Peculiarities of the lipid composition of sunflower wax

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Abstract

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Introduction. The purpose of this study is to determine the peculiarities of the lipid composition of sunflower wax separated from crude and refined oil.

Materials and methods. Waxes were separated from triacylglycerols by silica gel column chromatography. Wax compositions were determined by gas-liquid chromatography with flame ionization detection. The column was thermostatted in a given mode at temperatures from 170 to 380 °C.

Results and discussion. The article presents experimental data for determining the composition of waxes in crude and refined sunflower oil. Gas-liquid chromatography conditions described in the study methodology expanded the range of detection of long-chain waxes. New data have been obtained about sunflower oils consisting of waxes containing 50 or more carbon atoms.

Waxes with hydrocarbon chain lengths of 44-56 carbon atoms (C44 to C56) have been identified in crude sunflower oil. In crude sunflower oil, waxes with chain lengths ranging from 46 to 52 carbon atoms (C46, C48, C50, and C52) account for up to 61% of the total waxes. Identified waxes with even numbers of carbon atoms accounted for 82% of the total waxes. Crude sunflower oil was found to have the highest content of wax with 48 carbon atoms (C48) of chain length.

Refined sunflower oil showed a decrease in the content of wax compounds by an order of magnitude. The refining process removed waxes with shorter C44 and C46 hydrocarbon chains. Refined sunflower oil contains trace amounts of waxes with chain lengths ranging from 48 to 54 carbon atoms (C48, C49, C50, C51, C52, C53, and C54). Moreover, the predominance of chains with even numbers of carbon atoms over those with odd numbers of carbon atoms in refined sunflower oil is emphasized. Refined sunflower oil is dominated by waxes with chain lengths of 50, 52 and 54 carbon atoms (C50, C52, and C54).

The acid and iodine values, the saponification number and the wax melting point have been analyzed, which are in line with an identified composition of sunflower wax.

Conclusion. The dependence of wax content on the chain lengths with even and odd numbers of carbon atoms both in crude and refined sunflower oil is parabolic.

Introduction

Given the ever-increasing production and consumption of sunflower oil year after year, studies of oil-related substances, such as waxes, and their composition is a matter of current interest (Oseyko et al., 2020; Oseyko et al., 2021). The applications of the separated wax are determined by its composition and properties. Sunflower wax is a natural thickener, structurant, and the wax derivatives can be used as emulsifiers, surface-active compounds, components of protective coatings of food and drugs, etc.

Wax is a natural product of a plant or animal origin and consists of compounds of different compositions, mainly from esters derived from higher carboxylic acids and higher alcohols (Oseyko¹ et al., 2019; Oseyko¹ et al., 2021). Wax is insoluble in water but readily soluble in organic solvents and hot alcohol. Substances whose chemical properties are close to the ones of waxes, but do not contain esters, are called waxy analogues or waxy compounds, such as paraffin. The composition of wax depends on the source and method of production (Romanovska, 2006). Beeswax is distinguished by the following extraction methods: melting, mechanical pressing, and chemical extraction. Soy wax separated from soybean oil during refining is promising (Oseyko, 2006).

Currently, there is growing interest in the lipid and blend composition of plant materials, namely the composition, quality, functional and health-improving and pharmacological properties of food products, additives and drugs (Oseyko et al., 2017; Oseyko et al., 2019; Farinon et al., 2020). Awareness of the properties of multicomponent lipid products of natural origin, in particular, waxes, substantiates their applications. The versatility of methods for obtaining waxes and controlling the composition and physicochemical parameters of lipid products gives an impetus to the search for optimal methods for obtaining waxes and the study of their physicochemical parameters.

Among other sources of wax are lipid-containing materials, such as used filter powder after filtering frozen oil (Omelchenko et al., 2016), tank sediments self-precipitated during oil storage, and sunflower husks (Kleiman et al., 1969). Sunflower wax extraction methods are labor-intensive, resource-intensive, and time-consuming. These factors explain the currently insufficient information on the composition of sunflower wax lipids, and at the same time, its limited use in various fields of human activity. A review of the literature revealed an understudied composition of sunflower wax.

The purpose of this study is to determine the peculiarities of the lipid composition of sunflower wax separated from crude and refined oil.

Materials and methods

The study materials are crude sunflower oil and sunflower oil refined by classical refining process (Oseyko, 2006) (neutralization, freezing, bleaching, deodorization), as well as sunflower wax obtained by freezing and filtering from the used perlite powder. Samples of oils and used filter powder were obtained from the Kyiv Margarine Factory “Olkom”.

Methodology for the preparation of test samples

Before the chromatographic analysis of waxes, oil samples were passed through a silica gel column to purify the wax from related substances (Patent UA114235C2, Sposib khromatohrafichnoho vyznachennia voskopodibnykh rechovyn, M.I. Oseiko, I.V. Levchuk, V.A. Kishchenko, T.I. Romanovska, 2017).

The wax was eluted with a mixture of n-hexane/chloroform in a ratio of 7:3 (Patent UA114235C2, 2017).

The resulting eluate was boiled down to dry residue under vacuum at 60 °C and dissolved in 1 cm³ of chloroform (Patent UA114235C2, 2017).

2.0 x 10⁻³ cm³ (2 µl) of the prepared wax sample was added to the GC column (Gordon, 1990).

Study methodology

Wax composition analysis

Wax compositions were determined by gas-liquid chromatography with flame ionization detection (Gordon, 1990). Wax content of oils is determined by gas-liquid chromatography at the capillary column's maximum thermostating temperature of 320 – 380 °C (Kishchenko, 2014; Levchuk et al., 2016).

Wax separation

Wax separation was carried out in a silica column with a diameter of 0.32 mm and a length of 15 m filled with a chemically inert liquid stationary phase (5% diphenyl 95% dimethyl polysiloxane). The stationary phase does not volatilize at column operating temperatures, has a low viscosity, and forms a continuous, uniform film. Nitrogen flowing at a rate of 20 cm³ per minute served as the mobile phase that moved the wax sample through the column. The samples were passed through a GC column at temperatures starting from 170 °C and holding time of 1 minute with a heating rate of 6 °C per minute up to 380 °C and holding time of 20 minutes. The evaporator temperature was maintained at about 370 °C and that of the detector at 400 °C. The flame ionization detector was operated at the hydrogen flow rate of 30 cm³/min and air flow rate of 350 cm³/min. (Gordon, 1990).

Processing of results

The composition and content of waxes in the test samples were determined with three repeated measurements using the Agilent Technologies Chemstation software (Gordon, 1990; <https://www.agilent.com>).

Removal of frozen sunflower wax

The wax separated during freezing with an auxiliary filter material (filter perlite powder) was heated in a wax melter with a woven sieve in a cabinet dryer at a temperature of 80 °C within 3 hours until the wax was completely melted. The resulting filtered wax was crystallized at room temperature and analyzed.

Determination of Physicochemical Parameters of Sunflower Wax

For sunflower wax extracted by freezing, free fatty acid content (acid value), hydrolysable ester content (saponification value), unsaturated hydrocarbon chain content (iodine value) were determined by conventional titrimetric methods, and the wax melting point was determined using an open capillary tube (Mank et al., 2018).

Results and discussion

Lipid composition of sunflower wax

The study of the composition of waxes has some difficulties associated with the breaking of the separated multicomponent wax fraction into constituent elements in a thin layer of a capillary column and their detection using a detector. The composition of waxes is determined with a flame ionization detector and tracking substances (separated and purified substances) or mass spectrometry with a subsequent search in a mass spectral database of the compounds that may be present in test samples (Gordon, 1990). According to their properties, waxes belong to substances with a melting point of up to 80 °C (Gunstone, 2002). During the introduction of the sample, the temperature of the evaporator is maintained at about 370 °C and that of the flame ionization detector at about 400 °C so that the wax sample is transferred and distributed in the mobile gaseous phase (Gordon, 1990). The use of high separation and detection temperatures is due to the physical properties of the test substances.

The prepared sunflower wax samples were analyzed by gas-liquid chromatography using a flame ionization detector. Tables 1 and 2 present experimental data for determining the composition of waxes in sunflower oil.

Table 1

Composition of crude and refined sunflower oil wax, mg/g (n=3, P≥0.95)

Name of oil sample	C44	C46	C48	C50	C52	C54	C56	Other*	Total
Crude	0.028	0.046	0.059	0.055	0.050	0.036	0.011	0.064	0.349
Refined	0.001	0.001	0.002	0.003	0.003	0.003	0.001	0.006	0.020

Other* – hydrocarbon chains with an odd number of carbon atoms C43, C45, C47, C51, and C53 combined

Table 2

Composition of crude and refined sunflower oil wax with an odd number of carbon atoms, mg/g (n=3, P≥0.95)

Name of oil sample	C43	C45	C47	C49	C51	C53
Crude	0.007	0.007	0.009	0.012	0.015	0.014
Refined	-	0.001	-	0.002	0.002	0.001

Tables 1 and 2 show that oil samples mainly contain waxes with even numbers of carbon atoms in the hydrocarbon chains. Waxes with hydrocarbon chain lengths of 44-56 carbon atoms (C44 to C56) have been identified in crude sunflower oil. In crude sunflower oil, waxes with chain lengths ranging from 46 to 52 carbon atoms (C46, C48, C50, and C52) account for up to 61% of the total waxes. Refined oil is mainly characterized by hydrocarbon chains with even numbers of carbon atoms (C48, C50, C52, and C54), with waxes containing odd numbers of carbon atoms constituting the minority.

Refined sunflower oil showed a decrease in the content of long-chain wax compounds by an order of magnitude. The refining process probably removes waxes with shorter C44 and C46 hydrocarbon chains. Refined sunflower oil mainly contains waxes with chain

lengths ranging from 48 to 54 carbon atoms (C48, C49, C50, C51, C52, C53, and C54). That said, the predominance of chains with even numbers of carbon atoms over those with odd numbers of carbon atoms in refined sunflower oil is outspoken.

The thermostating temperature of the capillary gas-liquid chromatographic column of 380 °C and the detection mode enable the detection of waxes with hydrocarbon chain lengths of up to 56 carbon atoms (C56). Most waxes have been found to contain even numbers of carbon atoms, with the even-carbon-numbered waxes in the test sample accounting for 82% of the total number of waxes.

The conditions for the preparation of a sample for analysis and the gas-liquid chromatography temperature conditions described in the study methodology expanded the range of detection of long-chain waxes. Compared to studies (Carelli et al., 2002), we obtained new findings on the presence of waxes with hydrocarbon chain lengths of 50 carbon atoms and more in sunflower oil.

Tables 1 and 2 show that the dependence of wax content on the chain lengths with even and odd numbers of carbon atoms both in crude and refined sunflower oil is parabolic. Crude sunflower oil was found to have the highest content of wax with 48 carbon atoms (C48) of chain length. Refined sunflower oil is dominated by waxes with chain lengths of 50, 52 and 54 carbon atoms (C50, C52, and C54).

In a study (Carelli et al., 2002; Chalapud et al., 2016), oil waxes were preliminarily separated using wet silica gel with 2% moisture content. A mixture of n-hexane/diethyl ether in a ratio of 8.5:1.5 served as the wax eluent. Sudan dye is added to the column together with the oil in order to determine whether the wax fraction has been eluted from the silica gel since waxes are separated in the first place, followed by the dye, and finally triacylglycerols. The wax was separated using a gas-liquid chromatograph with a capillary column (11 m long, 0.32 mm in diameter) and 0.52 µm thick film of the liquid stationary phase containing 5% diphenyl and 95% dimethylpolysiloxane. The temperature in the column was increased from 80 to 200 °C at a rate of 30 °C/min, maintained at 200 °C for 1 minute, and then increased to 340 °C at a rate of 3 °C/min. The composition of the separated wax fraction was determined with a flame ionization detector at a temperature of 350 °C. The composition of waxes in crude oil containing 0.995 mg/g of waxes is given in Table 3.

Table 3

Chromatographically determined composition of waxes in crude sunflower oil
(Carelli et al., 2002), in % of total wax content

Sample	C36	C38	C40	C42	C44	C46	C48	C37	C39	C41	C43
Oil	13.0	4.2	11.5	7.5	7.0	11.2	13.9	9.2	3.4	14.3	4.8

Comparison of the data in tables 1 and 2 with the data in table 3 (Carelli et al., 2002) showed a significant effect of the temperature conditions of the chromatographic separation on the identification of waxes. Our data are related to the data (Carelli et al., 2002) in terms of the content of waxes with carbon atom numbers ranging from 44 to 48 (C44, C46, and C48). We believe that our inability to detect waxes containing more than 50 carbon atoms was due to the temperature in the chromatographic column maintained at 340 °C. The identified wax composition (Carelli et al., 2002) is incomplete as regards the presence of long-chain waxes with even and odd carbon numbers.

The wax fraction separated in a silica gel column was subjected to saponification (Carelli et al., 2002). The saponified sample was further extracted with diethyl ether to obtain

a hydroalcoholic fraction with fatty acids and a diethyl ether fraction with fatty alcohols. Following the conversion of fatty acids to methyl esters and fatty alcohols to trimethylsilyl ethers, they were subjected to the gas-liquid chromatographic analysis. The composition of fatty acids and fatty alcohols in the wax fraction is given in Table 4.

Table 4
Composition of fatty acids and fatty alcohols in the wax fraction of crude sunflower oil
(Carelli et al., 2002), wt% of the total amount

Fatty acids	wt%	Fatty alcohols	wt%
C14:0	1.4	C16	4.3
C16:0	9.8	C18	23.1
C16:1	1.2	C19	18.4
C18:0	4.9	C20	2.0
C18:1	18.6	C22	7.5
C18:2	44.0	C23	0.6
C18:3	1.3	C24	11.9
C20:0	4.3	C25	1.8
C20:1	1.0	C26	9.3
C21:0	0.2	C27	0.5
C22:0	9.7	C28	8.3
C22:1	0.7	C29	0.6
C24:0	0.1	C30	7.9
C26:0	0.9	C32	3.8
C27:0	0.2		
C28:0	1.0		
C29:0	0.3		
C30:0	0.4		

Table 4 shows that the wax fraction of sunflower oil mainly contains the following fatty acids: linoleic (C18:2), oleic (C18:1), palmitic (C16:0), behenic (C22:0). Among fatty alcohols, the wax fraction mainly contains octadecanol (C18), nonadecanol (C19), and tetracosanol (C24).

Sunflower oil waxes were subjected (Reiter et al., 2001; Broughton et al., 2018) to the gas-liquid chromatographic analysis using a flame ionization detector together with mass spectrometry of three different oil samples. The results were presented as a percentage of the total wax content. The content of C42:0 wax ester determined using a flame ionization detector in the range of 6.2-6.9% correlates to the content determined using mass spectrometry (6.1%). The content of C44:0 wax ester determined with a flame ionization detector is in the range of 3.8-12.0%, and the content determined using mass spectrometry is 1.9%. The content of C46:0 and C46:1 wax esters determined with a flame ionization detector was 4.1 and 5.7%, respectively, and the content determined using mass spectrometry was 3.5%. Among other wax esters were phytol esters with one of the fatty acids C18:1, C18:0, C20:0, C22:0, C24:0, and geranylgeranyl esters with one of the listed fatty acids C18:1, C18:0, C20:1, C20:0, C22:0, C24:0 (Reiter et al., 2001). The inability to detect long-chain waxes in oil was probably due to the fact that the maximum temperature of the capillary column during gas-liquid separation did not exceed 350 °C.

Tank sediments and sunflower seed shells (husks) serve as significant reserve sources of sunflower wax extraction. The wax content of tank sediments of sunflower oil and oil from sunflower husks was determined (Redondas et al., 2020; Kleiman et al., 1969). The wax composition was identified by gas-liquid chromatography in the temperature range of 100-400 °C using a flame ionization detector. Data on the composition of the detected waxes in the tank sediments of sunflower oil and oil from sunflower husks are given in Table 5.

Table 5
Composition of waxes in the tank sediments of sunflower oil and oil from sunflower husks
(Kleiman et al., 1969), in % of the total wax content

Wax carbon chain length	Wax content of tank sediments	Wax content of oil from sunflower husks
C42	4.0	5.1
C43	0.8	-
C44	21.2	16.0
C45	2.3	1.3
C46	23.9	19.4
C47	1.9	1.4
C48	15.4	15.5
C49	1.7	1.8
C50	8.4	12.6
C51	0.2	0.9
C52	7.7	10.0
C53	0.7	traces
C54	4.8	7.4
C55	0.6	-
C56	3.7	4.4
C57	-	-
C58	1.4	2.1
C59	0.5	-
C60	0.7	1.2
C61	-	0.7
C62	-	0.2

The composition of the wax determined in sunflower oil according to tables 1 and 3 and in sunflower wax from tank sediments according to table 5 shows a general trend in terms of the content of substances with a chain length of 44, 46, 48 carbon atoms (C44, C46, C48). However, sunflower oil has the highest content of wax with C48 carbon atoms, while tank sediment mainly contains wax with C46 carbon atoms. We proceed from the assumption that this is due to the oil storage conditions and duration, the conditions of formation of tank sediments and the polydispersity of the system, which, in addition to acylglycerols and waxes, has a significant amount of condensed moisture and phospholipids exhibiting surface activity. In this kind of system, even and odd carbon numbered wax esters containing C50 and more carbon atoms are more stable due to intermolecular interactions with triacylglycerols and related oil substances.

Sunflower oil tank sediments are predominantly composed of waxes with even carbon numbered hydrocarbon chains, namely C44, C46, C48, C50, C52, C54, and C56, which combined account for 76.6% of the total number of waxes identified.

Physicochemical parameters of sunflower wax samples

The wax sample separated from perlite filter powder was white, malleable and non-brittle. After melting and separating from perlite, the wax sample had a uniform matte straw color, was oily to the touch, and had a uniform and malleable structure. Physicochemical parameters of the wax are given in Table 6.

Table 6

Physicochemical parameters of the sunflower wax

Indicator	Wax sample	Hexane-extracted wax (Omelchenko et al., 2016)
Acid value, mg KOH/g	12.2 ± 0.6	7
Saponification value, mg KOH/g	111.5 ± 1.5	109
Iodine value, mg KOH/g	10.8 ± 0.4	8
Melting point, °C	69.0 ± 0.5	81

Physicochemical parameters in table 6, namely the iodine value, indicate that the wax esters of the wax sample mainly contain saturated acyls. The data are consistent with the data in table 3 (Carelli et al., 2002) and findings (Omelchenko et al., 2016). Table 4 (Carelli et al., 2002) shows that the crude sunflower oil wax, in addition to saturated fatty acids, has been found to contain unsaturated fatty acids, such as palmitoleic (C16:1), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), gadoleic (C20:1), and erucic (C20:1). Our data (Table 6) correspond to the data (Table 4) in terms of the wax iodine value, i.e. the degree of unsaturation of the fatty acid radicals of the wax compounds.

Sunflower wax contains ester bonds that undergo hydrolysis under the action of alkalis or acids, as indicated by the saponification value and acid value. The acid value indicates an acceptable free fatty acid content.

The melting point of the wax sample was below the melting point of the hexane-extracted wax. We assume that this fact can be explained by the duration of the processes of wax extraction from the used filter powder. Extraction is carried out using the exhaustive extraction technique by Soxhlet (Mank et al., 2018) and the whole process takes about 24 hours. Extraction of the wax sample by melting, filtering and crystallization took 6 hours. The shorter heating time of the material and the fatty acid profile of the wax esters may have resulted in a lower melting temperature of the wax sample.

Conclusion

The peculiarities of the lipid composition of sunflower wax separated from crude and refined oil have been determined.

1. In crude sunflower oil, waxes with chain lengths ranging from 46 to 52 carbon atoms (C46, C48, C50, and C52) account for up to 61% of the total waxes. Identified waxes with even numbers of carbon atoms accounted for 82% of the total waxes.

2. Refined sunflower oil showed a decrease in the content of wax compounds by an order of magnitude. The refining process removed waxes with shorter C44 and C46 hydrocarbon chains. The refined oil has been found to contain trace amounts of waxes.
3. The dependence of wax content on the chain lengths with even and odd numbers of carbon atoms both in crude and refined sunflower oil is parabolic. Crude sunflower oil was found to have the highest content of wax with 48 carbon atoms (C48) of chain length. Refined sunflower oil is dominated by waxes with chain lengths of 50, 52 and 54 carbon atoms (C50, C52, and C54).
4. The acid and iodine values, the saponification number and the wax melting point have been analyzed, which are in line with an identified composition of sunflower wax.

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