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Qualitative and Quantitative Assessment of Fatty Acids of Hazelnut by GC-TOF/MS

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Abstract

On the basis of gas chromatography coupled with time-of-flight mass spectrometry, we assessed the constituents and relative quantities of fatty acids extracted by supercritical carbon dioxide in seeds of hazelnut. Hazelnut seeds contain four fatty acids (palmitic, stearic, oleic, and linoleic acids). The content of unsaturated fatty acids is more than 92.9% in hazelnut seed oil. Oleic acid, which constitutes 76.1%, has a high boiling point and low volatility. Hazelnut oil has good storage stability and is recommended as senior edible oil for health and the food industry. Our study reveals the important contribution of hazelnut in the production of bioactive oils and compounds that prevent obesity, cancer, coronary disease, and many other human health as well as pharmaceutical challenges.

Keywords: hazelnut, fatty acid, gas chromatography coupled with time-of-flight mass spectrometry, supercritical carbon dioxide extraction

1. Introduction

The expanding population and improved living standards have increased the demand for edible vegetable oils. In 2016/2017, the global consumption of vegetable oils amounted to 168.53 million metric tons, compared to just 71.7 million metric tons in 1995/1996 [1]. The fastest increase has occurred in China, which in 2014 was 31.67 million metric tons, about 3.2 times greater than the consumption in 1996 [2]. Over the past decade, obesity rates, cerebrovascular, coronary disease, and cancers have increased dramatically [3–5]. This is because the changes in diets and lifestyles resulting from industrialization and market globalization have increased rapidly. However, a general improvement in the standard of living often has been

accompanied by unhealthy dietary patterns and insufficient physical activity to maintain an optimal energy balance and a healthy weight. The net result has been increased prevalence of diet-related chronic diseases.

Fatty acids form the building blocks of lipid molecules, contribute to the structure of cell membranes and hormones, and provide cells with energy [6]. Palmitic acid (C16:0) and stearic acid (C18:0) are common saturated fatty acids (SFA) in edible oils and are thought to raise blood cholesterol and low-density lipoprotein (LDL) levels, leading to many diseases [7]. Monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are considered a healthy source of dietary fat for humans. Originally, the American Heart Association (AHA) recommended a fatty acid balance of approximately 1:1.5:1 ratio of SFA:MUFA:PUFA [8]. Oleic acid (an omega-9 MUFA) is defined as “conditionally essential,” because it can be synthesized *in vivo*. Any amount of omega-9 is beneficial [9]. Linoleic acid (an omega-6 PUFA) is a desirable and an essential fatty acid, as humans cannot synthesize double bonds in the n-6 positions of their hydrocarbon chains.

Hazelnut (*Corylus avellana*) is the second most popular nut worldwide, and it is distributed in several areas of Europe and Asia [10–14]. Hazelnut seeds are high energy food rich in fats as well as proteins. Seed oil contents range from 41.96 to 63.73% of hazelnut kernel dry weight [11], and fatty acids of hazelnut are similar in composition to those of olive oil. The nutrition and health benefits of UFA in hazelnut oils can reduce or prevent cancer, cardiovascular, and autoimmune diseases, and have anti-ulcerogenic, regenerating, and anti-inflammatory properties [3, 10–13]. Hazelnut seeds contain high concentrations of bioactive compounds (such as tocopherols, polyphenolics, neolignans, and 1,1-diphenyl-2-picrylhydrazyl radical) [10–14]. These are valuable sources of phytonutrients, fiber, and antioxidants [15].

Few studies have compared the fatty acid composition of seed oils extracted by supercritical carbon dioxide extracting method (SCDE) and commercially prepared product oils. In this chapter, we (1) extracted seed oils using SCDE, (2) identified the constituents and relative contents of fatty acids in the extracted oils and in products purchased from Dihao company in China, using gas chromatography coupled with time-of-flight mass spectrometry (GC-TOF/MS), and (3) compared the differences in fatty acids between extracted oils and commercially prepared product oils. Finally, we also discuss the beneficial functions of these oils and provide useful information for producing this bioactive oil that reduces or prevents obesity, cancer, coronary disease, and many other human health as well as pharmaceutical challenges.

2. Materials and methods

2.1. Chemicals and reagents

Carbon dioxide gas (99.999%) was purchased from Airichen (Dalian, China). Hexane, methanol, and methylene chloride ($\geq 97\%$, GC grade) were obtained from Honeywell (Ulsan, Korea). The boron trifluoride and fatty acid methyl ester mix was obtained from Sigma-Aldrich (Steinheim, Germany).

2.2. Materials

We collected seeds of hazelnut hybrids (*Corylus heterophylla* × *C. avellana*) from Liaoning Provinces of China (**Figure 1A**). Oils from seeds were extracted by SCDE (**Figure 1C**). Seeds were stored in closed plastic bags in dark at 4°C. Commercially prepared seed oils were provided by Dihao (Liaoyang, China) company (**Figure 1C**).

2.3. Oil extraction by supercritical carbon dioxide

Seed samples were powdered using laboratory plant grinder and air-dried [16]. Ground sample (100 g) was then placed into the extraction kettle of SFT-110 supercritical carbon dioxide extracting device from Supercritical Fluid Technologies, Inc. (Newark, USA). The pressure parameter and temperature of kettle heating were set at 5500 PSI and 60°C, respectively. The carbon dioxide flow and extraction time followed was 18 mL·min⁻¹ [17].

2.4. Determination of fatty acids

Fatty acid profile was determined as fatty acid methyl esters (FAME) by gas chromatography. The methyl esters were prepared according to Wang et al. [18] and Sanchez-Salcedo et al. [6] with some modifications. Twenty milligram of oil was added to a test tube, followed by the addition of 2 mL of n-hexane and 5 mL of methanol-potassium hydroxide solution (1 M). The mixture was placed in a blender shock with water bath at 60°C for 30 min. After the reaction, 10 mL of boron trifluoride (BF₃) in methanol was added to the mixture, and the samples were left at 60°C for 30 min. FAME were then extracted using saturated sodium chloride solution (2 mL) and n-hexane (2 mL) through vigorous shaking for 1 min. Top layer was transferred into a vial and stored at -20°C.

The fatty acid compositions were analyzed using a Clarus 680 GC coupled with AxION iQT TOF/MS system (PerkinElmer, Shelton, USA). The system was equipped with Agilent J&W DB-23 capillary column (60 m × 0.25 mm × 0.25 μm). The flow rate of carrier gas (Helium) was 1 mL·min⁻¹ with a split mode (1:20). The temperature program was started at 50°C, raised

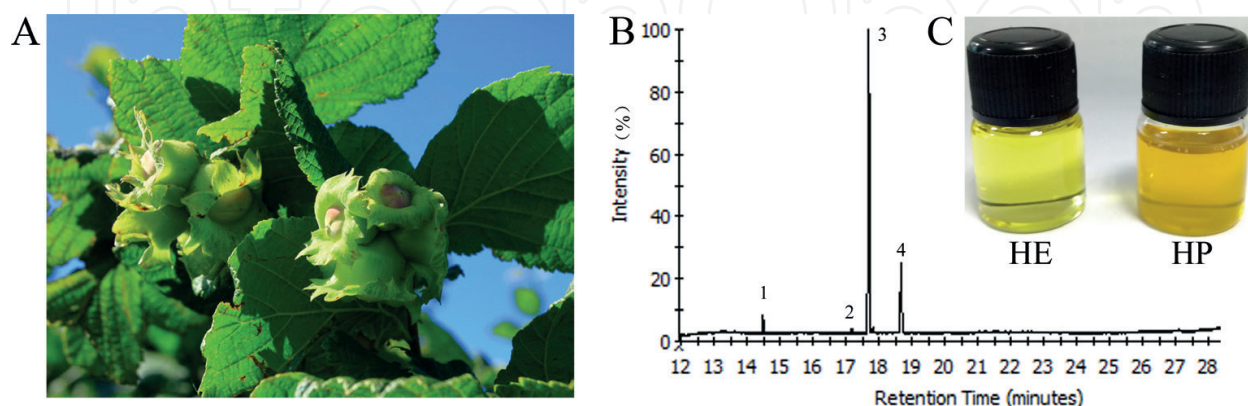


Figure 1. Fruits (A), fatty acid composition (B), and oils (C) of hazelnut. HE, oils extracted from seeds of hazelnut; HP, commercially prepared product oil of hazelnut seed.

to 200°C at 15°C·min⁻¹ up, and finally to 230°C for 10 min. The temperature of EI ion source was 230°C, and the injection volume was 1 µL. Fatty acids were identified based on the mass spectra of 37 FAME standards. Fatty acid composition was expressed using peak area normalization method. All the analyses were conducted in three replicates.

2.5. Statistical analysis

The results were expressed as mean ± standard deviation ($n = 3$). The p -value ≤ 0.05 was used to denote significant differences between mean values determined by one-way analysis of variance (ANOVA). All statistical analyses were performed using SPSS Statistics 20.0 software (IBM SPSS Statistics 20.0, Armonk, NY, USA) [19].

3. Results and discussion

3.1. Hazelnut species and distribution in China

C. America, *C. avellana*, *C. colurna*, and *C. mandshurica* are widely distributed species in the world. The world's hazelnut production is mainly covered by two main market players (Turkey and Italy). Turkey is the major hazelnut (*C. avellana*) producing country, supplying 65% of the world's total production. However, USA, Azerbaijan, Georgia, China, Iran, Spain, France, Kirgizstan, Poland, and Croatia are smaller but significant producers [15, 20]. *C. mandshurica*, also known as pilose hazelnut, is an economically and ecologically important species in China [21]. There is currently more than 4 million acres of natural hazel groves in northeastern China alone. Zong et al. [21] applied 10 polymorphic simple sequence repeat (SSR) markers to evaluate the genetic diversity and population structure of 348 *C. mandshurica* individuals among 12 populations in China and found that there was obvious genetic differentiation among populations from Northeast China to North China. The hybrid varieties of *C. heterophylla* and *C. avellana* have been widely planted in North China because of the cold resistance and high yield, which are superior to *C. avellana* in terms of unsaturated fatty acid content and antioxidant activity [22, 23].

3.2. Fatty acid composition in hazelnut seed oils

Hazelnuts are a high energy food with functional fats and proteins, which are the main components of the hazelnut kernel. The lipid portion represents a major determinant of kernel flavor, particularly following roasting [11]. We determined four fatty acids in hazelnut seed oils by GC-TOF/MS (**Figure 1B**). Oleic acid was the main fatty acid followed by linoleic acid, and palmitic and stearic acids were also measured in hazelnut oil. Hazelnut oil has low concentrations of SFA, and palmitic and stearic acids (4.7 and 2.4%, respectively) were quantified in hazelnut oil. Palmitic acid and SFA concentrations of oils extracted by the method of SCDE were significantly higher than in the commercially prepared product (**Table 1**).

Oleic acid (C18:1) concentrations were 76.1 and 74.5% in oils extracted from seeds of hazelnut (HE) and commercially prepared product oil of hazelnut seed (HP, **Table 1**), respectively.

Koksal et al. [20] investigated the oleic acid contents (74.2–82.8%) among 17 different hazelnut varieties grown in the Black Sea Region of Turkey. Because of its high percentage of oleic acid, hazelnut oil is stable edible oil and considered beneficial for a healthy diet. Oleic acid naturally exists in many plant and animal products and is considered one of the healthiest sources of dietary fat. It can reduce ratios of LDL/HDL and triglycerides in the blood, prevent coronary heart disease, hypertension, cerebrovascular disease, arteriosclerosis, stomach ache, and burn injuries; a high MUFA diet is recommended in diabetes mellitus patients [24].

PUFA are precursors of potent lipid mediators, important structural components of cell membranes, and play an important role in inflammation regulation and cell function [25]. Omega-6 fatty acids are necessary for healthy brain function, skin and hair growth, bone density, energy production, and reproductive health. Meat, eggs, and nut-based oils are the main dietary sources of omega-6 fatty acids [26]. The linoleic acid (one of omega-6 fatty acids) concentrations in HE and HP oils was 16.8 and 19.7%, respectively (**Table 1**). The linoleic acid concentration of HP oil was significantly higher than HE oil. Bacchetta et al. [11] found the percentage of linoleic acid ranged from 5.91 to 19.01% among 75 European hazelnut germplasm oil samples and detected its content was inversely correlated with oleic acid, because oleic acid is the precursor of linoleic and linolenic acids [11]. Because of the high level of oleic and linoleic acids, the composition of TUFA was more than 92%

Fatty acids	HE	HP	Dif.	Std.	Student <i>t</i>
Palmitic acid (C16:0)	4.73 ± 0.03	3.33 ± 0.22	1.40	0.16	0.71**
Stearic acid (C18:0)	2.40 ± 0.08	2.39 ± 0.14	0.01	0.11	0.07
Oleic acid (C18:1)	76.07 ± 0.79	74.54 ± 0.62	1.53	0.71	2.66
Linoleic acid (C18:2)	16.80 ± 0.84	19.74 ± 0.98	-2.94	0.91	3.96*
SFA	7.13 ± 0.05	5.72 ± 0.36	1.41	0.26	6.72*
MUFA	76.07 ± 0.79	74.54 ± 0.62	1.53	0.71	2.66
PUFA	16.80 ± 0.84	19.74 ± 0.98	-2.94	0.91	3.96*
TUFA	92.87 ± 0.05	94.28 ± 0.36	-1.41	0.26	6.72*
MUFA/SFA	10.67 ± 0.04	13.03 ± 0.72	-2.39	0.51	5.79*
PUFA/SFA	2.35 ± 0.14	3.45 ± 0.39	-1.11	0.29	4.72**

Data expressed as mean ± standard error (n = 3); nd, not detected; HE, oils extracted from seeds of hazelnut; HP, commercially prepared product oil of hazelnut seed; SFA, saturated fatty acids, are the sum of palmitic and stearic acid; MUFA, monounsaturated fatty acids, are the sum of stearic, eicosenoic, erucic, and nervonic acids; PUFA, polyunsaturated fatty acids, are the sum of linoleic and linolenic acids; MUFA/SFA, monounsaturated/saturated fatty acids ratio; PUFA/SFA, polyunsaturated/saturated fatty acids ratio; Dif., difference between oils extracted from seeds and commercially prepared product oils; Std, standard deviation (n = 3)

**P* < 0.05.

***P* < 0.01.

Table 1. Fatty acid composition (weight % of total fatty acids) and comparison of the mean values (%) of fatty acids between the oils extracted from seeds and the commercially prepared product oils.

(**Table 1**). Ciemniewska-Zytkiewicz et al. [15] quantified the concentrations of TUFA and oleic acid as 94.01 and 80.25%, respectively, in hazelnut seed oil, which were higher than the previous reports.

3.3. Fatty acid ratios in hazelnut seed oils

Fatty acids and their ratios affect lipid oxidation and physiological functions of oils and fats [7, 8]. Hazelnut oils have low concentrations of SFA and high concentrations of MUFA. The main sources of SFA in food are animal products (such as milk, meat, salmon, and egg yolks) and some plant products (such as chocolate and cocoa butter, coconut, and palm kernel oils). In modern time, people can easily consume sufficient SFA. SFA are thought to raise total cholesterol (TC) and low-density lipoprotein (LDL), which are undesirable to human health. But certain SFA (as consumed in our daily diet) have beneficial effects on the ratio of LDL to high-density lipoprotein (HDL) [7]. Recently, Souza et al. [27] and Mancini et al. [28] showed that saturated fat intake was not associated with mortality, cardiovascular disease, coronary heart disease (CHD), ischemic stroke, or type 2 diabetes, whereas trans fats were associated with mortality, total CHD, and CHD mortality, probably because of higher levels of intake of industrial trans fats than ruminant trans fats. No trans fatty acid occurred in hazelnut oil, and it had high MUFA/SFA (10.67) and low PUFA/SFA ratio (2.35) in HE oil (**Table 1**). So hazelnut oil has a high boiling point and low volatility and can improve the nutritional quality and shelf-life of processed foods [11]. Hazelnut oil has good storage stability and is recommended as senior edible oil for health and the food industry.

In addition, the abundant tocopherol, sterol, phenolic compounds, fiber, and antioxidants such as Vitamin E were also determined [29]. Hazelnut oil is suitable for cooking, salad oils, and for the manufacture of margarine. Meanwhile, a high level of MUFA and a low quantity of SFA in hazelnut oil enhance its usefulness in food as well as oleochemical applications [29].

4. Conclusions

Hazelnut seed oil contains four fatty acids, and the content of unsaturated fatty acid is more than 92.9% in hazelnut seed oil. The oleic acid concentration is 76.1%, which has a high boiling point and low volatility. Hazelnut oil is recommended as senior edible oil for health and the food industry. Our study reveals the important contribution of hazelnut for producing bioactive oils and compounds that reduce or prevent obesity, cancer, coronary disease, and many other human health as well as pharmaceutical challenges.

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