INVESTIGATION OF A CO_2 EXTRACT OF THE FRUIT OF Capsicum annuum

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Capsicum annuum L., family Solanaceae originates from Mexico and Guatemala. In the USSR it is cultivated as an annual plant mainly in the Ukraine, the Caucasus, the Crimea, and Central Asia [1]. The sharp and burning taste of the fruit of this red pepper is due to the presence in it of a special alkaloid – capsaicine.

In existing methods for the isolation of extracts (oleoresin) from the fruit of C. annuum, ethanol, acetone, diethyl ether, and other solvents are used [2, 3]. We have performed an investigation after the extraction of the fruit of the red pepper with liquid carbon dioxide, the advantages of which include the relatively low boiling point (thanks to which the possibility of the thermal degradation of the biologically active substances is excluded), the absence of the necessity for using a special heat carrier for eliminating traces of solvent, and nonpolarity, which provides the possibility of the extraction of the essential oils, alkaloids, and other components not volatile with steam.

The alkaloid capsaicine present in red pepper stimulates the mucous membrane. This action of capsaicine is intensified by its capacity for subliming at relatively low temperatures. Because of this, an industrial production of the extract from red pepper which involves grinding is difficult.

We have developed a method for isolating extract of red pepper permitting these defects to be eliminated [4].

EXPERIMENTAL

A CO_2 extract of red pepper was obtained on a pilot batch extraction unit. The fruit was first extracted in the whole state. The extract obtained contained capsaicine (3.75-6.0%), essential oil (2.6-3.9%), and carotenoids (11.6-36.7%). Then the pepper was ground and was re-extracted. The extract contained 20.0-37.9% of fatty oil, 0.8-2.0% of capsaicine, 0.5-4.2% of one volatile component of the essential oil, and 0.2-0.7% of carotenoids. The influence of the temperature on the efficiency of extraction of capsaicine from red pepper was studied. Extraction was performed with liquid carbon dioxide at 21°C ($P = 59.9$ kg/cm^2) and 28°C ($P = 70.2$ kg/cm^2). The temperature was stabilized by means of a thermostat.

The comminuted raw material was passed through smooth rolls. The thickness of the flake was 0.16 mm and its moisture content 7%. Figure 1 shows the dependence of the amount of residual capsaicine on the duration of the extraction process at various temperatures. Curve 1 corresponds to the temperature of 21°C ($P = 70.2$ kg/cm^2).

It follows from Figs. 1 and 2 that the capsaicine is extracted more intensively at 21°C, i.e., at a temperature more remote from the critical parameters of the extractant ($t = 31$°C, $P = 74.96$ kg/cm^2). The amounts of capsaicine and the fractions were found after predetermined intervals of time by the spectrophotometric method, and the initial amount of capsaicine in the raw material by exhaustive extraction of the spectrometry of the extracts.

The physicochemical and organoleptic indices of the extracts – the density, the refractive index, the amount of essential oil, and the solubility in ethanol – were determined by the appropriate methods of

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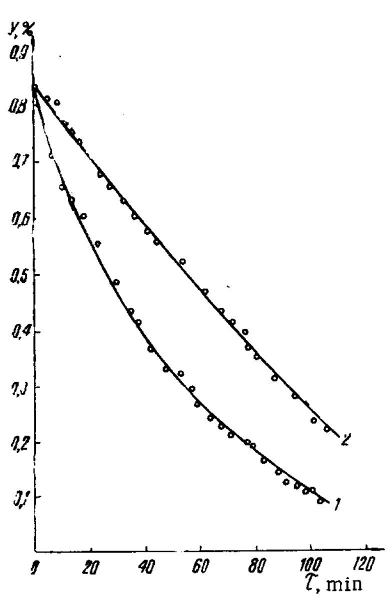

Fig. 1

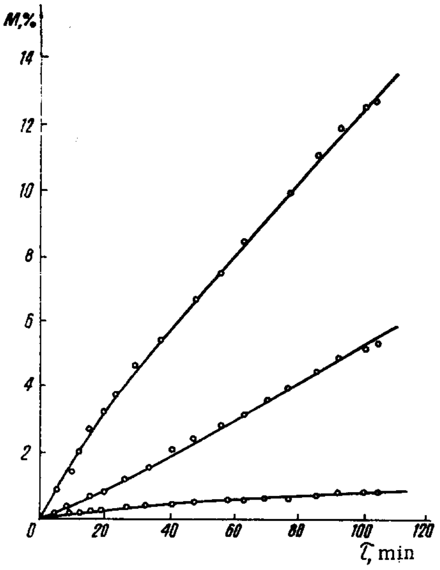

Fig. 2

Fig. 1. Dependence of the residual amount of capsaicine on the time of the extraction process at different temperatures (y is the residual amount of capsaicine, %, and τ is the time of extraction, min); 1) at t = 21°C; 2) at t = 28°C.

Fig. 2. Dependence of the extraction of extractive substances (I), fatty oil (2), and capsaicine (3) from the fruit of the red pepper on the time of extraction (t = 21°C, P = 59.9 kg/cm^2); M is the yield of extractive substances, % on the absolutely dry material of the sample, and τ is the time of extraction, min.

GOST [All-Union State Standard] 14618, 2–69–GOST 14618, 12–69 [5], the acid no. by the method for dark oils [6], the viscosity by Höppler's method, and the capsaicine content by the method described below.

Isolation of Capsaicine. A solution of 50 g of the extract in 100 ml of petroleum ether was extracted with 80% aqueous ethanol (3 × 80 ml). The methanolic extracts were washed with petroleum ether (4 × 50 ml) and treated with diethyl ether. The ethereal fractions were dried with Na_2SO_4, and the residue after the evaporation of the ether consisted of crystals of crude capsaicine, which were purified by means of a column of alumina (activity grade II). Impurities were eliminated by the passage of the solvent system isopropanol–petroleum ether (bp up to 40°C)–water (20 : 45 : 5) (3 × 50 ml), and pure capsaicine was eluted with 20 ml of ethanol–methanol (9 : 1) until the eluates gave a negative reaction with diazobenzenesulfonic acid in 8% Na_2CO_3 solution.

The pure capsaicine obtained had mp 65.5–66.0°C. Its purity was determined on an SF-4 A spectrophotometer in the ultraviolet region. The UV spectrum of capsaicine shows maxima at 230, 260, and 280 nm.

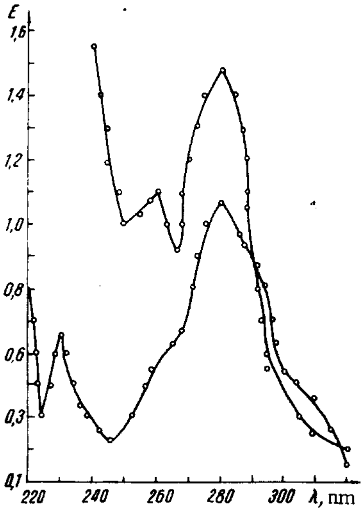

Fig. 3. Spectral characteristics of capsaicine isomers (1, 2) in ethanolic solution.

Figure 3 shows the spectral characteristics in the UV region of eluates of capsaicine on a column obtained with various solvents. It can be seen from the graph that capsaicine consists of isomers which are responsible for the burning taste of the fruit, as has been shown by a number of authors [7].

Capsaicine gives a characteristic green color with NH_4VO_3 in acetone in the presence of HCl (sp. gr. 1.19). We have used this reaction to develop a quantitative determination of capsaicine by a colorimetric method. A sample of the CO_2 extract weighing about 0.1–0.2 g was dissolved in 20 ml of acetone with the addition of 10 ml of deodorized kerosene and 10 ml of 5% aqueous NaCl solution, and the mixture

TABLE 1. Characteristics of the CO_2 Extracts

Index	Extract from fruit	Extract from hull	Essential oil from the fruit
Density at 20°C, g/cm^3	0,9428–0,9442	0,9232–0,9258	0,8263—0,8280
Refractive index at 20°C	1,4745–0,4752	0,4809–1,4819	1,4845—1,4852
Solubility in 96% ethanol	Partial	Partial	1 : 1
Acid No., mg KOH	48.4	11,2	8,0
Ester No., mg KOH	74,9	32,6	10,6
Capsaicine content, %	3,5–4,2	4,5–5,5	2,6
Hoppler viscosity at 20°C, cP	39,24	18,42	—

TABLE 2. Fatty-Acid Composition of the Extracts

Acid	CO_2 extract of the fruit of the red pepper		CO_2 extract from the seeds of the fruit		Diethyl ester extract from seeds of the fruit		Hexane extract from the seeds of the fruit	
	acid	amount, %	acid	amount, %	acid	amount, %	acid	amount, %
Unidentified	—	1,46	—	—	—	—	—	—
Lauric	$C_{12}:0$	1,09	—	—	—	—	—	—
Myristic	$C_{14}:0$	4,02	$C_{14}:0$	0,24	$C_{14}:0$	0,34	$C_{14}:0$	0,22
Palmitoleic	$C_{16}:1$	1,46	$C_{16}:1$	0,32	$C_{16}:1$	0,13	$C_{16}:1$	0,11
Palmitec	$C_{16}:0$	26,44	$C_{16}:0$	18,87	$C_{16}:0$	14,11	$C_{16}:0$	16,36
Stearic	$C_{18}:0$	0,73	$C_{18}:0$	1,41	$C_{18}:0$	2,34	$C_{18}:0$	1,53
Unidentified	—	1,09	—	—	—	—	—	—
Oleic	$C_{18}:1$	18,49	$C_{18}:1$	10,16	$C_{18}:1$	9,64	$C_{13}:1$	9,27
Linoleic	$C_{18}:2$	32,49	$C_{18}:2$	69,00	$C_{18}:2$	73,55	$C_{13}:2$	72,51
Linolenic	$C_{18}:3$	12,72	—	—	—	—	—	—

was shaken for 40 min. The residue after the evaporation of the aqueous layer was extracted with 15 ml of acetone. To the extract was added 0.5 ml of a 1% solution of ammonium vanadate in acetone solution, and the mixture was shaken for 20 sec and photometered with a red filter on an FÉKM-56 photoelectric colorimeter. For analyzing capsaicine in the initial material, 2 g of ground pepper was weighed out and extracted with 20 ml of acetone, and then the amount of capsaicine was determined by the method described above.

The amount of capsaicine was also determined from the optical density of solutions using a calibration curve. The calibration curve was plotted with known amounts of capsaicine. The Lambert–Beer law is observed at concentrations of capsaicine in the sample ranging from 0.05-0.2 mg/ml. By this method we found the amount of capsaicine in the CO_2 extract to be 3.5-4.2% and in the initial raw material, 0.6-0.8%.

The amount of capsaicine in the extract was calculated from the formula

$$X = \frac{a \cdot 100}{p}, \ \%,$$

where a is the amount of capsaicine determined from the optical density with a calibration curve, mg; and p is the weight of the extract, g.

The composition of red-pepper fruit and of extracts from it obtained by means of organic solvents have been little studied. We have developed a procedure for investigating their composition. A sample of CO_2 extract (1.5-2.0 g) was treated with 5 N aqueous HCl to precipitate basic compounds in the form of the hydrochloride. After this, the solution was brought to pH 7-8 with 5% NaOH, and the basic compounds were extracted with diethyl ether [8].

Then the free organic acids were removed with 2% aqueous sodium bicarbonate solution. The phenols were extracted with 5% aqueous NaOH solution, and the alkaline solution was acidified with 3 N HCl and extracted with ether. To detect phenols we used the color reaction of coupling with a diazotized sulfanilic acid with the formation of an azo dye. Phenols gave a coloration ranging from red-yellow to red-brown [9]. Carbonyl compounds were determined after the elimination of the basic compounds, acids, and phenols. The residue from the ethereal solution of the extract was passed through Amberlite IRA-400 resin in the bisulfite form (HSO_3^-) to isolate the carbonyl compounds. The latter were eluted from the resin with 1 N NaCl. The bisulfite derivatives were decomposed with sodium bicarbonate. After extraction with petroleum ether, the carbonyl compounds were concentrated under vacuum. The presence of carbonyl compounds was established from the formation of precipitates with a saturated solution of 2,4-dinitrophenylhydrazine in 2 N HCl.

The alcohols were isolated in the form of the phthalates with phthalic anhydride by a known method [9]. The residual neutral fraction of the extract was treated in the n-hexane—90% methanol system cooled to 5-10°C. The red-orange color of the neutral fraction of the red-pepper extract is due to carotenoid pigments. On treatment with this system of solvents, before complete decoloration the last portions of methanol extracted yellow xanthophylls, which belong to an inactive form of the carotenoids. A hexane fraction contained carotenoids in the biologically active form (α- and β-carotenes). The chemical composition of CO_2 extract of red pepper was determined by a method that we have developed:

Main Components	Content, %*	Main Components	Content, %*
Carbonyl compounds, including	3.54	Alcohols, including	7.24
benzaldehyde	1.2	linalool	4.6
hexanal	1.1	Bound fatty acids of triglycerides	41.4
furfural	0.8	Carotenoids, including	20.2
Basic compounds	0.76	α-carotene	1.2
Free organic acids	4.2	β-carotene	5.6
Phenolic compounds, including	8.06	xanthophylls	10.68
capsaicine	4.2	Water-soluble compounds	2.08
Hydrocarbons, including	11.5	Other compounds	1.02
limonene	5.3		
ocimene	4.2		

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* The figures given are the averages of three experimental determinations.

The fatty-acid composition of the extract of the fruit of the red paper was studied by the GLC method. The acids were chromatographed in the form of the methyl esters on a gas chromatograph. The esters were separated under the following conditions: "Tsvet" chromatograph IV, column 2 m, temperature 180-185°C, solid support Chromosorb 800-100 mesh, stationary phase 15% of poly(ethylene succinate); flame-ionization detector. The relative retention volumes of pure markers were used for identification. Results obtained are given in Table 2.

SUMMARY

The composition of a CO_2 extract of the fruit of the pepper Capsicum annuum L. with respect to the main classes of compounds has been studied. A method has been developed for the extraction and quantitative determination of capsaicine.

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