The Quality of Oils Produced From Local Hot Pepper Seeds

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Annotation. Alongside with the use of fresh cut hot pepper in daily consumption, there is also feasible obtaining various new products from the pepper in production areas. Moreover, pepper (Capsicum annuum L.) seeds can be used as additional raw materials. Because of research conducted in recent years, it has been established that the oils obtained from pepper seeds have the potential to become a source of protein due to their rich composition and nutritional value. According to the qualitative analysis (nutritional, chemical, antioxidant properties) of pepper seed oil, it has been established that it is a high-quality edible oil and fits perfectly as a component for use in the food industry and other industries, namely pharmaceutical, chemical, cosmetic industry. The literature review presented in this article revealed the high quality of oils extracted from the seeds of two peppers (Mumtoz and Tillarang peppers) grown in one region (Bukhara region, Uzbekistan), which can lead the pharmaceutical and cosmetic industry to develop new products based on economics.

1. Introduction

Vegetables that are part of the nutrition of the world's population, including diet food, are distinguished by their rich nutritional value. Pepper is a plant vegetable belonging to the Solanaceae family. Peppers that grown around the world vary in size, shape, color, and hotness. All peppers belong to the Capsicum species, and the most widely cultivated is Capsicum annuum L. Lately, more resources are being used to transform agricultural by-products into new, highly valuable products or ingredients [1]. Capsicum annuum is a native species originating from southern North America, spread to Central America and South America, and has a history of cultivation for more than 400 years [2]. There are different forms of the fetuses, which differ in size and taste, as well as names related to their etymology. E.g., in American English, non-hot varieties are called sweet peppers, while those high in capsaicin (which contributes to the peppery or hot taste) are called hot peppers or chili peppers. In British English, sweet varieties are called capsicum, hot varieties are called chili, while in Australian English, and Indian English, Capsicum is used mainly for bell pepper (block form) and for hotter varieties, i.e. chili [3]. Capsicum annuum L. is one of the most studied species in the Capsicum genus [4]. Asian producers account for 70% of the world production of Capsicum annuum L. (pepper, chili pepper) [5]. In terms of annual production in 2021, as shown in Figure 1, the world's top three producers of various peppers are India, Thailand, and China. Therefore, it is very important to make effective use of the large amount of pepper processing waste.

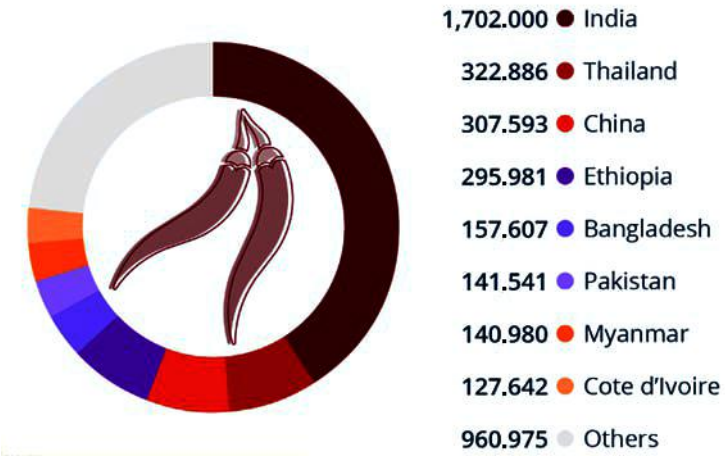

Fig.1.Top producers of chilies and pepper (green) for 2021

In accordance to the literature data, fresh pepper is a source of vitamin C, provitamin A, as well as carotenoids, phenolic acids and flavonoids. These nutrients are beneficial to human health as they have been shown to protect against certain cancers, prevent ulcers, boost the immune system, prevent cardiovascular disease, and protect against age-related diseases and cataracts [6, 7].

Particularly, red hot pepper has a high antioxidant potential due to its high total phenolic and flavonoid content [8]. Pepper has long been used in the food industry according to its culinary and industrial uses: fresh or cooked (salads), processed (dehydrated products, pickled products, spices, soups, for flavoring and coloring). The data in Fig. 2 shows that pepper will be among the top eight vegetables in terms of annual global production in 2021.

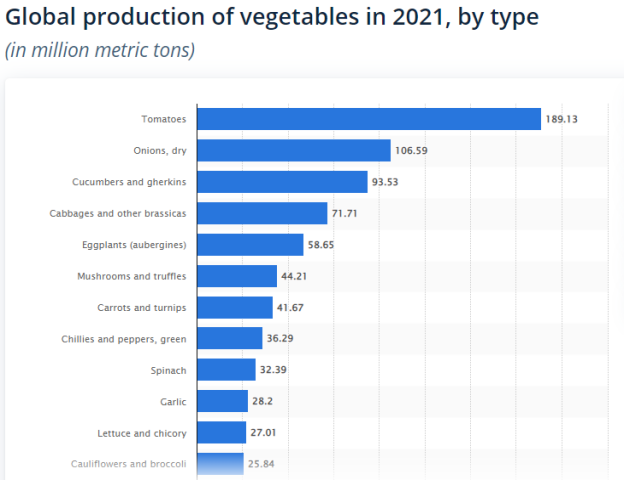

Fig. 2. Global production of vegetables (in million metric tonnes) for 2021 [5].

In a recently published article [9], many biologically active compounds and pigments in red pepper seed oil were evaluated as having strong antioxidant activity. The purpose of this review is to reveal literature data on the quality of two pepper seed oils grown in one region (Bukhara, Uzbekistan). “Pepper seed oil” is a product obtained from pepper seeds by various extraction methods.

Results of presented samples

№ 1 – “Mumtoz” pepper

№ 2 – “Tillarang” pepper

Determination of humidity and volatile substances [10]. The seeds were manually cleaned from the down, crushed, thoroughly mixed and scattered in a thin layer on the board. About 5 g of raw material was taken from different places of the mixed mass for each determination. The analysis was carried out in double replication. The crushed sample was transferred into preliminarily dried and weighed bottles and, having closed the lids, was weighed on an analytical balance. Samples were dried in an oven at 100-105°C, first for 2 hours and then for 30 minutes to constant weight.

Constant weight was considered achieved when the difference between weighing did not exceed 0.001 g.

The moisture content of raw materials in % (X) was calculated by the formula:

$$X = \frac{(P_1 - P_2).100}{P}$$

where: P_1 is the weight of bottle with sample before drying, in g

P_2 is the weight of the bottle with the sample after drying, in g;

P is weighed sample, in g

Determination of oil content of seeds. Determination of the oil content of seeds was carried out by the standard method of exhaustive extraction with gasoline according to option 2 [11].

The seeds were dried in an oven at a temperature of 105°C for 2 hours, ground in a coffee grinder, the grinding was thoroughly mixed with a spatula, and two weighed portions of 10 g each were taken from the mixed mass on an analytical balance.

Extraction cartridges from filter paper were weighed on an analytical balance. A portion of crushed seeds was placed in extraction cartridges, covered with a small layer of cotton wool, and the edges of the cartridges were wrapped and placed in the extractor of the Soxhlet apparatus. Clean flasks were attached to the extractor, which were preliminarily dried for an hour at 100-105°C, weighed, placed in a desiccator and kept in it until cooling. Through a water cooler, using a small flask, the required amount of pre-distilled extraction gasoline (boiling point 72-76°C) was poured into the extractor.

Oil extraction was carried out for 20-22 hours. A test for the completeness of extraction was carried out after 12 h by checking a drop of the extract on filter paper for the absence of a greasy spot after the extract had dried on it. After complete extraction of the oil, the receiving flasks with the oil extract were disconnected from the Soxhlet apparatus, and the gasoline was distilled off on a rotary evaporator. The remaining gasoline was removed by drying the oil in an oven at a temperature of 100-105°C to constant weight. The first weighing was carried out after 1 hour of drying, the next - every 30 minutes. Drying was considered complete if the difference between the last two weighing was 0.0002–0.0004 g.

Oil content in seeds in % (X) was calculated by the formula:

$$X = \frac{(P_1 - P_2).100}{P}$$

where: P_1 is the weight of the flask with oil, in g,
P_2 is the weight of the empty flask, in g,
P is sample of seeds, in g

To convert the oil content of seeds to dry matter, the following formula was used

$$X_2 = \frac{X_1 * 100}{100 - H}$$

where: X_1 is seed oil content per wet substance;

X_2 is oil content of seeds on dry matter;

H is moisture content of seeds.

Determination of the content of carotenoids. The analysis was carried out by the spectrophotometric method on a Cary-60 spectrophotometer (Germany). Preparation of a potassium dichromate standard sample solution was the following. A portion of 0.090 g of potassium dichromate ($K_2Cr_2O_7$, GOST 4220-75) was dissolved in distilled water in a volumetric flask with a capacity of 250 m^3, the volume of the solution was brought up to the mark with distilled water. One ml of this solution corresponds in color to the content of 0.00208 mg of β-carotene. A weighed portion of 0.5 g of oil (accurate to 0.0001 g) was dissolved in a small amount of hexane in a 25 ml volumetric flask, and the volume of the solvent was adjusted to the mark. The optical density (D) of this solution was measured on a spectrophotometer at a wavelength of λ 440 nm (cell thickness 10 mm). The content of the total carotenoids (X, mg%) in terms of β-carotene was calculated by the formula:

$$X = \frac{0{,}00208 \cdot D_1 \cdot 25 \cdot 100}{a \cdot D_0}$$

where: 0.00208 is the amount of β-carotene corresponding to the color of 1 ml of a standard solution of a potassium bichromate sample.

D_0 is the optical density of the standard sample solution;

D_1 is the optical density of the test solution;

25 is dilution amount, cm^3;

a is hinge, g.

The obtained indicators are summarized in Table 1.

Table 1.

Chemical characteristics of two samples of hot pepper seeds

Indicator	**Content**	
	No. 1	**No. 2**
Moisture and volatile substances, % of seed weight Carotenoids in oil, mg%	8.21	8.10
Oil content, taking into account moisture content, %	16.44	14.20
Oil content on dry matter, %	17.91	15.45
Carotenoids in oil, mg%	14.20	8.65

Determination of the composition of fatty acids. The analysis was carried out by gas chromatography (GC). Fatty acids (FA) were isolated from the oil by the recommended method [12-17]. About 5 g of oil was weighed into the flask, 30 ml of methanol and 5 ml of a 50% aqueous solution of KOH were added to the oil, an air condenser was attached, and the contents were boiled for 1 h until complete saponification. After cooling, the contents of the flask were quantitatively transferred to a separating flask, where 20 ml of warm distilled water was first poured, then 5 ml of ethanol was added, and another 20 ml of cold distilled water was added. The reaction flask was rinsed with 50 ml of extraction gasoline (boiling point 72-76°C), which was poured into a separating flask after its contents had cooled to room temperature. The separating flask was closed with a stopper, shaken for 1 min (periodically removing gasoline vapors through the tap) and left to stand until the liquid was separated in the flask. The soap solution was poured into the flask, and the extract of unsaponifiable substances was transferred to another separating flask. The extraction of the soap solution with gasoline was repeated six more times, using 50 ml of gasoline each time.

After the removal of unsaponifiable substances, a drop of methyl orange and portions of 50% sulfuric acid were added to the soap solution in a separating funnel to decompose the soap until a pink color of the solution appeared. The released FAs were extracted by extraction with diethyl ether (3 times, 10–15 ml each). The ethereal extracts were combined, washed with distilled water (3-5 times, 10-15

ml) until the washings were neutral with methyl orange. The absence of a pink color in the wash water indicated the complete removal of sulfuric acid from the solution. The ethereal solution of fatty acids was dried over anhydrous sodium sulfate, transferred to a dry round-bottomed flask, and evaporated on a rotary evaporator in a weak vacuum of a water jet pump. The isolated FAs were converted into methyl esters (ME) by treatment with an ethereal solution of diazomethane.

The obtained FA methyl esters (FAMEs) were purified by preparative thin layer chromatography (PTLC) on plates with a fixed layer of silica gel in the hexane: ether solvent system (8:2). The FAME zone on the sorbent was developed in J_2 vapor, the zone was cleaned from the plate, and desorbed from silica gel by repeated elution with chloroform. The chloroform eluates were combined, evaporated on a rotary evaporator, the residue was dissolved in hexane and analyzed on a gas chromatograph.

The analysis was carried out on an Agilent 8860 GC gas chromatograph with a flame-ionization detector, using a Supelco 100m x 0.25mm capillary column with SP^{tm} -2560 phase, helium carrier gas, column-programming temperature from 140°C to 250°C. FAs were identified by comparing the retention times of the peaks with those of the standard sample of a mixture of 37 fatty acid methyl esters (Supelco® 37 component FAME mix, Sigma-Aldrich, USA). The results obtained are given in Table 2.

Table 2. Fatty acid composition of 2 hot pepper seed samples, GC, % of total fatty acids

Fatty acid	Content	
	№1	**№2**
Myristic, 14:0	0.11	0.12
Palmitic, 16:0	10.87	11.77
Palmitoleic, 16:1	0.20	0.16
Stearic, 8:0	2.58	2.93
Oleic, 18:1 ω9	8.46	8.94
Linoleic, 18:2 ω6	76.63	75.18
Linolenic, 18:3 ω3	0.08	0.03
Arachic, 20:0	0.33	0.38
Eicosenic, 20:1 ω11	0.24	Trays
Behenic, 22:0	0.25	0.26
Lignoceric, 24:0	0.25	0.23
$\sum_{\text{saturated FA}}$	14.39	15.69
$\sum_{\text{unsaturated FA}}$	85.61	84.31

Essential oils (EOs) from seeds were obtained by hydrodistillation from air-dry raw materials for 3 h using a glass flask and a Clevenger nozzle. The obtained EOs are a pale yellow mobile liquid with a specific odor, which was stored at 4°C in sealed ampoules before analysis. Essential oils were analyzed on an Agilent 5975C inert MSD/7890A GC chromato-mass spectrometer. Separation of the components was carried out on an Agilent HP-INNOWax column (30m×250μm×0.25μm) in the temperature regime: 60°C (2 min) – 4°C/min to 220°C (10 min) – 1°C/ min to 240°C (10 min). The volume of the introduced sample was 0.2 μl, and the flow rate of the mobile phase (H_2) was 1.1 ml/min. The components were identified based on the comparison of the characteristics of the mass spectra with the data of electronic libraries by chromato-mass spectrum. The results for the essential oil composition of the two samples are shown in Table 3.

Table 3. The composition of the etheric oil in Tillarang and Mumtoz pepper seeds obtained by hydrodistillation

№	Compounds	RI*	RT**	% Tillarang	% Mumtoz
1	*trans*-2-Heptenal	1290	5.342	0.38	-
2	1-Pentadecene	2174	27.808	-	2.42
3	Tricosane	2286	30.266	0.77	-
4	Cyclohexadecane	2323	31.036	-	0.43
5	*trans*-2-Hexenal	2324	31.049	0.37	-
6	Tetracosane	2385	32.336	1.19	-
7	1-Heptadecene	2425	33.145	0.76	-
8	Vanillin	2450	33.662	-	0.29
9	7-Tetradecene	2474	34.218	-	0.40
10	Cyclotetradecane	2479	34.225	0.52	0.45
11	Pentacosane	2484	34.341	1.29	-
12	1-Octadecene	2527	35.189	4.84	4.38
13	Cyclopentadecane	2581	36.230	1.98	1.04
14	Myristic acid	2615	36.877	1.81	1.13
15	1-Heptadecene	2628	37.136	-	0.43
16	Pentadecanoic acid	2670	37.912	3.17	3.30
17	Tetradecyl trichloroacetate	2685	38.203	-	0.92
18	Cycloeicosane	2732	39.063	0.54	0.46
19	Cyclotridecane	2785	40.021	0.66	-
20	Palmitic acid	2825	40.739	40.64	31.22
21	1-Tridecene	2840	41.011	1.00	-
22	(-)-Isopulegol	2863	41.418	-	0.22
23	Palmitelaidic acid	2901	42.084	-	0.48
24	Margaric acid	2924	42.544	-	1.17
25	Cyclododecane	2955	43.158	-	2.46
26	Stearic acid	3029	44.749	3.10	2.52
27	Oleic acid	3052	45.306	3.37	3.20
28	Elaidic acid	3058	45.454	1.53	4.11
29	Linoleic acid	3104	46.599	10.67	16.89
Σ				**78.59**	**77.92**

The results of the analysis showed that the composition of the etheric oil of Tillarang pepper seeds consists of 19 components, among them palmitic acid (40.64%), linoleic acid (10.67%), 1-octadecene (4.84%), oleic acid (3.37%), pentadecanoic acid (3.17%), stearic acid (3.10%), myristinic acid (1.81%), cyclopentadecane (1.98%), elaidic acid (1.53%), pentacosan (1.29%), tetracosan (1.19%).), 1-tridecene (1.00%) and other substances were found in relatively large quantities. The general number of substances listed was 74.59% of the total amount, and 4.00% of the total amount was less than 1% of the amount.

The composition of the etheric oil of Mumtoz pepper seeds consists of 21 components, among them palmitic acid (31.22%), linoleic acid (16.89%), 1-octadecene (4.38%), elaidic acid (4.11%), pentadecanoic acid (3.30%), oleic acid (3.20%), stearic acid (2.52%), cyclododecane (2.46%), 1-pentadecene (2.42%), margaric acid (1.17%), myristinic acid (1.13%), cyclopentadecane (substances such as 1.04% were found to be found in relatively large amounts). The general number of substances listed was 73.84% of the total amount, and 4.08% of the total amount was less than 1% of the substances.

Conclusion

Reviewing the data on the composition of the oils extracted from the seeds of Mumtaz and Tillarang peppers in this article allowed comparing their differences. Oil content, carotenoid content, fatty acid composition and content, etheric oil composition and content in pepper seed oils (grown in the Bukhara region of the Republic of Uzbekistan) differed significantly between Mumtoz and Tillarang varieties, but the content of moisture and volatile substances and there was almost no difference. Thus, pepper seed oil can be considered as a very valuable product and ingredient in the development of new and sustainable products. Application of pepper seed oil in food products has promising potential in gastronomy worldwide due to its well-received sensory attributes. Nevertheless, its use may also depend on the social background of consumers.

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