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Composition and tocopherol, fatty acid, and phytosterol contents in micro-endosperm ultra-high oil corn

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SUMMARY: China has developed a new oil crop, micro-endosperm ultra-high oil corn, approved by the government in 2011 and named Huajian No. 1 (HJ-1). This study analyzed the nutrients in HJ-1 cold-pressed whole-seed oil, their composition and contents in tocopherols, fatty acids, and phytosterols and compares them with those of seven selected vegetable oils. HJ-1 oil contained α-, β-, γ-, and δ-tocopherol, with contents of 433.25 ± 0.13, 26.27 ± 0.08, 570.69 ± 0.27, and 38.41 ± 0.005 mg/kg, respectively, the highest nutritional values among the vegetable oils studied, except for soybean and palm oils. Gas chromatography was used for fatty acid analysis and seven were detected, with the main ones being palmitic, oleic, and linoleic acids. In HJ-1, the ratio of oleic to linoleic acid was close to 1:1, and β-sitosterol, campesterol, and stigmasterol were present with contents of 254.20 ± 0.11, 108.91 ± 0.19, and 105.67 ± 0.58 mg/kg, respectively.

***KEYWORDS**: Fatty acids; Micro-endosperm ultra-high-oil corn; Phytosterols; Tocopherols*

RESUMEN: ***Composición y contenido de tocoferoles, ácidos grasos y fitoesteroles en micro-endospermo de maíz ultra alto.*** China ha desarrollado un nuevo cultivo de aceite, el de micro-endospermo de maíz ultra alto, aprobado por el gobierno en 2011 y denominado Huajian No. 1 (HJ-1). Este estudio analizó los nutrientes en el aceite de semilla entera prensada en frío HJ-1: su composición y contenido de tocoferoles, ácidos grasos y fitoesteroles y los compara con los de siete aceites vegetales seleccionados. El aceite HJ-1 contenía α, β, γ y δ-tocoferol, con un contenido de 433,25 ± 0,13, 26,27 ± 0,08, 570,69 ± 0,27, y 38,41 ± 0,005 mg/kg, respectivamente, los valores nutricionales más altos entre los aceites vegetales estudiados, excepto aceites de soja y palma. La cromatografía de gases se utilizó para el análisis de ácidos grasos: se detectaron siete, los principales fueron los ácidos palmítico, oleico y linoleico. En HJ-1, la proporción de ácido oleico a linoleico fue cercana a 1:1, y β-sitosterol, campesterol y estigmasterol estaban presentes con contenidos de 254,20 ± 0,11, 108,91 ± 0,19 y 105,67 ± 0,58 mg/kg, respectivamente.

***PALABRAS CLAVE:** Ácidos grasos; Fitoesteroles; Micro-endospermo de maíz ultra alto; Tocoferoles*

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1. INTRODUCTION

Oils are among the most important nutrients for humans. According to the United States Department of Agriculture statistics, from 2013 to 2017, the average annual production of vegetables oils was 178.81 million metric tons, while the average annual consumption was 175.20 million metric tons (USDA Foreign Agricultural Service Oilseeds, 2018). However, China, a populous country, still needs to import a large amount of edible oils every year to fill the gap in the availability of edible oils. In 2016, the self-sufficiency rate of edible oils in China was only 32.3%, with an external dependence of 67.7%. Therefore, the current status of the Chinese vegetable oil supply is not encouraging. The development of new oil species is needed to meet the demands of global population growth and bio-energy development. HJ-1, the first micro-endosperm ultra-high oil corn without transgenics, was approved by the Chinese government in 2011 (approval no. Guishenyu 2011017). HJ-1 is a new type of oilseed crop cultivated by Guangxi University and Nanning Guifuyuan Agriculture Co. Since 1994, Professor Wu Zikai of Guangxi University and his team have made great breakthroughs in the breeding of Micro-endosperm ultra-high oil corn (Qin *et al*., 2005; Wu *et al*., 2006; Dai *et al*., 2009). The typical characteristics of HJ-1 are a seed oil content exceeding 20% at corn seed maturity, a small endosperm, and an embryo weight ratio ≥ 40%, which means that HJ-1 can be used directly for oil extraction without the need to extract the corn embryo.

HJ-1 is universally suitable for planting, with a yield of 4200 kg/ha and a dry seed oil content of 27%, which would make it suitable for large-scale use. Regarding oil extraction, the embryo-to-weight ratio of HJ-1 can reach more than 53%, which not only allows the complicated process of embryo lifting to be avoided, but also improves the utilization rate of the raw materials. Therefore, as a new oil seed, analyzing the nutrient content of HJ-1 oil is important.

Tocopherol and phytosterol contents are important indices for measuring the nutritional value of corn oil. Natural tocopherols comprise four different structures, namely α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol, of which α-tocopherol exhibits the highest biological activity (Shi *et al*., 2014). Tocopherols can reduce the speed of oil oxidation and remove free radicals. Phytosterols are important components of plant cell membranes, and mainly comprise β-sitosterol, stigmasterol, and campesterol. Furthermore, phytosterols have comprehensive biological activities, such as antioxidation, anti-inflammation, and anticancer activities. Related studies have shown that phytosterols can inhibit the absorption of low-density lipoprotein cholesterol, resulting in a reduction in total cholesterol in the blood (de Jong *et al.*, 2003). Phytosterols cannot be synthesized by the body so are obtained solely through the diet.

This study aims to analyze the composition and contents in tocopherols, fatty acids, and phytosterols in HJ-1 oil, compare them with those of seven common vegetable oils, and explore the nutritional value of HJ-1 oil.

2. MATERIALS AND METHODS

2.1. Materials

HJ-1 oil, obtained by drying HJ-1 seeds to a moisture content of about 8% then pressing the whole seeds using a screw press, was kindly provided by Nanning Guifuyuan Agriculture Co. (Model YZYX130, Guangxin Cereals and Oils Machinery Manufacturing Co., Mianyang, China). The HJ-1 corn came from the Nanning, Wuming and Tanluo regions of China. Three samples were randomly selected from these three different regions for pressing the whole corn kernels to produce HJ-1 oil. The pressed HJ-1 corn oil was then directly tested without any processing.

2.2. HPLC analysis of tocopherols

Samples (1.00 g) were mixed with BHT (0.1 g) in a 25-mL brown volumetric flask, with a mobile phase (10 mL; 90% hexane + 10% BHA/MTBE–THF–methanol (20:1:0.1), *v*/*v*) which was freshly prepared before each use. After dissolving the sample by vortexing, the solution was made up to 25 mL with the mobile phase and then shaken. The solution was then filtered through a 0.22-μm membrane filter into brown autosampler vials before injecting into the HPLC system, following the ISO 9936 method (ISO, 2016).

An HPLC system (Model 1200, Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an amino column (150 mm × 3 mm × 1.7 μm) and a fluorescence detector was used for tocopherol analysis. Chromatographic analysis was performed using the following conditions: column temperature, 30 °C; mobile phase, 90% hexane + 10% BHA/MTBE–THF–methanol (20:1:0.1), *v*/*v*; flow rate, 0.8 mL/min; excitation wavelength, 294 nm; emission wavelength, 328 nm; and injection volume, 10 μL.

2.3. GC analysis of fatty acids

Samples (0.2 g) were added to a 2% NaOH–methanol solution (8 mL) and refluxed over an 80 ± 1 °C water bath until the oil droplets disappeared. Boron trifluoride–methanol (7 mL, 15%) was then added into the top of the reflux condenser and reflux was continued for 2 min. The reflux condenser was rinsed with a little water and the reaction flask was removed and rapidly cooled to room temperature. After adding *n*-heptane (20 mL), the mixture was shaken for 2 min and saturated aqueous NaCl was added. After allowing the layers to separate, 5 mL of the *n*-heptane extract (upper layer) were transferred to a 25-mL test tube and anhydrous Na_2SO_4 (approx. 4 g) was added, followed by shaking for 1 min and allowing to stand for 5 min. The dried extract was then transferred to a sample vial for determination according to the ISO 12966-2 method (ISO, 2017).

Gas chromatography (GC; 7890B, Agilent Technologies Inc.) was performed using a capillary column (100 m × 0.25 mm × 0.2 µm) with high polarity polypolar cyanopropyl siloxane as the stationary phase. GC was performed under the following conditions: sampler temperature, 270 °C; detector temperature, 280 °C; carrier gas, nitrogen; split ratio, 100:1; and injection volume, 1.0 µL. The GC temperature program was as follows: initial temperature, 100 °C for 13 min; increased to 180 °C at 10 °C/min and held at 180 °C for 6 min; increased to 200 °C at 1 °C/min for 20 min; and finally, increased to 230 °C at 4 °C/min and held at 230 °C for 10.5 min.

2.4. HPLC analysis of phytosterols

Samples (2 g) were added to a NaOH–ethanol solution (20 mL, 2%) and refluxed for 1 h. Distilled water (100 mL) was then added into the top of the condenser and the reaction mixture was shaken. This saponification mixture was then transferred into a separatory funnel, extracted several times with petroleum ether (100 mL), and the solvent was evaporated. The resultant unsaponifiable matter was then dissolved with ethanol to a volume of 10 mL and filtered through a 0.45-µm filter for determination (Ito *et al.*, 2017).

Phytosterol analysis was performed using an HPLC system (Agilent Technologies Inc.) equipped with a UV detector using an IODS column (250 mm × 4.6 mm × 5 µm, Agilent Technologies Inc., Palo Alto, CA, USA). HPLC analysis was performed under the following conditions: mobile phase, methanol; volume temperature, 25 °C; flow rate, 1.0 mL/min; injection volume, 10 µL; and detection wavelength, 210 nm.

2.5. Data treatment

All measurements were performed in triplicate and results are shown as means ± standard deviation.

3. RESULTS AND DISCUSSION

3.1. Tocopherol composition and content

Vitamin E is an essential human nutrient because of its important physiological functions and it is obtained solely through foods and supplements (Burton *et al.*, 1983). Vegetable oils, which contain 700–1900 mg of tocopherols per kg of oil, are the main source of tocopherols in the human diet (Gliszczyńska-Świgło and Sikorska, 2004). Unlike other vitamins, vitamin E contains tocopherols and tocotrienols, which are closely related fat-soluble components (Azzi and Stocker, 2000). Tocopherols are mainly found in corn, soybean, and olive oils, while palm, rice bran, and barley oils are rich in tocotrienols. Therefore, this study determined the levels of four tocopherols in HJ-1 oil and compared them with those found in seven other selected edible vegetable oils (Table 1).

Tocopherols in HJ-1 oil were dominated by α-tocopherol and γ-tocopherol, as also observed in

Table 1. Composition and content of tocopherols in HJ-1 oil and other selected oils.

Sample	α-Tocopherol (mg/kg)	β-Tocopherol (mg/kg)	γ-Tocopherol (mg/kg)	δ-Tocopherol (mg/kg)
HJ-1	433.25±0.13	26.27±0.08	570.69±0.27	38.41±0.005
Corn oil	505.3	59	442	ND*
Sunflower oil	671.0	40.0	26.0	ND
Soybean oil	557.3	53.0	599.5	118.3
Rapeseed oil	418.5	64.3	328.0	12.0
Olive oil	172.5	ND	ND	ND
Palm oil	223.5	131.5	131.5	137.5
Camellia oil	247.44	46.05	ND	11.65

*ND - not detected. The data for camellia oil were cited from Ge *et al.*, (2017), with others from Ergönül and Köseoğlu (2014). All data came from different samples and were measured three times in parallel. Results are shown as means ± standard deviation. Sunflower, corn, soybean and rapeseed oils were obtained from Altınyağ Inc. (İzmir, Turkey), using a chemical refining process; palm oil from Orkide Oil Industry Inc. (İzmir, Turkey), using a physical refining process; olive oil samples TARİŞ Inc. (İzmir, Turkey), using physical refining; and camellia oil from Anhui Province, China, using aqueous enzymatic extraction.

corn, rapeseed, and soybean oils. Furthermore, HJ-1 oil contained 38.41 ± 0.005 mg/kg of δ-tocopherol, which was not detected in corn oil. δ-Tocopherol was also detected in soybean, rapeseed, palm, and camellia oils. Jung *et al.*, (2016) have previously shown that the presence of δ-tocopherol is beneficial for maintaining the stability of other tocopherols with a certain moisture content. Therefore, the nutrients in HJ-1 might be more stable than those in corn oil. Table 1 also shows that the main tocopherol in olive and corn oils was α-tocopherol, but that the contents of all four tocopherols in olive oil were lower than those in HJ-1. However, the contents of the four tocopherols in soybean oil were higher than those in HJ-1 oil.

3.2. Fatty acid composition

Edible vegetable oil plays a significant role in the human diet, providing energy for growth and metabolism. Furthermore, many essential fatty acids, such as linoleic and linolenic acids, cannot be synthesized in the human body. Therefore, data on fatty acid compositions and contents are essential for evaluating oil quality. In the present study, the fatty acid composition of HJ-1 oil was determined and compared with those of seven other edible vegetable oils (Table 2).

Seven kinds of fatty acids were detected in HJ-1 oil, of which palmitic (C16:0), oleic (C18:1-9c), and linoleic (C18:2-9c12c) acids were the main components, as also observed in normal corn oil. The oleic acid (C18:1-9c) content in HJ-1 oil was higher than that of linoleic acid (C18:2-9c12c), while the opposite was observed for normal corn oil. Furthermore, the unsaturated fatty acid content in HJ-1 oil was approximately 83.56%, which was higher than that of corn oil (79.85%).

Olive oil is rich in unsaturated fatty acids, which has played a key role in its global recognition as a superior oil. Table 2 shows that the fatty acid composition of camellia oil is similar to that of olive oil. Compared with the other selected oils, the most outstanding feature of HJ-1 oil was an oleic acid to linoleic acid ratio close to 1:1. Among saturated fatty acids, long-chain saturated fatty acids (such as palmitic acid 16:0) are prone to raising serum cholesterol. Therefore, people tend to reduce the intake of saturated fatty acids in the diet. Many scholars have proposed reasonable ingestion ratios for saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. For example, the Ministry of Health and Welfare of Japan advocates a 1:1.5:1 ratio of saturated fatty acids/monounsaturated fatty acids/polyunsaturated fatty acids, with the World Health Organization and the Food and Agriculture Organization of the United Nations proposing a fatty acid ratio of 1:1:1. Therefore, HJ-1 oil could be suitable for use either alone or as a raw material in blended oils.

3.3. Phytosterol composition

Phytosterols, such as β-sitosterol, campesterol, and stigmasterol, are natural ingredients found in plant cell membranes and have high contents in vegetable

Table 2. Composition and content of fatty acids in HJ-1 oil and other selected oils.

	Sample	HJ-1	Corn oil	Sunflower oil	Soybean oil	Rapeseed oil	Olive oil	Palm oil	Camellia oil
Fatty acids	16:0	12.85±0.07	12.69±0.92	5.73±0.00	10.04±0.23	5.01±0.01	14.36±0.66	43.5	10.45±0.02
	16:1	ND*	0.09±0.01	ND	ND	0.28±0.00	0.86±0.13	0.20	0.12±0.01
	17:0	ND	0.10±0.00	ND	ND	ND	0.17±0.05	ND	0.093±0.01
	18:0	3.18±0.005	0.82±0.09	5.81±0.03	4.70±0.92	1.70±0.00	4.06±0.39	4.30	4.38±0.06
	18:1	43.00±0.04	30.11±3.39	27.70±0.07	23.4±0.51	61.77±0.04	65.38±2.11	39.8	75.05±0.15
	18:2	39.56±0.006	49.05±2.62	59.52±0.06	52.90±0.20	21.05±0.03	12.27±1.92	10.20	8.57±0.04
	18:3	0.76±0.006	0.60±0.12	ND	6.90±1.21	8.59±0.02	0.98±0.21	0.30	0.19±0.01
	20:0	0.47±0.006	0.44±0.12	0.35±0.01	0.40±0.23	0.45±0.01	0.64±0.04	0.20	0.17±0.03
	20:1	0.24±0.00	ND	ND	0.20±0.01	0.95±0.01	0.03±0.02	ND	0.61±0.01
	22:0	ND	0.07±0.00	0.84±0.05	0.30±0.30	0.23±0.01	0.19±0.03	ND	ND
	22:1	ND	ND	ND	0.00±0.00	<0.1	0.48±0.15	ND	0.29±0.00

*ND - not detected. The data for sunflower oil and rapeseed oil were cited from Roche *et al.*, (2016), and others from He *et al.*, (2017), Makeri *et al.*, (2015), Ozkan *et al.*, (2017), Gong *et al.*, (2013), and Zhang *et al.*, (2011). All data came from different samples, measured three times in parallel and results are shown as means ± standard deviation. Sunflower oil and rapeseed oil were obtained from NK-Syngenta Seed (hybrid variety Santiago II) and Ringot Cie, Toulouse, France, respectively, from dried ground seeds using an automatic Soxhlet extractor; palm oil from mesocarp and exocarp of palm fruitlets (Danzhou of Hainan Province in China) by using mechanical expression; olive oil from Gaziantep-Nizip Province Turkey, using an oleodosor system; soybean oil extracted by n-hexane (Giant Hypermarket); corn oil purchased directly from Beijing supermarkets; and camellia oil from Guangdong province, China, obtained by conventional solvent extraction.

TABLE 3. Composition and content in phytosterols in HJ-1 oil and other selected oils.

Sample	β-Sitosterol (mg/100 g)	Campesterol (mg/100 g)	Stigmasterol (mg/100 g)
HJ-1	254.20±0.11	108.91±0.19	105.67±0.58
Corn oil	661.7	195.72	50.45
Sunflower oil	268.00	53.51	31.81
Soybean oil	175.60	58.05	56.10
Rapeseed oil	341.50	155.01	8.04
Olive oil	216.36	10.58	3.20
Palm oil	34.03	19.81	7.74
Camellia oil	52.06	21.52	14.14

The data for Palm oil were cited from Sampaio KA *et al*., (2017) and others from Han *et al*., (2007). All data came from different samples and were measured three times in parallel and results are shown as means ± standard deviation. Palm oil was kindly provided by Agropalma S/A, Amazon Refining Company (Belém, PA, Brazil). Other vegetable oils were obtained from Beijing, China.

oils, nuts, seeds, and cereals. Phytosterols are mainly found as free sterols or sterol esters of fatty acids in vegetable oils. Many studies have confirmed the ability of phytosterols to reduce blood cholesterol levels (Yuan *et al*., 2017). The present study measured the contents of three phytosterols in HJ-1 oil and compared them with those in seven other selected edible vegetable oils (Table 3).

The composition and content of phytosterols among different types of vegetable oils varied greatly. Table 3 shows that β-sitosterol was the dominant phytosterol in HJ-1 oil, as also observed in the other seven oils, accounting for more than 50% of the total phytosterols. The β-sitosterol and campesterol contents in corn oil and rapeseed oil were higher than those in HJ-1 oil, especially the β-sitosterol content in corn oil, which was about 2.6 times that in HJ-1 oil. The stigmasterol content in HJ-1 oil was the highest among the oils analyzed, at approximately 105.7 mg/100 g, which was about twice that in corn oil. Unlike other vegetable oils, the contents of the three phytosterols in HJ-1 oil were very similar, indicating a relatively balanced content compared with the other seven oils. According to Roche *et al*., (2010), sunflower seeds are a major source of natural phytosterols. The β-sitosterol content in HJ-1 oil was equivalent to that in sunflower oil, while the contents of campesterol and stigmasterol were much higher than those in sunflower oil. Therefore, HJ-1 oil shows great potential for industrial applications.

4. CONCLUSIONS

HJ-1 oil is derived from whole corn kernels, without the traditional embryo extraction process, which effectively reduces processing costs. The contents of four vitamins E in HJ-1 oil were much higher than those in olive oil. No δ-tocopherol was detected in traditional corn oil but the contents of α-tocopherol and β-tocopherol in HJ-1 oil were lower than in traditional corn oil. The content of unsaturated fatty acids in HJ-1 oil was higher than that in traditional corn oil. Unlike traditional corn oil, the content of oleic acid in Huajian I was higher than its linoleic acid content. Like other vegetable oils, the phytosterols in HJ-1 were mainly β-sitosterol. Although the total amount of the three phytosterols in HJ-1 was not as high as in traditional corn oil, the content of stigmasterol in HJ-1 was twice as high.

ABBREVIATIONS

HJ-1, Huajian No. 1; HPLC, High performance liquid chromatography, GC; Gas chromatography; BHT, 2,6-Di-*tert*-butyl-4 methylphenol; BHA, Butylated hydroxyanisole; MTBE, Methyl *tert*-butyl ether; THF, Tetrahydrofuran

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