

Composition and Physicochemical Properties of Corn Wax

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Abstract

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Abstract: Corn wax, as a kind of vegetable wax, is a by-product of the corn oil industry. In this study, the corn wax was purified from the corn oil refineries. The composition and physicochemical properties of corn wax were studied to provide theoretical guidance for the production and application of corn wax. The results showed that the purity of corn wax was higher than 95%, and the corn wax ester ranged from C42 to C58, with C46, C44, and C48 being predominant. The fatty acid carbon atom of corn wax was in the range of 16-30, with higher percentages of C20, C22, and C24. The fatty alcohol of corn wax was in the range of C20-C32 and dominated by C22, C24, C30, and C23. The physicochemical properties of corn wax were also determined and compared with other vegetable and animal waxes to get a more comprehensive understanding of corn wax. The results showed that corn wax was a green and healthy natural resource with great potential for application in food, health care, and medicine.

Keywords: Vegetable wax; Corn wax; Compositions; Physicochemical properties

Introduction

Corn or maize (*Zea mays L.*) is an important cereal crop and is widely grown worldwide (Wang & Hu, 2021). China and United States are the major producers and consumers. The global corn production and consumption in 2020 were 1115 and 1140 million tons, respectively. While the production and domestic consumption of China were 260 and 285 million tons, respectively. Due to the relatively large germ of corn, corn contains higher oil content than other commercial cereals. Therefore, corn might be used as a source of vegetable oil as well as a staple food.

Corn oil contains high levels of unsaturated fatty acids (86%), and the percentage of linoleic acid is about 60%. In addition, corn oil is recognized as an ideal healthcare oil due to the highest level of unsaponifiable matters (1.3%-2.3%) (Stamenković et al., 2020). As the principal unsaponifiable substances, tocopherol and phytosterols play a significant role in regulating the level of cholesterol and preventing cardiovascular diseases (Marinho et al., 2019). The nutritional benefits force the popularity of corn oil, and the global corn oil market is projected to reach 7.2 billion US dollars by the year 2022. Apart from the desirable nutritional components, crude corn oil also contains some undesirable ingredients such as waxes, pigments, and free fatty acids. These components are needed to be removed by appropriate refining processes. As an ester of a long-chain acid and long-chain alcohol, wax could crystallize at low temperatures and cause turbidity in oils. Wax content in corn oil is in the range of 150-500 mg/kg. To ensure the physical stability of corn oils at low temperatures, a dewaxing or winterization process is required to remove wax from corn oil.

Vegetable wax is an essential industrial resource. It can regenerate and can be used as a polishing agent, protective agent, and emulsifier for manufacturing fiber. The processing and the application of vegetable wax such as carnauba wax (CW), sunflower wax (SW), rice bran wax (RBW), flax wax, and lacquer wax have gradually become research hotspots. Vali et al. (2005) have studied the composition of purified RBW, and the results showed that the physicochemical characteristics of the purified RBW were comparable to CW, which made RBW could be used as a potential substitute for CW. RBW could be used as a pastry release agent, chewing gum plasticizer, or dibble coating to enhance the shelf-life of fruit (Abhirami et al., 2020). Lozhechnikova et al. (2017) developed a simple method to produce water-based wax dispersions using CW particles and zinc oxide nanoparticles. Oregel-Zamudio et al. (2017) combined the candelilla wax (CLW) edible film with *Bacillus subtilis* for the first time and found that it could effectively extend the shelf life of strawberries. Patel et al. (2015) identified the critical gel concentration of three vegetable waxes, and use a rheological characterization to gain new insights into the gelation behavior of natural waxes.

As a by-product of the corn oil refining industry, the production of corn wax presents a gradual increasing trend. However, the properties of corn wax were rarely studied. In industry, the utilization rate of crude corn wax was shallow. Only a tiny part of the crude corn wax was utilized after rough processing, and the rest was directly discarded, resulting in a tremendous waste of resources. Therefore, it is necessary to study the composition and physicochemical properties to understand the actual value of corn wax to guide industry production. Crude corn wax contains neutral fats, waxes, phospholipids, free fatty acids, pigments, and proteins. In this study, corn wax was isolated and purified from crude corn wax obtained from corn oil refineries. Then the compositions of chain fatty esters, fatty acids, and fatty alcohols were analyzed by the GC method. The physicochemical properties were also characterized to provide data support for the comprehensive utilization of corn wax.

Materials and Methods

Materials and chemicals

The crude corn wax samples (n=30) were supplied by Sanxing corn industry technology Co., Ltd. (Shandong, China). Potassium hydroxide, sodium hydroxide, potassium iodide, phenolphthalein, soluble starch, potassium dichromate, cyclohexane, sodium thiosulfate, thiourea, ascorbic acid, and n-hexane were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). Chloroform, hydrochloric acid, nitric acid, perchloric acid, sulfuric acid, and glacial acetic acid were purchased from Luoyang Chemical Reagent Factory (Luoyang, China). Iodine monochloride was purchased from Chron Chemical (Chengdu, China). Hexane was of chromatographic purity, and the other reagents were of analytical purity.

Purification of corn wax from the crude corn wax

Corn wax was purified by solvent defatting and bleaching, according to the previous paper. Crude corn wax (180 g) was dissolved in acetone (900 mL), and the mixture was agitated and refluxed at 50°C for 70 min in a water bath. Then the mixture was cooled to room temperature, and the insoluble wax was filtrated off by a water flow filter. The solvent of defatted wax was removed by evaporation.

Bleaching must be carried out to remove the pigments and other impurities in the defatted wax. The composite adsorbent made up of active carbon and clay at a ratio of 4.5:1 (w/w) was used as the optimal adsorbent. The defatted corn wax was put into a 100 mL three-port flask, and the adsorbent was added to the flask at the dosage of 21% (w/w). The defatted wax was decolorized under vacuum at 89°C for 30 min. Then the mixture was filtered immediately at 89°C for 120 min through a Buchner funnel. The filtrate was poured into a mold and cooled to room temperature. Finally, the refined corn wax product could be obtained.

Gas chromatography of wax ester

The purified corn wax (0.05 g) and n-hexane (6 mL) were placed in a 10 mL test and shaken until the wax was completely dissolved. Then mixture sample (1 mL) was used to analyze the corn wax esters composition. The corn wax esters composition was determined by gas chromatography (GC) analysis using Agilent 7890A and a flame ionization detector (Agilent, USA). The separation of wax esters was carried out on DB-1HT dimethyl-polysiloxane non-polar column (28 m × 250 µm × 0.1 µm). The initial column temperature was 150°C and held for 1 min, then increased to 270 at a rate of 25/min. Finally, the column temperature was increased to 360 at a rate of 5/min and maintained for 10 min. The temperature of the injection and detector was 300 and 350, respectively. The injection volume was 1 µL in split mode at a ratio of 20:1. Fatty acid esters were identified based on the retention time of the standard and quantified by the peak area normalization method.

Saponification of wax ester

Saponification was carried out to release the fatty acid and fatty alcohol of wax ester according to a previous study with a little modification. Wax (1.0 g) and 30% KOH in isopropanol (40 mL) were mixed in a 100 mL flask. The mixture was refluxed at 100°C for 6 h, and vacuum evaporated to remove the solvent. Then 50 mL ethyl acetate was added and mixed with the residue under stirring at 50 for 2 h. The filtrate (fatty alcohols) and filter cake (fatty acid salts) were obtained by filtering. The filter cake was washed with ethyl acetate (3 × 20 mL), and the filtrate was collected to analyze the composition of fatty alcohols. The filter cake was acidified with 30 mL 30% HCL at 50 for 1 h. 20 mL distilled water was added to the acidified mixture, and the fatty acids were extracted with ethyl acetate. The combined extract was washed with water to neutral pH, and the ethyl acetate layer was dried over anhydrous sodium sulfate. The ethyl acetate was removed by vacuum evaporation, then the fatty acids were derivatized with boron trifluoride for GC analysis. The filtrate was dried over anhydrous sodium sulfate, and the composition of fatty alcohols was also analyzed by GC.

GC condition for fatty acids analysis

The fatty acid composition was determined as methyl ester by GC analysis using Agilent 7890A and a flame ionization detector (Agilent, USA). The fatty acid methyl esters were separated on an HP-88 chromatographic column (100 m × 250 µm × 0.2 µm). The temperature was increased from 100 to 240°C at a rate of 3/min and maintained for 40 min at 240degC. The injection volume was 1 µL. The temperature of the injection and detector was both 250. The nitrogen was used as the carrier gas, and the split ratio was 10:1. The fatty acid methyl esters were identified by the retention time and quantified by the peak area normalization method.

GC condition for fatty alcohols analysis

Fatty alcohols analysis was carried out on Agilent 7890A equipped with a flame ionization detector (Agilent, USA), and chromatographic column DB-1HT (28 m × 250 µm × 0.1 µm) was used to separate fatty alcohols. Analyses were performed under the following temperature program: the initial temperature was 100 and held for 1 min; increased to 220 at a rate of 50°C/min; increased to 290°C at a rate of 15°C/min; increased to 320°C at a rate of 40°C/min and held for 6 min; increased to 360°C at a rate of 8°C/min and held for 10 min. The injection volume was 1 µL. The temperature of the injection and detector was 300°C and 350°C, respectively. The nitrogen was used as the carrier gas, and the split ratio was 10:1. The fatty alcohols were identified by the retention time and quantified by the peak area normalization method.

Differential scanning calorimetry (DSC)

Corn wax (10 mg) was placed in DSC, and the temperature was increased from 30°C to 90°C at a rate of 30°C/min. The sample was cooled to 20°C at a rate of 5°C/min and held for 20 minutes to allow complete crystallization of the corn wax. Then the temperature was increased to 90°C at a rate of 5°C/min, and the nitrogen flow rate was 40 mL/min. The temperature at the beginning of the first endothermic effect on the melting curve and the shoulder temperature after the last endothermic peak were used to characterize the melting range of the system.

Physicochemical properties of corn wax

The water and volatiles content (AOCS Ac 2-41), ash content (AOCS Ca 11-55), acid value (AOCS Cd 3a-63), peroxide value (AOCS Cd 8-53), iodine number (AOCS Cd 1-25), saponification number (AOCS Cd 3b-76), lead content (GB 5009.12-2010), arsenic content (GB 5009.11-2014) and phosphorus content (AOCS Ca 12-55) of corn wax were determined according to standard methods.

Acetone insoluble in corn wax

0.30 g corn wax (m_1) was mixed with 10 mL acetone in a 15 mL test tube. The mixture was kept at 40°C for 1 h. Then the mixture was centrifuged and the acetone layer was discarded. The mixture was washed with acetone and centrifuged several times until the acetone layer was colorless. The acetone was removed by vacuum evaporated, and the residue was placed in an oven at 100°C to dry to a constant weight (m_2). The acetone-insoluble content (AI) and the purity of the corn wax were calculated as follows.

$$AI (\%) = \frac{m_2}{m_1} \times 100$$

$$Purity (\%) = AI (\%) - PC (\%)$$

AI (%): the acetone-insoluble content of corn wax. PC (%): the phospholipid content of corn wax.

Statistics Analysis

The data were expressed as mean values \pm standard deviations of triplicate experiments and analyzed by one-way analysis of variance (ANOVA) using IBM SPASS Statistics 20. The significance of the difference between samples was determined by Duncan's multiple range test ($p < 0.05$).

Results and Discussion

Composition of wax esters

Corn wax was isolated and purified from crude corn wax obtained from corn oil refineries. The purity of the corn wax was determined, and the results were shown in Fig. S1. The purities of corn waxes produced from different crude corn wax samples ($n=30$) under the same purification process conditions were in the range of 95.29% to 98.71%.

The chromatogram of the corn wax ester was shown in Fig. S2A, and the quantitative results were presented in Table 1. The wax esters of corn wax ranged from C42 to C58. Most of the wax esters in corn wax were even carbon chains, which were similar to the SW, and the wax recovered from sunflower oil winterization waste (Chalapud et al., 2017; Redondas et al., 2020). C44 (17.44%-30.63%) and C46 (24.99%-30.12%) were the predominant wax esters of corn wax ester followed by C48 (12.22%-18.06%). The carbon numbers of the wax esters from refined canola oils ranged from C36 to C56, with C36, C44, C46, and C48 being the most common (Hu et al., 1993). However, C28 and C30 were the major esters in sorghum wax (Harron et al., 2017; Hwang et al., 2002). Doan et al. (2017) have researched the waxes ester distribution of some natural waxes and found that the carbon chain lengths of SW, RBW, bees wax, CLW, and berry wax were in the range of C44-C50, C44-C52, C40-C48, C34-C52, and C34-C38, respectively. Compared with these waxes, the carbon chain length of corn wax ester was longer and it might affect its physicochemical properties, such as melting point. Wax esters have a variety of important biological functions, such as protecting fruits from desiccation, UV light, and pathogens (Kalscheuer et al., 2006). Wax esters could also represent an excellent source for

biodiesel production, as wax esters have a higher energy density than the triacylglycerol-type vegetable oils (Iven et al., 2016). They could be used as an important economical oil in the medicine, cosmetics, and food industries. At present, wax esters are mainly synthesized by expensive chemical precursors or extracted from plant jojoba oil, which leads to insufficient supply and high cost. As wax ester was the main component of corn wax, corn wax has great potential for producing wax esters.

Composition of fatty acids of wax esters

The composition of fatty acids released from wax ester was shown in Fig. S2B and Table 2. The fatty acids carbon atom numbers of corn wax esters were between 16 and 30, which was similar to RBW. The fatty acid carbon numbers of RBW were between 16 and 32 (Dassanayake et al., 2009). The fatty acids of corn wax esters were mainly saturated, with C20:0 (27.96%-34.56%), C22:0 (28.83%-31.74%), and C24:0 (14.22%-17.61%) being predominate. Although these fatty acids also existed in common peanut oil, rapeseed oil, etc., the content was much lower than corn wax. The main unsaturated fatty acids were C18:1, C18:2, and C18:3, but their percentages were very low. The content of saturated fatty acids of corn wax ester was around 90%. It was similar to the lacquer wax, in which the total amount of saturated fatty acids was 83.62% (Chen et al., 2014). Because of the higher percentage of saturated fatty acids in corn wax, corn wax has a strong antioxidant capacity.

The fatty acid carbon atoms of wax recovered from sunflower oil winterization waste were in the range of 14-34, with the C16, C18, C30, C32, and C34 fatty acids being the predominant (Chalapud et al., 2017). Another result showed that the fatty acids of SW were in the range of 14-30 carbon atoms with higher percentages of C18:1, C20:0, and C16:0 (Carelli et al., 2012). It might be mainly due to the different pretreatment and extraction process of the raw materials. Doan et al. (2017) have found that the fatty acid portion of RBW, SW, bees wax, CLW, carnauba Brazilian wax (CRBW), carnauba wild wax (CRWW) consisted mainly of components with even alkyl chains between 16 and 24 carbon atoms. The dominant fatty acid portion of bees wax were C16 and C18. Meanwhile, CRBW and CRWW were rich in C24, C16, and C18. The montan wax esters consisted of fatty acids with longer chains (C24:0-C32:0) (Tada et al., 2014). It has been reported that bees wax and CW contained straight-chain fatty acids of even carbon numbers exclusively (Asperger et al., 1999). The composition of fatty acids varied in different raw materials. Suitable materials could be selected according to the actual needs.

Composition of fatty alcohols of corn wax esters

As presented in Fig. S2C and Table 3, the corn wax ester was composed of fatty alcohols in the range of C20-C32 with higher percentages of docosanol (C22), tetracosanol (C24), triacontanol (C30), and tricosanol (C23). Their percentages were 10.81%-24.28%, 10.63%-21.68%, 6.35%-14.74%, and 3.69%-14.71%, respectively. The percentage of octacosanol (C28) in corn wax ester was 4.4%-10.98%. Octacosanol, as a promoter of calcitonin formation with extremely low side effects, is an ideal natural food additive. Octacosanol is also a natural anti-fatigue substance and could enhance endurance, regulate motor nerve function, protect the liver, and reduce cholesterol and blood lipids contents (Taylor et al., 2003). In addition, triacontanol (C30) is a plant growth regulator. It could promote carbon and nitrogen metabolism and increase the storage and accumulation of ATP to increase crop production. Triacontanol also has inhibitory effects on various solid tumors, such as liver cancer, bowel cancer, and lung cancer. At present, wheat germ oil was used as the raw material to prepare octacosanol, while the bees wax and RBW were used as the materials to prepare triacontanol. Corn wax also contains a considerable amount of octacosanol (4.4%-10.98%) and triacontanol (6.35%-14.74%) and could be used as one of the raw materials for preparing these fatty alcohols. It is of great significance to explore the high value-added substances of corn wax and extend its potential application areas.

The fatty alcohols of corn wax were mainly saturated, and the even-numbered carbon fatty alcohols had a higher percentage than odd-numbered carbon fatty alcohols. The fatty alcohols composition of RBW and wax obtained from refined canola oil were in the range of C24-C38 and C16-C30, respectively (Dassanayake et al., 2009; Hu et al., 1993). While the fatty alcohol composition of SW ranged from C18 to C34. C24, C26, and C28 constituted majority parts (Carelli et al., 2012; Chalapud et al., 2017). The composition of corn

wax was summarized in Table 4.

The melting point of corn wax

Corn wax was a mixture of wax esters formed by higher fatty acids and higher monohydric alcohols. Its melting temperature could hardly be expressed as an accurate temperature value but described as the melting point range. As shown in Table 5, the melting point of corn wax was in the range of 71°C-84°C. It was wider than the melting point of SW (69.02°C-75.13°C) (Redondas et al., 2020). It has been reported that the melting point of CW derived from different subspecies of carnauba tree was 79.2°C-84.2°C, and the wax had eutectic behavior when exposed to elevated temperatures (De Freitas et al., 2019). The melting point of RBW was 77°C-79°C, while the melting points of different grades of CW and sorghum wax were 78°C -86°C and 77°C -85°C, respectively. The melting point of CLW was 69°C - 73°C (Navarro-Guajardo et al., 2017).

The main factors affecting melting temperature were the carbon chain length, the unsaturation grade, and the placement of the ester bond of the wax ester. It was known that the melting temperatures of wax ester increased with the elongation of the carbon chain length. Unsaturated fatty acids could melt at lower temperatures with less energy (Chalapud et al., 2017). The melting point of CW was relatively higher than other general vegetable waxes, so CW was often added to other waxes to increase their melting points. Due to the broader range of corn wax melting points, corn wax could also be a kind of additive to improve the crystallization characteristics of other waxes.

Chemical properties of corn wax

For a more comprehensive understanding of corn wax, the basic chemical properties of corn wax were determined, and the results were summarized in Table 6.

The water content could directly affect the quality, storage, and transportation stability of corn wax, as the water could react with the residual neutral oil and accelerate the hydrolysis of the wax. Among the 30 samples, the water contents were all less than 0.05%, and the lowest was close to 0.01% (Fig. 1A). The difference in water content between the samples might be attributed to the difference in raw materials, the preparation process, and the refining process.

The ash content reflects the content of inorganic substances in corn wax. Vegetable waxes used for food were needed to meet the limitation requirements of ash content to avoid inorganic contamination. For example, the requirement of ash content for CW was less than 0.25% proposed by the Chinese national standard. As presented in Fig. 1B, the ash content of corn wax was in the range of 0.001%-0.009%, and it was much lower than the requirement for CW.

The acid value indicates the content of free fatty acids in wax. As shown in Fig. 1C, the acid values of corn waxes were all less than 2 mg/g, and there were significant differences among different samples ($p < 0.05$). The differences might be due to the varying degree of deacidification of various raw materials, and the preparation and refining processes of corn wax could also affect its acid value. Doan et al. (2015) have studied the acid values of RBW, SW, bees wax, CLW, CRBW, CRWW, and found that the acid values of these waxes were 13.25 mg/g, 5.00 mg/g, 19.10 mg/g, 12.62 mg/g, 5.88 mg/g, 6.34 mg/g, respectively. The acid value of CW and sorghum wax were in the range of 2-10 mg/g and 10-16 mg/g, respectively (Hwang et al., 2002). Compared with other kinds of waxes, the acid value of corn wax was lower, and it indicated that the content of free fatty acids in corn wax was lower.

The peroxide value of corn wax ranged from 0.05 g/100 g to 0.22 g/100 g, as shown in Fig. 1D. It was lower than the requirement of Chinese national food safety standards for vegetable oil (0.25 g/100 g). The lower peroxide value indicated that the degree of oxidation of corn wax was low, and this might be due to the higher degree of saturation of corn wax. The difference in peroxide value between samples might be caused by the different raw materials and the degree of oxidation during processing.

The iodine number is a measurement of the degree of unsaturation. The result showed that the iodine number of corn wax was 5.17 g/100 g-8.86 g/100 g (Fig. 1E). It has been reported that the iodine number of RBW

was in the range of 8-15 g/100 g (Dassanayake et al., 2009). The limitation of iodine number for lacquer wax was less than 25 g/100 g put forward by the Chinese national standard, and the iodine number of corn wax was far below it. This could be attributed to the lower content of unsaturated fatty acids in corn wax.

The saponification number is related to the content of unsaponifiable matters, free fatty acids, monoglycerides, and diglycerides of wax. It can reflect the purity of wax. The lowest saponification number of corn wax was 69.16 mg/g, and the highest was 81.66 mg/g (Fig. 1F). The saponification numbers of RBW, SW, bees wax, CLW, CRBW, CRWW were 78.17 mg/g, 64.58 mg/g, 36.88 mg/g, 7.14 mg/g, 72.64 mg/g, 74.81 mg/g, respectively (Doan et al., 2015). The saponification numbers of CW and sorghum wax were 77-95 mg/g and 16-49 mg/g, respectively (Hwang et al., 2002). The limit value of saponification number for RBW was 70-95 mg/g proposed by the Chinese enterprise standard, and it was similar to corn wax.

As heavy metals, lead and arsenic pose a threat to human health and especially show a high risk to the nervous system. The lead and arsenic in vegetable waxes are generally from the additives used in the oil refining processes. The lead and arsenic contents of corn wax were found to be in the range of 0.04-0.10 ppm and 0.01-0.06 ppm, respectively (Fig. 2A, Fig. 2B). The lead and arsenic contents of corn wax were far below the China permissible limits for RBW and met the food safety requirements, providing security for the application of corn wax.

CONCLUSIONS

Corn wax was purified from the crude corn wax, and the compositions of corn wax esters, fatty acids, and alcohols were analyzed. Corn wax esters were between 42 and 58 carbon atoms, with C44 and C46 being predominant. C20:0, C22:0, and C26:0 were the primary fatty acids and C22, C24, C30, and C23 were the primary fatty alcohols. The physicochemical characteristics of the corn wax were studied, and it was found that the melting point of corn wax was in the range of 71°C-84°C. The lower contents of water, ash, and heavy metals represented that corn wax was of high quality and safety. By comprehensively studying the composition and physicochemical properties of corn wax, it was found that corn wax could be applied to food, health care, daily chemicals, and medicine as other vegetable waxes. This study could contribute to a comprehensive understanding of the properties of corn wax and provide guidance for the future development and utilization of corn wax.

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AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. All authors read and approved the final manuscript.

DECLARATIONS

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

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Fig. 1 The physicochemical properties of corn wax. **A** The water content of corn wax. **B** The ash content of corn wax. **C** The acid value of corn wax. **D** The peroxide value of corn wax. **E** The iodine value of corn wax. **F** The saponification value of corn wax

Fig. 2 The heavy metals content of corn wax. **A** The lead content of corn wax. **B** The arsenic content of corn wax

Fig. S1 The purity of corn wax

Fig. S2 The composition of corn wax. **A** The gas chromatogram of corn wax esters. **B** The gas chromatogram of corn wax fatty acids. **C** The gas chromatogram of corn wax fatty alcohols

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