

Physicochemical Properties and Nutritional Ingredients of Kernel Oil of *Carya cathayensis* Sarg

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Abstract

Carya cathayensis Sarg is distributed near Tianmu mountain areas in Zhejiang Province and Anhui Province, China. Kernel of *Carya cathayensis* Sarg, which is well-known for the daintiness and nutritional content, is popular and very delicious food in eastern China. In this paper, we investigated the physicochemical properties and nutrient gradients of the kernel oil of *Carya cathayensis* Sarg. The results showed that the kernel oil of *Carya cathayensis* Sarg contained 63% of fat, 9.1% of proteins, 3.6% of starch and 4.6% of soluble proteins (based ration of weight). The compositions of the fatty acids were very similar to olive oil, and the total unsaturated fatty acids were 94%. Meanwhile, the kernel oil of *Carya cathayensis* Sarg also contained some polyphenols such as vanillic acid, ferulic acid, 3-methoxybenzoic acid and quercetin. The content of total polyphenols was 9.7 µg/g. Furthermore, the kernel oil of *Carya cathayensis* Sarg also contained some trace amount of squalene, β -Sitosterol, Vitamin E.

Keywords

Carya cathayensis Sarg, Kernel Oil, Compositions, Fatty Acids

1. Introduction

Nuts are a wide variety of dried seeds, which are composed of inedible hard shells and edible seeds [1]. It is well known that nuts are rich in nutrient elements, e.g. high content of fats, abundant protein, and varieties of mineral substances [2]. Thus, nuts are very beneficial for reducing the risks of malnutrition and some chronic diseases such as cardiovascular and cerebrovascular diseases [3]. Due to their good nutrition and taste, nuts are very popular food and dietary

supplements [4], which could be added into chocolate, ice cream, snacks and so on. Most spices of nuts draw a lot of attention on processing and utilizations; however, some of them are still not well applied and studied.

Due to their high content of fat contents, nuts are good raw materials for processing edible oil, such as olive, coconut, peanut, palm and so on. These nuts could be utilized to producing oil [5] [6] [7]. And, these oils are composed of high contents of monounsaturated and polyunsaturated fatty acids which are beneficial for human's blood vessel and heart. The unsaturated fatty acids could decrease blood lipids, improve blood cycle, and prevent blood platelet aggregation [8]. In addition, these unsaturated fatty acids also could synthesize DHA, EPA and AA. Especially, DHA could improve children's remembrance and study skill [9]. Unsaturated fatty acids usually are composed of oleic acid, linoleic acid, linolenic acid and so on [10]. As we known, most of nuts have high content of oil, some species even as high as 70% - 80% (based on the whole kernels of the nuts weight). As the quality of people's life improving, the demand of eating healthy draws a lot of attention in daily life. Therefore, nuts oil which is composed of high content of unsaturated fatty acids could cater these trends.

Carya cathayensis Sarg is one of the very precious trees, the fruit of which is well-known for the daintiness and nutritional content. *Carya cathayensis* Sarg is distributed near Tianmu mountain areas such as Chun'an, Lin'an, Tonglu and Ning Guo, Shexian, Jixi in Zhejiang Province and Anhui Province [11]. The fruit of *Carya cathayensis* Sarg is a natural, green food and with great economic value. Fruits of *Carya cathayensis* Sarg have generated increasing interest as a healthy foodstuff to decrease the risk of heart disease [12]. Jiang *et al.* studied on the chemical constituents in peels of *Carya cathayensis* Sarg and identified many active substances such as amino acids, polysaccharide, flavonoid, saponin, volatile oil, cardiac glycoside, and alkaloid [13]. Chen *et al.* applied chromatography method to study total flavanone on the husk of *Carya cathayensis* Sarg through hot water extraction [14]. The husk was applied to hydrothermal carbonization to get liquid production as biofuel and had good heating value as commercial coals [15]. However, as so far, research of *Carya cathayensis* Sarg focused on the extraction technology of *Carya cathayensis* Sarg [11] [16] [17]. There are few studies and reports on the physicochemical properties and nutrition ingredients of *Carya cathayensis* Sarg.

In this paper, we focused on analyzing and explored the physicochemical properties and fatty acids composition and other nutritional ingredients of fruit of *Carya cathayensis* Sarg.

2. Experimental

2.1. Materials

The kernel of *Carya cathayensis* Sarg was purchased in Lin'an, Hangzhou Province China and store at 4°C for use. All chemicals and standards were purchased in aladin company (China), and all standards were bought in Sigmaaldrich company (USA).

2.2. Determination of Total Fatty Acids

The content of the total fatty acids was evaluated by Pritam method with slight modification [18]. The 3 g of samples in soxhlet extractor were extracted with petroleum ether at 90°C for 8 h. The extracted solutions were condensed to 1 - 2 mL with rotary evaporator under vacuum and dried at water bath till no solvents left. The residues were continually dried at 105°C for 1 h and weighted. And repeat dried step for several times till to constant weight (within 2 mg difference in followed two dried). The contents of fats were calculated as follows:

$$X = \frac{m_1 - m_0}{m_2} \times 100$$

X —Contents of fat in samples (g/100g)

m_1 —the weight of flask and fat (g)

m_0 —the weight of flask (g)

m_2 —the weight of samples (g)

100—conversion coefficient

2.3. Determination of Total Proteins

The Micro-Kjeldahl method was adopted for determination of total proteins with little modification [19]. With addition of 0.4 g copper sulfate, 6 g potassium sulfate and 20 mL sulfuric acid, 2 g of solid samples were digested at 420°C for 1 h, then cooled to room temperature. And 50 mL water were added for the evaluated. The total proteins were determined as follows:

$$X = \frac{(v_1 - v_2) \times c \times 0.0140}{m \times v_3 \times 100} \times F \times 100$$

X —Contents of proteins in samples

v_1 —Sulfuric acid volume consumed in samples (mL)

v_2 —Sulfuric acid volume in blank samples (mL)

C —Contents of sulfuric acid (mol/L)

0.0140—Mass of nitrogen that 1 mL sulfuric acid ($c = 1$ mol/L) consumed (g)

m —Mass of samples (g)

v_3 —Volume of digested (mL)

F —Conversion of Nitrogen to protein

100—Conversion coefficient

2.4. Determination of Ash [20]

Samples were firstly heated in oven till no smoking, then calcinated in muffle furnace at 550°C for 4 h and cooled to 200°C. The samples were weighted after put into drier for 30 min repeatedly, calcinated till the weight of the sample to constant weight (within 0.5 mg difference).

$$X = \frac{m_1 - m_2}{m_3 - m_2} \times 100$$

X —Ash content of samples

m_1 —Weight of crucibles and ash (g)

m_2 —Weight of crucibles (g)

m_3 —Weight of crucibles and samples (g)

2.5. Determination of Moisture Content [21]

The samples placed in weighting flasks were dried at 105°C for 3 h, then cooled to room temperature in drier. Followed, weighted and repeatedly dried every 1 h till the adjacent 2 times being within 0.0005 g of difference.

$$X = \frac{w_1 - w_2}{w_1 - w_0} \times 100$$

X —Contents of moisture

w_1 —Weight of wet samples and weighting flasks

w_2 —Weight of dried samples and weighting flasks

w_0 —Weight of dried weighting flasks

2.6. Determination of Composition of Fatty Acids [18] [22]

Methyl esterification: With addition of 2 mL benzene-petroleum ether (1:1 v/v) 0.1 g of oil samples solved in 10 mL tubes stood for 5 min, then added 1 mL 0.1 mol/L KOH of methanol solution. The mixture was stood for 10 minutes and then added saturated NaCl solution to 10 mL of constant volume.

Gas chromatographs spectrometry (GC) was conducted on a Hewlett-Packard (HP) 7890 GC coupled to a FID Detector using a 30 m × 0.32 mm id., 0.25 µm film thickness DB-WAXETR capillary column (J & W Scientific Inc., Folsom, CA) with nitrogen as carrier gas. (150°C for 2 min and then programmed to 190°C at 5°C/min and held for 20 min).

2.7. Determination of Starch

Starch standard was boiled in distilled water, cooled and made up to a measured volume with distilled water. So that a 2.0 mL aliquot contains 10 to 200 micrograms of starch. Aliquot in a colorimeter tube was treated with 4.0 mL of 0.1% anthrone in concentrated sulfuric acid (4 h to 9 days) rapidly added from a pipet or burette. The solution was mixed immediately and allowed to air cool. After approximately 10 to 15 min, the tube is cooled completely in a cold-water bath. At the same time one or more 2.0 mL starch standards (100 micrograms suitable) received the same treatment. The colorimeter was adjusted to zero with the reference blank and the samples and standards are then read. When a logarithmic scale colorimeter was used, concentrations were proportional to scale readings. The proportionality constant is determined by the standards used.

2.8. Determination of Polyphenols

1.0 mg/mL of the 23 kinds of standards solutions (0.5 mL) were prepared by dissolving the standards in methanol and diluted into different contents in methanol and acetic acid.

The diol-bonded phase cartridge was conditioned according to the method described by Garcia *et al.* (García, Yousfi, Mateos, Olmo, & Cert, 2001). 2.5 g of the oil samples were dissolved in 6 mL hexane. Then, the samples were loaded to the cartridges, and washed by 6 mL hexane, 3 mL of hexane/ethyl acetate (90:10, v/v), followed extracted with 10 mL of methanol and evaporated at 40°C under vacuum, dried away of the solvent to obtain the solid residues. The residues were dissolved in 2 mL methanol and water (1:1, v:v), finally filtrated through 0.45 µm membrane.

A filtered aliquot (20 µL) of the final colorless solution was injected onto the HPLC system (an Agilent Technologies 1100 liquid chromatographic system equipped with an SPD-10A UV detector). The column was a WondaSil C18 reverse column (4.6 mm × 250 mm, 5 µm particle size) maintained at 30°C. The gradient elution, at a flow rate of 1.0 mL/min, was achieved using the following mobile phases: water as solvent A, 0.2% acetic acid in water as solvent B. The solvent gradient was programmed as follows: from 10% (A) in 5 min, to 20% A in 10 min, to 30% A in 20 min, to 40% A in 35 min, to 60% A in 45 min, to 70% A in 55 min, to 100% A in 70 min, followed by 5 min of maintenance.

2.9. Determination of Squalen

Dissoved in 2 mL of benzene-petroleum ether (1:1, v:v), 0.1g kernel oil of *Carya cathayensis* Sarg was added into 2 mL of 0.4 M KOH in methanol solution. Followed the mixture was washed by saturated NaCl solution, and the upper layer was collected and centrifuged at 2000 r/min for 10 min.

Gas chromatographs spectrometry (GC) was conducted on a Hewlett-Packard (HP) 7890 GC coupled to a FID Detector using a 30 m × 0.32 mm id., 0.25 µm film thickness SP-2560 capillary column (J & W Scientific Inc., Folsom, CA) with nitrogen as carrier gas at a rate of 1.0 mL/min (160°C for 5 min and then programmed to 220°C at 5°C/min and held for 15 min).

2.10. Determination of Vitamin E [23]

1.0 g oil samples were dissolved in 10 mL hexane, and 1.5 mL of the mixture were transferred to 2 mL centrifuge tub. And centrifugated for 10 min at 10,000 r/min and filtrated by 0.45 µm organic membrane.

The HPLC system (Agilent1100, USA) used consisted of a G1311A liquid chromatograph pump, G1379A online degas unit, G1313A automatic injector with a 10 µL loop, and fluroescence detector. The data were collected using an Agilent1100 ChemStation system provided by Agilent Company. A special ZORBAX Eclipse XDB-C18 HPLC column (250 mm × 4.6 mm, 5 µm), (Agilent, USA), optimized for the separation of vitamin E, was used at ambient temperature of 30°C. The vitamin E elution was performed with a mixture of n-hexane and isopropanol in a ratio of 97:3 (v/v) at a flow rate of 1 mL/min, and UV absorbance of the effluent was monitored at 333 nm and scan time was 7 min. The vitamin E was quantified by comparing their integration values of peak area to a

calibrated standard curve.

2.11. Determination of Sterol

1.0 g oil sample dissolved in 2 mol/L KOH ethanol solutions was saponificated at 85°C water bath for 1 h. After saponification, the mixture was transferred into 500 mL funnel and added 50 mL saturated NaCl solution, and extracted 3 times with 50 mL petroleum ether, then washed the organic phase with deionized water to neutral. Followed, the organic phase was dried by anhydrous NaSO₄, removed solvent, dissolved in 10 mL n-hexane and filtrated for application.

Gas chromatographs spectrometry (GC) was conducted on a Hewlett-Packard (HP) 7890 GC coupled to a FID Detector using a 30 m × 0.32 mm id., 0.25 µm film thickness HP-5 capillary column (J & W Scientific Inc., Folsom, CA) with nitrogen as carrier gas at a rate of 1.0 mL/min. (160°C held for 1 min and then programmed to 280°C, held for 5 min then at rate of 5°C/min to 300°C and held for 15 min). The sterol was quantified by comparing their integration values of peak area to a calibrated standard curve.

3. Results and Discussion

Physiochemical Properties of Kernel Oil of *Carya cathayensis* Sarg

According to some studies, the fundamental physiochemical properties are very vital for industrial and. Fat, one of the three main macronutrients, is triglycerides. Fat is an important foodstuff and has structural and metabolic functions. As shown in **Table 1**, the content of *Carya cathayensis* Sarg was nearly 63%. Protein also one of the three main macronutrients as the nutrient substances is very important for catalyzing metabolic reactions, DNA replication, responding to stimuli and so on. From **Table 1**, the contents of total proteins were 9.14%. Starch is a kind of carbohydrates, which composed by glucose units. In our research, the kernel oil of *Carya cathayensis* Sarg contained 3.5% starch and 4.6% soluble proteins.

Fatty acid is a carboxylic acid with a long aliphatic chain. Fatty acid is widely existing in nature in form of triglycerides, phospholipids, and cholesterol esters.

Table 1. The compositions of kernel oil of *Carya cathayensis* Sarg.

Compositions	Contents
Fat (%)	62.58 ± 0.0312
Total protein (g/100g)	9.14 ± 0.1022
Starch (%)	3.55 ± 0.0439
Ash (g/100g)	0.5438 ± 0.0716
Moisture content (%)	7.17 ± 0.047
Soluble proteins (mg/g)	4.63 ± 0.0259

Data is presented as means ± standard deviations (n = 3).

Fatty acids are both important dietary sources of fuel for animals and they are important structural components for cells. In **Table 2**, 8 species of main fatty acids were investigated, among of which, oleic acid (67.4%) was highest content and followed by linoleic acid (24.95%) and linolenic acid (2.2%). These unsaturated acids were up to 90% in whole fat. Thus, oil of kernel oil of *Carya cathayensis* Sarg is very similar to olive oil which is known as main constituent of Mediterranean diet. Furthermore, Total saturated acids content of *Carya cathayensis* Sarg which is harmful were 7% *ca*.

Polyphenols are a structural class of phenol structural units. The characteristics of these phenol structures underlie the unique physical, chemical, and biological properties. As shown in **Table 3**, 5 kinds of polyphenols were detected and among of which, quercetin was 4.11 µg/g and 3-methoxybenzoic acid was 4.01 µg/g.

Squalene is initially obtained from shark liver oil, which is a precursor for synthesis cholesterol and steroid hormones in the human body. squalene plays an important role in topical skin lubrication and protection. In kernel oil of *Carya cathayensis* Sarg was nearly 0.25 µg/g. β -Sitosterol (beta-sitosterol) is a kind of phytosterols, which are white, waxy solids with a characteristic odor and

Table 2. The compositions of fatty acids of *Carya cathayensis* Sarg.

Fatty acids	Contents (%)
Palmitic acid	5.01 ± 0.43
almitoleic acid	0.08 ± 0.01
Stearic acid	0.007 ± 0.0003
Linoleic acid	67.40 ± 0.18
Linolenic acid	24.95 ± 0.31
Arachidic acid	2.20 ± 0.05
cis-11-Eicosenoic acid	0.13 ± 0.03
Total saturated acids	6.93 ± 0.21
Total unsaturated acids	93.55 ± 0.08

Data is presented as means ± standard deviations (n = 3).

Table 3. Polyphenols in *Carya cathayensis* Sarg seed oil.

Polyphenols	Contents (µg/g)
Vanillic acid	0.28 ± 0.032
Ferulaic acid;	0.09 ± 0.002
3-Methoxybenzoic acid	4.01 ± 0.009
Quercetin	4.11 ± 0.223
Apiin	0.98 ± 0.135
Total	9.65 ± 0.391

Data is presented as means ± standard deviations (n = 3).

Table 4. β -Sitosterol, Vitamin E, squalene content of kernel oil of *Carya cathayensis* Sarg.

Substances	Contents ($\mu\text{g/g}$)
Squalene	0.243 \pm 0.006
β -Sitosterol	0.743 \pm 0.059
Vatimin E	0.372 \pm 0.009

Data is presented as means \pm standard deviations (n = 3).

have potential to reduce benign prostatic hyperplasia (BPH) and blood cholesterol levels. Vitamin E is a group of eight fat soluble compounds. Vitamin E deficiency, can cause nerve problems. In our studies, the three trace contents in kernel oil of *Carya cathayensis* Sarg were 0.243 $\mu\text{g/g}$, 0.743 $\mu\text{g/g}$, 0.372 $\mu\text{g/g}$ respectively (see **Table 4**).

4. Conclusion

In this work, we have investigated the physiochemical properties and nutrient gradients of the kernel oil of *Carya cathayensis* Sarg. The results were shown that the kernel oil of *Carya cathayensis* Sarg contained 63% of fat, 9.1% of proteins, 3.6% of starch and 4.6% of soluble proteins (based ration of weight). The compositions of the fatty acids were very similar to olive oil, and the total unsaturated fatty acids were 94%. Meanwhile, the kernel oil of *Carya cathayensis* Sarg also contained some polyphenols such as vanillic acid, ferulic acid, 3-methoxybenzoic acid and quercetin. The content of total polyphenols was 9.7 $\mu\text{g/g}$. Furthermore, the kernel oil of *Carya cathayensis* Sarg also contained some trace amount of squalene, β -Sitosterol, and Vitamin E. In a word, the kernel oil of *Carya cathayensis* Sarg was a beneficial oil for human beings. And our future work will focus on development of product of the kernel oils of *Carya cathayensis* Sarg.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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