harz, respectively) [5,7]. Of particular interest is the study of the fatty acid composition of the oil in different Cucurbita species grown in different regions of the world, because the influence of climatic and soil features on the biochemical composition of crops has been proved.

The purpose of the study was to reveal differences in the studied parameters among representatives of the main cultivated Cucurbita species from the VIR collection grown under the conditions of AOS VIR, select accessions with a low hull and high oil content, and an optimal FAC. Thus, the VIR Cucurbita collection can be screened for accessions to be used when creating new oil cultivars of Cucurbita species with a high oil content and optimal FAC to expand the assortment of healthy, functional and therapeutic nutritional products.

2. Materials and Methods

2.1. Materials

The materials for the study comprised 142 seed samples from four cultivated Cucurbita species, namely C. pepo, C. maxima, C. moschata, C. mixta, and of two varieties of C. pepo, i.e., var. giromontia and var. melopepo (squash and pattypan squash, respectively), grown under conditions of AES VIR in 2015-16 and collected at the stage of full fruit ripeness (Table 1 and Table S1).

The alluvial-meadow soils are characterized by a low humus content (1.87%) in the upper horizon and a sharp decrease with depth. Ground water lies low, at a depth of 1.5–2.5 m. The plants were grown on two-row plots, with 10 plants in a row, totaling 20 plants per plot. The layout of plants was 2×1.4 m. Sowing was carried out on May 15–17 each year. The emergence of seedlings was noted on the 7–8th day. Artificial manual pollination was carried out with preliminary isolation of male and female flowers. The harvesting of fruits was carried out at the stage of full fruit ripeness. For seed separation, only fully ripe fruits typical for the variety were used. The used agricultural practices were in accordance with the VIR methodological guidelines [16]. The climate in the location of AES VIR (46.221782 N, 48.034153 E) is sharply continental, with pronounced seasonal changes.
Table 1. List of *Cucurbita* L. species from the VIR collection used as a research material.

| Species      | Variety          | Origin                                                   | Number of Accessions |
|--------------|------------------|----------------------------------------------------------|----------------------|
| *C. pepo*    |                  | Russia, Ukraine, USA, Canada, Germany, Portugal, France, Australia | 35                   |
| *C. pepo*    | var. *gironontia*| Russia, Ukraine, Georgia, USA, Bulgaria, Turkey           | 7                    |
| *C. pepo*    | var. *melopepo*  | Russia, France                                           | 4                    |
| *C. maxima*  |                  | Russia, Ukraine, USA, Canada, Germany, France, Argentina, Spain, Uzbekistan, Bulgaria, Turkmenistan, Congo, Peru, Turkey, China, Czech Republic, Syria, India, Japan, Hungary, Jamaica | 84                   |
| *C. moschata*|                  | Russia, Ukraine, Azerbaijan, Bangladesh, Japan, Canada, Zimbabwe | 8                    |
| *C. mixta*   |                  | Mexico                                                    | 4                    |

The growing season of 2015–2016 was warmer compared with the long-term average data (from 1980 to 2018, RIHMI-World Data Center) [17]. The hottest months were June 2015 and August 2016, with air temperatures exceeding the long-term average by 3.1 and 3.6 °C, respectively. The coldest one was June 2016, when the temperature was almost 1 °C below the long-term average. The growing seasons of 2015–2016, were more arid, with 14.8 and 11.9 mm less rainfall, respectively. The driest month during our research was May 2015, when the difference between the monthly average precipitation and the long-term data reached 20.3 mm (Table S2) [17].

2.2. Chemicals and Reagents

A standard mixture of fatty acid methyl esters was used (37 components, 47885U, Supelco, Bellefonte, PA, USA). Petroleum ether (chemically pure, 70–100 °C, Technical conditions (TU) 6-02-1244-83) and sodium hydroxide (chemically pure, GOST standards of the Russian Federation-4328-77) were products of EKOS-1 (Moscow Region, Russia), methanol and n-hexane were of high-performance liquid chromatographic grade, and were obtained from Merck KGaA (Darmstadt, Germany).

2.3. Sample Preparation

Each sample comprised 10 g of seed mix from three Cucurbit fruits. Each analysis was performed in two replications, and the obtained average values were statistically analyzed. Seeds were ground in a laboratory mill into flour (Lab. Mill-I, Labor Muszeriparimuv, Esztergom, Hungary) with a particle size passing through a sieve with cell size of 1.00 mm. Before milling, the seeds were dehulled manually, and the hull content was measured as proportion of the total weight of sample in percent by using the laboratory balance (Sartorius A 120S, Sartorius GMBN, Germany) [18]. Oil content was investigated by Soxhlet apparatus using petroleum ether as extraction agent, calculated as proportion of dry fat-free residue from 2 g of the milled sample, expressed as percent [18]. The five main fatty acids in cucurbit seed oil were detected by gas chromatography. One hundred milligrams of the milled sample powder was mixed with 1.5 mL of n-hexane, shaken periodically within 20 min, then centrifuged at 10,000× g for three min. The hexane fraction was evaporated to complete dryness in flowing nitrogen, then 0.5 mL 0.1 Mol solution of sodium hydroxide in methanol was added and heated for 15 min at 100 °C to obtain fatty acid methyl esters. After cooling, 0.5 mL of n-hexane was put in the tube and shaken vigorously, then the hexane fraction was transferred into the GC vial [18,19].
2.4. GC-MS Analysis

The GC analysis was carried out using the Crystallux-4000M gas chromatograph (“Meta-Chrome” Research and Production Company, Yoshkar-Ola, Russian Federation) with a flame ionization detector. Fatty acid methyl esters were separated on a Omegawax TM 250 polar column (polyethylene glycol, 30.0 m, 250.00 µm, 0.25 µm; USA) under the heating conditions from initial temperature of 170 °C, which was kept for 2 min, then increased to 220 °C at a rate of 3 °C/min, and kept at 220 °C for 5 min; the injection volume was 1.2 µL, and the helium flow rate was 1.5 mL/min.

2.5. Data Processing

Fatty acid identification was carried out using retention time of a standard mixture of fatty acid methyl esters. Chromatograms were processed using the UniChrom™ (2010, “New Analytical Systems”, Minsk, Belarus, www.unichrom.com) program. The content of fatty acid methyl esters was calculated by the method of internal normalization; the content of each fatty acid was expressed as percent of the total fatty acid content. FAI (fatty acids index) was calculated as SFA (saturated fatty acids) to USFA (unsaturated fatty acids) ratio. The results were processed using the STATISTICA 7.0 for Windows software package.

3. Results

One of the important indicators affecting the oil content in cucurbit seeds is the ‘seed hull to the total seed weight’ ratio, or the hull content. High hull content complicates seed processing, increases its cost, and reduces the yield of the final product, the seed kernel mass; however, this coat protects the seed from the effects of adverse environmental conditions and helps to maintain germination ability during the long-term storage. The oil content determines the nutritional value, taste and profitability of using a crop as a raw material for oil or other food production. The hull and oil content were determined for seed samples of *C. pepo*, *C. maxima* and *C. moschata*—the species most widely cultivated in the Russian Federation. The hull content ranged from 16.4 (% *C. maxima*, vk-1041 (vk means Temporary Catalog No.) to 56.3% (*C. moschata*, vk-1780), and the oil content from 40 (% *C. maxima*, k-4322 (k means Permanent Catalog No.) to 49.7% (*C. moschata*, k-2741). The average hull content values remained virtually unchanged over the years of the study for *C. pepo* and *C. moschata* (22.5 and 22.3; 34.2 and 32.6%, respectively). The hull content in *C. maxima* accessions was higher (27.6%) in 2015 compared with 2016 (22.7%). Among the studied accessions, the highest hull content values were recorded for *C. moschata* and the lowest ones for *C. pepo* (Table 2 and Table S3).

Low hull content (below 20%) was observed for the accessions of *C. pepo*: k-99 (France), vk-1658 (Germany), vk-1710 (Russia), vk-1741 (USA), vk-1748a (Portugal), of *C. maxima*: k-937 (Peru), k-4322 (India), vk-1041 (Turkey), and of *C. moschata*: k-3861 (Japan) (Table S3).

The oil content in the seeds of the studied *Cucurbita* accessions was above 40% and ranged from 40 to 49.7%. For all the studied accessions of *C. pepo*, *C. maxima* and *C. moschata*, the average oil content was lower in 2015 (44.1; 45.3; 43.4%, respectively) and higher in 2016 (46.1; 48.7; 47.1%, respectively). The maximum accumulation of oil was observed in *C. maxima*, while a lower oil content was recorded for *C. pepo* and *C. moschata*. The identified accessions with oil content above 48% included those of *C. maxima*: k-693 (USA), k-752 (Turkey), k-1407 (Russia), k-1642 (China), k-4272 (Argentina), k-4319 (Syria), k-4371 (Russia); and of *C. moschata*: k-2741 (Russia) (Table S3).
Table 2. Average values of hull, oil content and fatty acids profile 1 (percentage, mean values ± SD 2) in seeds of *Cucurbita* L. species from the VIR collection.

| Cucurbita Species and Varieties | Years of Study | Hull Content | Oil Content | C 16:0 | C 18:0 | C 18:1 | C 18:2 | C 18:3 | USFA 1 | SFA 1 | FAI 1 |
|--------------------------------|----------------|--------------|-------------|--------|--------|--------|--------|--------|--------|-------|-------|
| *C. pepo*                      | 2015           | 22.5 ± 3.2   | 44.1 ± 1.9  | 11.6 ± 2.4 | 3.3 ± 1.2 | 38.5 ± 6.7 | 46.4 ± 7.2 | 0.2 ± 0.3 | 85.1 ± 3.0 | 14.9 ± 3.0 | 0.2 ± 0.0 |
|                                | 2016           | 22.3 ± 3.5   | 46.1 ± 2.2  | 10.0 ± 2.6 | 2.7 ± 0.9 | 36.3 ± 8.8 | 50.9 ± 8.2 | 0.0 ± 0.1 | 87.3 ± 3.0 | 12.7 ± 3.0 | 0.2 ± 0.0 |
| Average                        |                | 22.4 ± 3.3   | 45.1 ± 2.3  | 10.8 ± 2.6 | 3.0 ± 1.1 | 37.4 ± 7.8 | 48.7 ± 8.0 | 0.1 ± 0.3 | 86.2 ± 3.2 | 13.8 ± 3.2 | 0.2 ± 0.0 |
| *C. pepo* var. giromontia      | 2015           | N/D          | N/D         | 11.8 ± 2.1 | 2.7 ± 0.9 | 32.3 ± 4.9 | 53.2 ± 5.6 | 0.1 ± 0.1 | 85.5 ± 1.8 | 14.5 ± 1.8 | 0.2 ± 0.0 |
|                                | 2016           | N/D          | N/D         | 12.7 ± 3.7 | 2.1 ± 0.7 | 34.3 ± 6.1 | 50.5 ± 6.8 | 0.4 ± 0.8 | 85.2 ± 3.4 | 14.8 ± 3.4 | 0.2 ± 0.1 |
| Average                        |                | N/D          | N/D         | 12.2 ± 2.9 | 2.4 ± 0.8 | 33.3 ± 5.5 | 51.8 ± 6.2 | 0.2 ± 0.5 | 85.3 ± 2.6 | 14.7 ± 2.6 | 0.2 ± 0.0 |
| *C. pepo* var. melopepo       | 2015           | N/D          | N/D         | 7.5 ± 1.7  | 0.4 ± 0.3 | 27.1 ± 0.7 | 63.3 ± 3.2 | 1.8 ± 1.8 | 92.2 ± 2.0 | 7.8 ± 2.0  | 0.1 ± 0.0 |
|                                | 2016           | N/D          | N/D         | 7.2 ± 3.1  | 0.2 ± 0.2 | 26.5 ± 3.0 | 64.8 ± 3.0 | 1.3 ± 1.3 | 92.6 ± 3.2 | 7.4 ± 3.2  | 0.1 ± 0.0 |
| Average                        |                | N/D          | N/D         | 7.3 ± 2.3  | 0.3 ± 0.2 | 26.8 ± 2.0 | 64.1 ± 3.0 | 1.6 ± 1.5 | 92.4 ± 2.5 | 7.6 ± 2.5  | 0.1 ± 0.0 |
| *C. maxima*                   | 2015           | 27.6 ± 6.3   | 45.3 ± 1.8  | 14.0 ± 2.1 | 3.3 ± 1.7 | 24.8 ± 7.5 | 57.7 ± 7.8 | 0.2 ± 0.6 | 82.7 ± 3.3 | 17.3 ± 3.3 | 0.2 ± 0.1 |
|                                | 2016           | 22.7 ± 6.1   | 48.7 ± 1.7  | 14.5 ± 2.5 | 3.6 ± 1.0 | 24.0 ± 7.5 | 57.9 ± 7.4 | 0.0 ± 0.1 | 81.9 ± 2.5 | 18.1 ± 2.5 | 0.2 ± 0.0 |
| Average                        |                | 25.1 ± 6.7   | 47.0 ± 2.5  | 14.2 ± 2.2 | 3.5 ± 1.4 | 24.4 ± 7.5 | 57.8 ± 7.6 | 0.1 ± 0.4 | 82.3 ± 3.0 | 17.7 ± 3.0 | 0.2 ± 0.0 |
| *C. moschata*                 | 2015           | 34.2 ± 17.5  | 43.4 ± 2.3  | 10.0 ± 2.6 | 2.7 ± 0.9 | 36.3 ± 8.8 | 50.9 ± 8.2 | 0.0 ± 0.1 | 87.3 ± 3.0 | 12.7 ± 3.0 | 0.2 ± 0.1 |
|                                | 2016           | 32.6 ± 15.0  | 47.1 ± 2.3  | 14.7 ± 3.2 | 4.4 ± 0.6 | 24.6 ± 5.9 | 56.4 ± 6.2 | 0.0 ± 0.0 | 81.0 ± 3.3 | 19.0 ± 3.1 | 0.2 ± 0.1 |
| Average                        |                | 33.4 ± 15.7  | 45.2 ± 3.0  | 14.7 ± 2.6 | 4.3 ± 1.0 | 26.7 ± 6.1 | 54.3 ± 6.7 | 0.0 ± 0.1 | 81.0 ± 3.1 | 19.0 ± 3.1 | 0.2 ± 0.1 |
| *C. mixta*                    | 2015           | N/D 3        | N/D         | 15.5 ± 2.1 | 6.0 ± 1.4 | 29.6 ± 12.1 | 48.7 ± 10.4 | 0.2 ± 0.1 | 78.5 ± 3.0 | 21.5 ± 3.0 | 0.3 ± 0.1 |
|                                | 2016           | N/D          | N/D         | 14.0 ± 0.8 | 5.4 ± 1.0 | 25.1 ± 2.9 | 55.4 ± 3.2 | 0.2 ± 0.1 | 80.6 ± 0.7 | 19.4 ± 0.6 | 0.2 ± 0.0 |
| Average                        |                | N/D          | N/D         | 14.8 ± 1.7 | 5.7 ± 1.2 | 27.4 ± 8.5 | 52.0 ± 8.0 | 0.2 ± 0.1 | 79.6 ± 2.3 | 20.4 ± 2.3 | 0.3 ± 0.0 |

Identified FAs: C 16:0 (palmitic), C 18:0 (stearic), C 18:1 (oleic) and C 18:2 (linoleic); 1. The content of each FA is expressed as % of the total identified FAs; USFA = unsaturated fatty acids; SFA = saturated fatty acids; FAI = fatty acids index; 2. SD = standard deviation; 3. N/D = not determined.
Although the observations show the influence of weather conditions on the above parameters, it was not possible to establish reliable relationships from a two-year study. Among the identified FAs, palmitic (C 16:0), stearic (C 18:0), oleic (C 18:1) and linoleic (C 18:2) ones were dominant in the cucurbit seed oil. The other FAs amounted to less than 1%. Eighty or more percent of FAC in cucurbit seed oil was presented by two acids: oleic and linoleic, the values of which in the studied accessions ranged from 13.6 to 49.6% and from 33.5 to 69.3%, respectively (Table S3).

The average values of the palmitic, stearic, oleic, linoleic and linolenic (C 18:3) acids content in the seed oil of C. maxima, C. pepo var. melopepo practically did not change in different years of the study. Insignificant fluctuations were noted in FA values for the accessions of C. pepo, C. moschata, C. mixta, C. moschata and C. pepo var. giromontia. In 2015, C. moschata and C. mixta had a higher content of oleic (36.3; 29.6%), and C. pepo var. giromontia of linoleic (53.2%). In 2016, a higher content of palmitic acid (14.7%) was noted for C. moschata, and of linoleic acid (50.9; 56.4 and 55.4%) for C. pepo, C. moschata and C. mixta accessions, respectively (Table 2).

Differences in FAC were observed between accessions of different Cucurbita species. The highest values of the total saturated FAs were observed for the accessions of C. mixta (20.4%), which is due to the highest content of palmitic (14.9) and stearic (5.7%) acids in the oil of this species. Almost the same values of palmitic acid content were recorded for C. maxima (14.2%). The accessions of C. pepo var. melopepo were characterized by the lowest values of the total SFA (7.6%), palmitic and stearic acids (7.3 and 0.3%, respectively), and the highest of USFA (92.4%). The latter is due to the content of monounsaturated oleic, polyunsaturated linoleic and linolenic FAs (26.8; 64.1 and 1.6%, respectively). The most significant oleic acid content (37.4%) was observed in the accessions of C. pepo, while the minimum (24.4%) was noted for those of C. maxima (Table 2).

High contents (over 89%) of USFA were displayed by the accessions of C. pepo, namely k-327 (89.5%; Ukraine), k-2955 (89.7%; Ukraine), k-3000 (89.5%; Ukraine), k-3589 (93.2%; Ukraine), k-4031 (91.7%; Ukraine), vk-344 (91.6%; Ukraine), vk-1710 (89.1%; Russia), vk-1728 (89.6%; USA); of C. maxima: k-998 (89.2%; China); of C. pepo var. giromontia: k-4648 (89.9%; Russia); and of C. pepo var. melopepo: k-7 (94.4%; France), k-4514 (93.6%; Russia), k-4517 (93.0%; Russia) (Table S3). Such FA content is suitable for external medicinal products. The FA index (FAI) characterizes the proportion of saturated and unsaturated acids in vegetable oils. It is important for oil usage in pure or blended form [20]. Among the studied accessions, FAI ranged from 0.06 (1/17) in C. pepo var. melopepo (k-7; France) up to 0.32 (1/3) in C. maxima (k-4449; Russia), as the greater part of FAC is represented by the unsaturated oleic and linoleic FAs (Table S3).

The lowest FAI values (0.06; 0.07 and 0.07, respectively) were recorded for the accessions of C. pepo var. melopepo: k-7 (France), k-4514 and k-4517 (Russia); therefore, they can be recommended for blending with vegetable oils with a high SFA content (Table S3).

Levels of oleic acid exceeding that of linoleic were found for accessions of C. pepo vk-333 (49.1 and 38.3; Ukraine), vk-1186 (47.5 and 37.65; USA), k -4361 (47.8 and 36.45; Portugal) and for C. maxima k-1437 (49.5 and 33.50%; Japan), so these samples could be promising for the food industry (Table S3). The statistical processing revealed significant differences in biochemical parameters between the accessions of Cucurbita species and subspecies taken into the study.

The results of the analysis of variance (Figure 1, Table S4) confirm the above. The accessions of C. mixta were noted among the others for high values of SFA (palmitic and stearic) and their total, as a consequence (Figure 1a,b,f). The groups of C. maxima and C. moschata were also characterized by a considerable amount of palmitic acid (Figure 1a). The oil of C. pepo seeds contained significantly less linoleic acid compared with other Cucurbita species (Figure 1d). The values of linolenic acid content in C. maxima, C. mixta, C. moschata, C. pepo and C. pepo var. giromontia were equally low (Figure 1e). The insignificant content of the main PUSFA (polyunsaturated FA) explains the small values of the total UFAs content in C. mixta, C. moschata and C. maxima (Figure 1g). On the contrary, the accessions of C. pepo var. melopepo differ from other groups by low content of palmitic, stearic and total SFAs, and by high content of linoleic, linolenic acids, and the total USFAs (Figure 1a–g).
The results of the analysis of variance (Figure 1, Table S4) confirm the above. The accessions of C. pepo var. melopepo does not form a separate group. Due to its low content of palmitic, stearic and total SFAs, the content of linolenic acid did not have sufficient factor loading to affect the difference in high oil values (Figure 1a–g). The groups of C. moschata, C. pepo var. melopepo differed from other groups by high content of linoleic, linolenic acids, and the total USFAs (Figure 1a–g). 

Figure 1. ANOVA diagrams for parameters of the studied seeds of C. pepo var. melopepo, C. moschata, C. pepo var. giromonti, and those of C. mixta with 25% reliability. With this approach, C. pepo var. melopepo does not form a separate group. The analysis of principal components showed that the first factor (palmitic and stearic acids content, total USFA, total SFA, FAI (54.6% of explained variability) separated the accessions of C. moschata, C. pepo var. melopepo, C. moschata, C. pepo var. giromonti, and C. mixta. Due to its low content of USFAs, the group of C. pepo var. melopepo did not form a separate group. The classification function, which was significantly different for the contents of PUFA and SFA, helped to separate the accessions of C. moschata, C. pepo var. melopepo, C. moschata, C. pepo var. giromonti, and C. mixta. The analysis of variance of oil content showed no significant differences between the accessions of C. pepo var. melopepo, C. moschata, C. pepo var. giromonti, and those of C. mixta. The insignificant content of the main PUSFA (polyunsaturated FA) explains the small values of the content of linoleic acid compared with other groups. The analysis of variance of hull content made it possible to separate the accessions of C. moschata, C. pepo var. melopepo, C. moschata, C. pepo var. giromonti, and C. mixta. The classification function, which was significantly different for the contents of PUFA and SFA, helped to separate the accessions of C. moschata, C. pepo var. melopepo, C. moschata, C. pepo var. giromonti, and C. mixta. 

Figure 1. Cont.
The accessions of *C. pepo* stand out for the amount of the monounsaturated oleic acid, while the seed oil of *C. maxima*, *C. moschata*, *C. mixta* and *C. pepo* var. *melopepo* display its deficiency (Figure 1c). The analysis of variance of oil content showed no significant differences between the accessions of *C. moschata* and *C. pepo*, while *C. maxima* was significantly different in high oil values (Figure 1i). On the contrary, the results of hull content analysis made it possible to separate the accessions of *C. maxima* and *C. pepo* from *C. moschata* (Figure 1j).

The analysis of principal components showed that the first factor (palmitic and stearic acids content, total USFA, total SFA, FAI (54.6% of explained variability) separated *C. pepo* var. *melopepo* from other accessions. The second factor (oleic and linoleic FA content, 26.9%) helped to separate *C. maxima* and *C. pepo* accessions. *C. pepo* var. *giromontia* occupied an intermediate position between *C. maxima* and *C. pepo*, others (*C. moschata* and *C. mixta*) did not form distinct groups. Due to its low values, the content of linolenic acid did not have sufficient factor loading to affect the difference between various groups of accessions (Figure 2a, Table S5). Another set of factors (Figure 2b, Table S6) that includes the first factor (total SFA, total USFA, palmitic, stearic FA content and FAI; 41.9% of explained variability), the second factor (oleic and linoleic FA and hull content; 25.1%), and the third factor (oil and linolenic FA content; 12.6%) confirmed the difference between the accessions of *C. maxima*, *C. pepo* and *C. moschata*, which formed separate groups. The classification function, which took into account the influence of almost all the main parameters, except for the total SFA and USFA, made it possible to separate the accessions of *C. maxima* and *C. pepo* var. *giromontias* from the other studied ones with a 91% reliability, those of *C. pepo* with 70%, and those of *C. mixta* with 25% reliability. With this approach, *C. pepo* var. *melopepo* does not form a separate group.

**Figure 1.** ANOVA diagrams for parameters of the studied seeds of *Cucurbita* species from the VIR collection: content of (a) palmitic, (b) stearic, (c) oleic, (d) linoleic, (e) linolenic acids; (f) total SFA (saturated fatty acids); (g) total USFA (unsaturated fatty acids); (h) FAI (fatty acids index) in seed oil; (i) seed oil content; and (j) hull content.
The discriminant analysis model included the most significant parameters, that is, the content of oleic and stearic acids at \( p = 0.000 \), of oil \( (p = 0.004) \) and of hull \( (p = 0.028) \). As a result, of statistical processing of these data, the studied accessions of \( C. \) maxima, \( C. \) pepo, and \( C. \) moschata formed separate groups. Moreover, an analysis of coincidence of the assumed and real group affiliation of the studied accessions showed that the matches (or ‘correct decisions’) for \( C. \) maxima equaled 87.9\%, for \( C. \) pepo it was 76.5\%, for \( C. \) moschata about 75.0\%, and in totality, the number of correct decisions was 82.8\% on an average (Table S7). The canonical discriminant analysis of the obtained results made it possible to specify the differentiation of the studied \( Cucurbita \) accessions. Two ‘informatively valuable’ variables, the first Root and second Root, were identified (Figure 3, Tables S8 and S9). The first variable (Root 1) included values of oleic acid and oil content, for which strong and medium correlation coefficients were established \((-0.841, 0.539, \text{respectively})\) (Table S9). The second variable (Root 2) included the values of stearic acid and hull content with a correlation of medium strength \((0.739 \text{ and } 0.526, \text{respectively})\). The first canonical variable differentiated the accessions of \( C. \) pepo and \( C. \) maxima, while the second one separated \( C. \) maxima and \( C. \) pepo from \( C. \) moschata.

Figure 2. Factor analysis of hull, oil content and FA composition in the studied seeds of \( C. \) pepo, \( C. \) maxima, \( C. \) moshata, \( C. \) mixta, \( C. \) pepo var. giromontia and \( C. \) pepo var. melopepo (a) and \( C. \) pepo, \( C. \) maxima, \( C. \) moshata, (b) accessions from the VIR collection.

Figure 3. The discriminant analysis of hull, oil content and FA composition in seeds of \( C. \) pepo, \( C. \) maxima and \( C. \) moshata from the VIR collection.
4. Discussion

A comparison of the results from this study (Table 2) with those obtained earlier shows that the seed oil content in *C. pepo* from the VIR collection was slightly higher than that in *C. pepo* (45.1 and 41.5%, respectively) from Tabriz, a northwestern region of Iran [21]. The cucurbit seed oil in *C. pepo* from this region was characterized by a higher content of stearic and linolenic (8.67 and 0.68%, respectively), and by a lower content of linoleic FA (39.84%) in comparison with *C. pepo* accessions from VIR (Table 2). The values of palmitic and oleic FAs were almost the same (10.68 and 38.42%). A comparison of the palmitic and stearic FAs content (14.82 and 6.68%, respectively) in *C. pepo* seed oil from the central regions of Tunisia [12] and our data shows that the content of SFAs in the accessions grown in the Astrakhan Province was lower (Table 2). The content of polyunsaturated linoleic and linolenic FAs in *C. pepo* accessions (VIR) was also slightly lower compared to that in Tunisian accessions (50.9 and 0.2%, respectively). On the contrary, the content of oleic acid in *C. pepo* accessions (VIR) was higher (Table 2) compared with the 25.8% given by Bardaa et al. [12]. According to the results of Akin et al. [22], who studied *C. pepo* in central Anatolia (Turkey), the content of palmitic, stearic, linoleic and linolenic acids was higher (11.95; 5.28; 53.23; 0.41%, respectively), and of oleic acid lower (27.56%) than in similar accessions from VIR (Table 2). According to the studies of Tsaknis et al. [15], the oil of *C. pepo* seeds from the western part of Greece (Evros) was noted to have a higher content of palmitic and stearic acids (12.70 and 6.0%, respectively), a lower of linoleic (42.10%), and almost the same content of oleic and linolenic acids (38.10 and 0.2%, respectively), compared with *C. pepo* accessions from VIR. The FA profile obtained in this study for the accessions of *C. maxima* significantly differs from the data of Reziga et al. [3,14]. The seeds grown under the conditions of North Africa (Tunisia) had a lower content of linoleic (45.00%) and a higher content of palmitic, stearic and oleic acids (16.2; 8.42 and 29.52%, respectively). FAC of seed oil of *C. maxima* from the central region of Italy (Umbria) was characterized by a higher content of stearic, oleic and linolenic acids (5.8; 41.4 and 0.2%, respectively) and by a lower (37.0%) content of linoleic acid [23] compared with the results of the present study. The palmitic acid content obtained by Montesano et al. [23] and in this study practically coincided (14.2%). According to the data by Tsaknis et al. [15] on the accessions of *C. maxima* grown in Greece, the content of stearic and oleic acids was higher (5.70 and 37.20%, respectively), that of palmitic and linoleic acids lower (12.80; 42.80%), and that of linolenic almost the same (0.1%) compared with *C. maxima* accessions from VIR (Table 2).

A high (50% or more) content of linoleic acid in *C. pepo* seed oil demonstrates good wound healing activity [12]. According to our results, the content of this FA in *C. pepo* accessions was slightly lower (48.7), while the highest was found for *C. pepo* var. *melopepo* (64.1%). The content of linoleic acid exceeded 50% (Table 2) in the seed oil of other *Cucurbita* species, which suggests that the oil of the studied accessions can be used for treating wound surfaces both in pure form and as a component of complex wound healing medications [5,12,13].

Thus, the accessions of *C. pepo* and *C. maxima* had significant differences in seed oil FAC. The SFA values for the accessions from the VIR collection were mainly lower. Higher values of linoleic and lower of oleic acid were observed in *C. maxima* accessions from VIR. No stable trends with respect to USFA were observed in *C. pepo* accessions. In concordance with the data from this study, the content of oleic acid was either higher than that cited by other researchers, or the same, while the content of linoleic acid was mainly higher.

5. Conclusions

The performed study, followed by statistical analysis, made it possible to reveal differences between *Cucurbita* species from the VIR collection with respect to the main parameters, such as the content of oil, its FAC, and hull content, as well as to identify the informatively significant parameters that differentiate the accessions of various *Cucurbita* species (the content of hull, oleic and stearic acids). We failed to establish a reliable influence of weather factors on the composition of cucurbit seed oil. However, in the face of the global climate change, it is relevant to monitor the dynamics of changes in
the main economically important characters and biochemical parameters of the main crops, which include representatives of the Cucurbitaceae family. Using the accessions from the VIR collections as an example, we can evaluate changes in the nutritional value parameters of cucurbit seed oil.

Thus, the study of Cucurbita L. accessions from the VIR collections was promising for identifying sources combining valuable biochemical composition and productivity when grown in various ecological and geographical zones. These results will make it possible to use the potential of species and varieties of the genus Cucurbita L. to the maximum extent for various purposes, including functional, dietary nutrition and natural ingredients for medicines.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/1/1491/s1, Table S1: List of accessions of Cucurbita L. species from the VIR collection used as a research material; Table S2: The monthly average of temperature (°C) and precipitation (mm) for the AOS VIR in 2015-2016; Table S3: Fatty acid profile (% of total identified FA), oil and hull content (% of dry weight) in the studied Cucurbita L. accessions from VIR collection, mean values ± SD; Table S4: Results of ANOVA for parameters of studied Cucurbita L. accessions from the VIR collection; Table S5: Factor structure of variation for parameters of the studied C. pepo, C. maxima, C. moschata, C. mixta, C. pepo var. gironontia and C. pepo var. melopepo accessions from VIR collection (analysis of principal components); Table S6: Factor structure of variation for parameters of C. pepo, C. maxima, C. moschata accessions from VIR collection (analysis of principal components); Table S7: The discriminant analysis model. Classification matrix for C. maxima, C. pepo, C. moschata accessions; Table S8: Classification functions for studied C. maxima, C. pepo, C. moschata accessions; Table S9: Structure of canonical roots for C. maxima, C. pepo, C. moschata accessions.

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Abbreviation
AES—Astrakhan Experiment Station of VIR, FA—fatty acids, FAC—fatty acid composition, SFA—saturated fatty acids, USFA—unsaturated fatty acids, PUSFA—polyunsaturated fatty acids, FAI—fatty acids index.

References

1. Ryndin, A.Y. Physical Methods for Grain Quality Determination: Analysis of Sources. Bulletin NGIEI 2013, 12 (31). Available online: https://cyberleninka.ru/article/n/fizicheskie-metody-opredeleniya-kachestva-zerna-analiz-istochnikov (accessed on 18 February 2020). (In Russian)
2. FAOSTAT. Statistics Database; FAO: Rome, Italy, 2019; Available online: http://www.fao.org (accessed on 24 April 2020).
3. Rezig, L.; Chouaibi, M.; Ojeda-Amador, R.M.; Gómez-Alonso, S.; Salvador, M.D.; Fregapane, G.; Hamdi, S. Cucurbita maxima Pumpkin Seed Oil: From the chemical properties to the different extracting techniques. Not. Bot. Horti Agrobot. Cluj-Napoca 2018, 46, 663–669. [CrossRef]
4. FGBNU “Rosinformagrotekh”. State Register for Selection Achievements Admitted for Usage (National List). Vol. 1. “Plant Varieties” (Official Publication); FGBNU “Rosinformagrotekh”: Moscow, Russia, 2019; 516p. (In Russian)
5. Piskunova, T.M.; Mutyeva, Z.F. The VIR collection as a source of initial material for promising trends in squash and pumpkin breeding. Veg. Crops Russ. 2016, 3, 18–23. Available online: https://www.vegetables.su/jour/article/viewFile/280/267.pdf (accessed on 18 March 2020). (In Russian) [CrossRef]
6. Ayyildiz, H.F.; Topkafa, M.; Kara, H. Pumpkin (Cucurbita pepo L.) seed oil. In Fruit Oils: Chemistry and Functionality; Ramadan, M., Ed.; Springer: Cham, Switzerland, 2019. [CrossRef]
7. Piskunova, T.M. Squash Collection of VIR: Stages of Organization, Ways of Mobilization and Genetic Potential. Fruit Growing and Viticulture of South Russia 2015, 36(06). Available online: http://journal.kubansad.ru/pdf/15/06/10.pdf (accessed on 18 March 2020). (In Russian)

8. Yao, Y.; Liu, W.; Zhou, H.; Zhang, D.; Li, R.; Li, C.; Wang, S. The relations between minor components and antioxidant capacity of five fruits and vegetables seed oils in China. J. Olio Sci. 2019, 68, 625–635. [CrossRef] [PubMed]

9. Caili, F.; Huan, S.; Li, Q. A review on pharmacological activities and utilization technologies of pumpkin. Plant Foods Hum. Nutr. 2006, 61, 70–77. [CrossRef] [PubMed]

10. Nishimura, M.; Ohkawara, T.; Sato, H.; Takeda, H.; Nishihira, J. Pumpkin seed oil extracted from cucurbita maxima improves urinary disorder in human overactive bladder. J. Tradit. Complement. Med. 2014, 4, 72–74. [CrossRef] [PubMed]

11. Orsavová, J.; Mišurcová, L.; Ambrozova, J.V.; Vicha, J.; Mlcek, J. Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. Int. J. Mol. Sci. 2015, 16, 12871–12890. [CrossRef] [PubMed]

12. Bardaa, S.; Ben Halima, N.; Aloui, F.; Ben Mansour, R.; Jabeur, H.; Bouaziz, M.; Sahnoun, Z. Oil from pumpkin (Cucurbita pepo L.) seeds: Evaluation of its functional properties on wound healing in rats. Lipids Health Dis. 2016, 15, 73. [CrossRef] [PubMed]

13. Safar, A. The role of pumpkin seed oil in healing of wounds in mice. J. Garmian Univ. 2019, 6, 555–565. [CrossRef]

14. Rezig, L.; Chouaibi, M.; Msaada, K.; Hamdi, S. Chemical composition and profile characterisation of pumpkin (Cucurbita maxima) seed oil. Ind. Crops Prod. 2012, 37, 82–87. [CrossRef]

15. Tsaknis, J.; Lalas, S.; Lazos, E.S. Characterization of crude and purified pumpkin seed oil. Grasas y Aceites 1997, 48, 267–272. [CrossRef]

16. Fursa, T.B.; Malinin, M.I.; Artyugin, Z.D. Study and Maintenance of the Collection of Melons and Gourds; Guidelines: Leningrad, Russia, 1988; p. 32. (In Russian)

17. RIHMI-World Data Center. Available online: www.meteo.ru (accessed on 2 September 2020).

18. Ermakov, A.I. Methods of Biochemical Research of Plants, 3rd ed.; Agropromizdat: Leningrad, Russia, 1987; 430p. (In Russian)

19. Grigoryev, S.V.; Shelenga, T.V.; Illarionova, K.V. Hempseed and cottonseed oils in the accessions from the vir collection as sources of functional food ingredients. Proc. Appl. Bot. Genet. Breed. 2019, 180, 38–43. [CrossRef]

20. Naghshineh, M.; Ariffin, A.A.; Ghazali, H.M.; Mirhosseini, H.; Mohammad, A.S. Effect of saturated/unsaturated fatty acid ratio on physicochemical properties of palm olein–olive oil blend. J. Am. Oil Chem. Soc. 2009, 87, 255–262. [CrossRef]

21. Ardabili, A.G.; Farhoosh, R.; Khodaparast, M.H. Chemical composition and physicochemical properties of pumpkin seeds (Cucurbita pepo subsp. pepo var. Styriaca) grown in Iran. J. Agric. Sci. Technol. 2011, 13, 1053–1063.

22. Akin, G.; Arslan, F.N.; Elmasa, S.N.K.; Yilmaz, I. Cold-pressed pumpkin seed (Cucurbita pepo L.) oils from the central Anatolia region of Turkey: Characterization of phytosterols, squalene, tocols, phenolic acids, carotenoids and fatty acid bioactive compounds. Grasas y Aceites 2018, 69, 232. [CrossRef]

23. Montesano, D.; Blasi, F.; Simonetti, M.S.; Santini, A.; Cossignani, L. Chemical and nutritional characterization of seed oil from Cucurbita maxima L. (var. Berrettina) Pumpkin. Foods 2018, 7, 30. [CrossRef] [PubMed]