

Original Paper

Extraction of Seabuckthorn Seed Oil and Analysis of Its Fatty Acid Composition

Ren Guangling¹

¹ Chengdu, Sichuan, Xihua University, 610039, China

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Abstract

Seabuckthorn seed oil was extracted by fat extraction method. The effects of quality of seabuckthorn seed, breaking time, soaking time and extraction solvent on the extraction rate were investigated by single factor experiment and response surface methodology. The fatty acids in seabuckthorn seed oil were determined. The research results show that the most suitable extraction conditions for using a fat extraction instrument are: seabuckthorn seed mass is 1.40 g, crushing time is 11 s, soaking time is 26 min, extraction time is 4 h, temperature is 70°C, and the extraction solvent is petroleum ether. The extraction rate can reach 6.56%; the extracted seabuckthorn seed oil mainly contains three main fatty acids: linoleic acid, linolenic acid and oleic acid, these three fatty acids are also the main sn-2 fatty acids.

Keywords

Seabuckthorn seed oil, Extraction, Fatty acid

1. Introduction

1.1 Sea Buckthorn

Seabuckthorn is a plant with strong vitality, which is drought-resistant, cold-resistant, barren and salt-tolerant. Seabuckthorn in China is mainly distributed in northwest, north and northeast provinces (Zhang, Jin, & Zhang, et al., 2018; Liu, Wen, & Zhou, et al., 2016). Seabuckthorn roots, stems, leaves, fruits, especially seeds contain a variety of bioactive substances, such as a variety of amino acids, protein, vitamins, fatty acids, alkaloids, flavonoids, trace elements and so on.

Seabuckthorn total flavonoids and vitamin C contained in seabuckthorn have the effects of anti-aging, whitening skin and regulating immune system (Chen & Jiang, 1990). Seabuckthorn contains substances that can inhibit inflammation and promote tissue regeneration. It contains a variety of amino acids, vitamins and trace elements, which can strengthen brain and promote children's growth and

development. Organic acids such as malic acid and oxalic acid in seabuckthorn can protect liver. Contains a variety of nutrients and phenolic compounds can promote digestion, bowel movement, seabuckthorn also has digestion and stagnation, spleen and stomach, soothing liver and qi (Shi, Wang, & Lu, 2017). The bioactive components of *Hippophae rhamnoides* have obvious anticancer and antitumor activities. It can inhibit human cancer cells and block carcinogenic factors. The multivitamins and carotenoids in seabuckthorn can effectively regulate the body fat metabolism, and have a good effect on simple obesity (Huang, Matthan, & Galluccio, 2020). Total flavonoids in seabuckthorn can reduce hypertension and hyperlipidemia, reduce blood viscosity, soften blood vessels, improve blood circulation, prevent arteriosclerosis, improve blood supply and oxygen supply to brain, and promote the healthy circulation of cerebrovascular system.

1.2 Seabuckthorn Seed Oil

Seabuckthorn seed is a by-product of seabuckthorn. Seabuckthorn seed oil is a brownish yellow to brownish red transparent oily liquid, which is made from seabuckthorn seeds by supercritical fluid extraction or subcritical low temperature extraction. It is a highly concentrated product of seabuckthorn active ingredients. It contains more than 140 bioactive components, such as flavonoids, organic acids, alkaloids, sterols, triterpenes and vitamins. Seabuckthorn seed oil is a kind of natural oil extracted from seabuckthorn seeds, which is a kind of high-quality edible and medicinal oil. The main component is unsaturated fatty acid, which can reduce blood fat, soften blood vessels, stabilize blood pressure and promote microcirculation after long-term consumption (Hou, Hui, & Li, et al., 2002; Chen & Jiang, 1990). Seabuckthorn seed oil can be used as raw material of health food, which is widely used in anti-oxidation, anti-tumor, ulcer repair, immunity enhancement, cardiovascular and cerebrovascular protection, liver protection, blood lipid reduction and so on. With the continuous development and innovation of seabuckthorn seed oil at home and abroad, seabuckthorn seed oil will show great international market potential. Efficient extraction of seabuckthorn seed oil is the research goal of food industry. This time, the extraction method of Soxhlet extraction was used to extract seabuckthorn seed oil, and related influencing factors were discussed to provide reference for production practice (Hou, Hui, & Li, et al., 2002).

1.3 Application of Seabuckthorn Seed Oil

Seabuckthorn seed oil, as a raw material of health food, has been widely used in anti-oxidation, anti-fatigue, liver protection and blood lipid reduction. There are 20 kinds of health foods made from seabuckthorn seed oil. Seabuckthorn seed oil, as a medicinal material, has obvious biological effects. It has strong anti-infection ability, quick healing and the function of promoting tissue regeneration. Seabuckthorn seed oil has good and stable anti-inflammatory and capillary blood circulation promoting effects (Dubey, Ramana, & Mishra, 2018). Seabuckthorn seed oil is also an important raw material for beauty and skin care. It is a complex of vitamins and bioactive substances, which can nourish the skin, promote metabolism, promote epithelial cell regeneration, repair the skin, maintain the acidic environment of the skin, and has strong permeability.

1.4 Efficacy of Seabuckthorn Seed Oil

Seabuckthorn seed oil is a kind of oil extracted from seabuckthorn seeds, which is rich in nutritional value, contains more amino acids and fatty acids, and has a variety of nutritional components, such as vitamin C, vitamin E, flavonoids, calcium ions and iron ions. Seabuckthorn seed oil can protect stomach and treat gastric ulcer. Seabuckthorn seed oil can prevent arteriosclerosis and reduce liver damage caused by excessive drinking and drugs. Seabuckthorn seed oil can limit the thickness of tear film, relieve the symptoms of red eyes, and has the effect of protecting eyesight. Seabuckthorn seed oil can reduce blood lipid, soften blood vessels, and whiten teeth. Seabuckthorn seed oil also has the effect of beautifying, which can help resist wrinkles and freckles and prevent the skin from being damaged by sunlight and ultraviolet rays. Eating seabuckthorn seed oil in moderation can help prevent cancer and cancer; It can accelerate the discharge of waste in the body, promote intestinal peristalsis and improve constipation; And has the function of promoting blood circulation and removing blood stasis, and can reduce the cholesterol content in the body (Hao, He, & Zhu, 2019).

1.5 Protective Effect on Cardiovascular and Cerebrovascular Diseases

In recent years, a series of studies on seabuckthorn seed oil have shown that it has significant protective effect on cardiovascular and cerebrovascular diseases. The research by Wu Ying et al. showed that seabuckthorn seed oil can significantly reduce the serum total cholesterol of high-fat rats (Wu & Wang, 2000), and can increase the serum high-density lipoprotein cholesterol content, and can also significantly improve the mouse auricle microcirculation and the rat mesenteric microcirculation. The above research shows that linolenic acid, tocopherol and phytosterol in seabuckthorn seed oil can reduce blood lipid, inhibit platelet aggregation, improve vascular microcirculation, etc., and have certain health care effects on cardiovascular and cerebrovascular diseases.

1.6 Effects on Gastrointestinal Tract

Studies have shown that seabuckthorn seed oil has obvious preventive and therapeutic effects on various experimental gastric ulcers, such as pylorus ligation, stress and reserpine, which are characterized by reduced ulcer incidence, ulcer index and gastric juice secretion. Seabuckthorn seed oil is rich in antioxidant β -sitosterol, which can protect gastrointestinal tract and relieve gastrointestinal ulcer symptoms.

1.7 Effect on Skin

Seabuckthorn seed oil is widely used in repairing various skin injuries, including eczema, burns, severe wounds which are difficult to recover, sun burns, radiotherapy and postoperative recovery after laser cosmetic surgery (ZEB, 2004). Seabuckthorn seed oil is rich in palmitoleic acid, which is a fatty acid in skin and can effectively treat local burns and scalds. Oral seabuckthorn seed oil capsule can effectively improve the average smoothness and maximum smoothness of skin and eliminate wrinkles.

1.8 Auxiliary Protection of Chemical Liver Injury

Seabuckthorn seed oil can inhibit the increase of glutamic pyruvic transaminase and glutamic oxaloacetic transaminase in liver injury induced by carbon tetrachloride, ethanol and acetaminophen,

and protect liver cell membrane. Seabuckthorn oil has auxiliary protective effect on chemical liver injury. This may be because seabuckthorn oil contains high content of tocopherols, carotenoids and other substances with strong antioxidant effect, which can reduce the activity of aspartate aminotransferase and alanine aminotransferase in blood and increase the activity of GSH in liver (Zang, Deng, & Cong, et al., 2015).

1.9 Alleviate Dry Eye

Although seabuckthorn seed oil can not directly affect the fatty acid composition of tear film lipid layer, the tocopherols, carotenoids and arachidonic acid derived from fatty acids in seabuckthorn seed oil may have certain anti-inflammatory and anti-cell differentiation effects on meibomian gland cells, thus having a certain effect on relieving dry eye symptoms.

1.10 Present Situation of Seabuckthorn Seed Oil

Seabuckthorn can be used as raw material in food processing to make various beverages, foods and wines, such as juice drinks, fruit wine, fruit vinegar, jam, various cakes and dairy products, etc. In medicine, medicine and health care, it has certain curative effects on cardiovascular and cerebrovascular diseases, lung, stomach, spleen, blood circulation, blood stasis, cervical erosion, burns and frostbite, etc. Seabuckthorn also has its unique value in light industry. Developed skin care and washing products that nourish skin, promote cell metabolism, promote epithelial tissue regeneration, resist allergy, resist bacteria, have strong permeability and protect natural skin color; Seabuckthorn branch is hard and can be used as raw material of plywood and other building materials. Seabuckthorn seed oil and fruit oil, as intermediates and raw materials of medicine, cosmetics and functional foods, have broad application fields and huge market potential (Zhang & Chen, 2010).

Since 1990s, the number of seabuckthorn seed oil processing enterprises in China has increased year by year. The main dosage form of seabuckthorn seed oil is oral liquid, which has the disadvantages of short shelf life, easy oxidation and rancidity, poor taste and low absorption and utilization rate. At present, the best method is to prepare powder oil by microencapsulation technology, which can overcome these defects. Although seabuckthorn oil powder oil technology has been successfully developed, it has not been transformed into productivity. Therefore, we should adopt a variety of effective cooperation methods to vigorously promote this technology.

In recent years, the main seabuckthorn extracts at home and abroad are seabuckthorn seed oil, seabuckthorn fruit powder, seabuckthorn fruit oil, seabuckthorn flavone, seabuckthorn dietary fiber and so on. If some flavonoids are dissolved into seabuckthorn seed oil by adopting appropriate technology, the efficacy of the oil can be improved. Flavonoids, as a recognized natural antioxidant, can replace synthetic antioxidants, which will be beneficial to the preservation of seabuckthorn seed oil, prevent oleic acid spoilage and peroxidation, prolong the shelf life of seabuckthorn seed oil and ensure the curative effect. Or adding a proper amount of Chinese herbal medicine extract containing natural antioxidants or peel residue extract containing flavonoids into seabuckthorn seed oil can also play the above role, and can save the tedious process of improving oil extraction, at the same time, it also

strengthens the efficacy of seabuckthorn seed oil (Zhang & Ni, 2001).

Seabuckthorn resources are abundant in China. At present, China is known as “Seabuckthorn Kingdom”, with seabuckthorn forest area reaching 1.4 million hectares, accounting for more than 95% of the total seabuckthorn area in the world. Drawing lessons from the experience of comprehensive utilization of seabuckthorn at home and abroad, as long as we invest time and energy and organize a strong scientific research team to comprehensively develop and utilize seabuckthorn plant resources in China, it is believed that the new generation of seabuckthorn products will bring huge economic and social benefits.

1.11 Soxhlet Extractor Method

Soxhlet extraction, also known as continuous extraction and Soxhlet extraction, is a method for extracting compounds from solid substances. Fat is widely found in the seeds and fruits of many plants. The determination of fat content can be used as an index to identify its quality. Soxhlet extraction method is usually used to determine the crude fat content in substances. Extraction method is widely used at home and abroad, among which Soxhlet extraction method is recognized as a classic method and the preferred standard method for grain and oil analysis in China (Dubey, Ramana, & Mishra, 2018). This method takes a long time, which is usually extracted by fat analyzer in laboratory.

That is to say, low boiling point organic solvent (ether or petroleum ether) is used for reflux extraction to remove the crude fat in the sample, and the crude fat content is calculated based on the weight difference between the sample and the residue.

Therefore, at present, it is mainly aimed at the optimization of Soxhlet extraction method, so as to get as much seabuckthorn seed oil as possible under the most suitable conditions.

The matters needing attention in Soxhlet extraction method are that the determination of sample, extractant and extraction organic solvent need dehydration. Sample size should be appropriate. We must attach great importance to the safe use of extraction solvents.

2. Materials and Methods

2.1 Test Materials and Reagents

Table 1. Main Materials and Reagents

Material name	Manufacturer
Fresh seabuckthorn seeds	Wild seabuckthorn seeds in Lvliang, Shanxi
Porcine pancreatic lipase (Type II)	Sigma Aldrich Company
Lipase AY “Amano” 30SD lipase	Amano Enzyme Products Co., Ltd., Japan
Formic acid (analytically pure)	Tianjin Dongtianzheng Fine Chemical Reagent Factory
2',7'- dichlorofluorescein (90%)	Sigma Aldrich Company
Normal hexane (chromatographically pure)	Aladdin biochemical technology co., ltd
Normal hexane (analytically pure)	Aladdin biochemical technology co., ltd

Sodium bisulfate (analytically pure)	Tianjin Zhiyuan Chemical Reagent Co., Ltd.
Anhydrous sodium sulfate (analytically pure)	Chengdu Kelon Chemical Reagent Factory
Methyl alcohol (analytically pure)	Tianjin kemio chemical reagent co., ltd.
Calcium chloride (99.9%)	Beijing Soleil Technology Co., Ltd.
Sodium chloride (analytically pure)	Chengdu Cologne Chemicals Co., Ltd.
Trizma (99.9%)	Sigma Aldrich Company
Muriatic acid (analytically pure)	Chengdu Cologne Chemicals Co., Ltd.
Sodium cholate (analytically pure)	Beijing Soleil Technology Co., Ltd.
Ether (analytically pure)	Chengdu Kelon Chemical Reagent Factory
Potassium hydroxide (analytically pure)	Tianjin Continental Chemical Reagent Factory
Absolute ethyl alcohol (analytically pure)	Chengdu Cologne Chemicals Co., Ltd.
TLC chromatographic plate	Germany merck kga
Petroleum ether (analytically pure)	Tianjin Fuyu fine chemical co., ltd
Chloroform (analytically pure)	Chengdu Cologne Chemicals Co., Ltd.
Muriatic acid (analytically pure)	Chengdu Cologne Chemicals Co., Ltd.
Sodium methoxide (0.5mol/L)	Sigma Aldrich Company
Boron trifluoride-methanol (14%)	Sigma Aldrich Company

2.2 Main Instruments and Equipment

Table 2. Main Instruments and Equipment

Equipment name	Equipment type	Manufacturer
Vortex mixer	VORTEX 3	Aika Instruments and Equipment Co., Ltd.(IKA China)
Heat collection type constant temperature magnetic stir bath	HWCL-3	Zhengzhou Great Wall Science, Technology and Trade Co., Ltd.
Centrifuge table low speed	TD-5M	Sichuan Shuke Instrument Co., Ltd.
Fume cupboard	1500*800*2350	Chengdu century ark co., ltd
Dark box type uv analyzer	ZF-20C	Shanghai Baoshan Gu Cun Electro-optical Instrument Factory
Low noise air pump	GA-5000A	Beijing Zhongxing Huili Technology Development Co., Ltd.
High purity hydrogen generator	GH-500	Beijing Zhongxing Huili Technology Development Co., Ltd.
Agilent gas chromatograph	7820A	Agilent technologies Inc
Ultrasonic cleaning machine	SB-5200 DTN	Ningbo Xinzhi Biotechnology Co., Ltd.
Electronic balance	JA2003	Shanghai Shunyu Hengping Scientific Instrument Co., Ltd.
Multifunctional crusher	MS-800A	Yongkang Sufeng Industry and Trade Co., Ltd.
Electric constant temperature blast drying oven	SFG-0.2B	Huangshi Hengfeng Medical Device Co., Ltd.
Fat meter	SOX406	Jinan Haineng Instruments Co., Ltd.

2.3 Extraction of Seabuckthorn Seed Oil

Seabuckthorn seed oil was extracted by Soxhlet extraction method.

Weigh the extraction cup with an electronic balance and record the weight of the cup. Accurately weigh a certain amount of seabuckthorn seed powder and put it into a filter paper bag, put the wrapped sample into a filter paper frame, and fix the filter paper frame on a magnet fixture. Measure 60 mL petroleum ether with a measuring cylinder and pour it into the extraction cup. Soak the filter paper frame in the extraction cup, and lift the filter paper frame to suspend it after soaking for a certain time. Open the condensed water, and set the parameters of the fat analyzer to start heating and extracting. The parameters were set as follows: the extraction temperature was 70°C and the extraction time was 4 hours.

After extraction, stop heating and remove the extraction cup. Recover petroleum ether. Shut down, cut off water and power. Put the removed extraction cup in a fume hood for a certain time until petroleum ether is completely volatilized. Weigh the extraction cup again to make it constant weight to obtain the total mass of the extraction cup and grease. Finally, the quality of oil was obtained and the extraction rate was obtained.

2.4 Single Factor Experiment on Extraction of Seabuckthorn Seed Oil

2.4.1 Effect of Seabuckthorn Seed Quality on Extraction Rate of Seabuckthorn Seed Oil

To explore the effect of seabuckthorn seed quality on the extraction rate of seabuckthorn seed oil. The crushing time of seabuckthorn seeds was set as 5 s, the extraction solvent was petroleum ether, the solvent was taken as 60 mL, soaking for 20 min, extracting for 4 h and the temperature was set at 70°C. Seabuckthorn seeds with the mass of 1 g, 2 g, 3 g, 4 g and 5 g were taken for experiments. Each group did two parallel experiments.

2.4.2 Effect of Crushing Time on Extraction Rate of Seabuckthorn Seed Oil

To explore the effect of crushing time on the extraction rate of seabuckthorn seed oil. 2 g seabuckthorn seeds were extracted with petroleum ether and 60 mL solvent, soaked for 20 min and extracted for 4 h at 70°C. The extraction experiments of seabuckthorn seed oil were carried out with crushing time of 3 s, 5 s, 7 s, 9 s, 11 s and 13 s respectively.

2.4.3 Effect of Reagent Types on Extraction Rate of Seabuckthorn Seed Oil

To explore the effect of reagent types on the extraction rate of seabuckthorn seed oil. Take 2 g seabuckthorn seeds, crush for 5 s, take 60 mL solvent, soak for 20 min, extract for 4 h, and set the temperature at 70°C. Three reagents, ether, n-hexane and petroleum ether, were used to extract seabuckthorn seed oil.

2.4.4 Effect of Soaking Time on Extraction Rate of Seabuckthorn Seed Oil

To explore the effect of soaking time on the extraction rate of seabuckthorn seed oil. 2 g seabuckthorn seeds were crushed for 5 s, extracted with petroleum ether and 60 mL of solvent for 4 h, and the temperature was set at 70°C. The extraction experiments of seabuckthorn seed oil were carried out with soaking time of 10 min, 20 min, 30 min and 40 min respectively.

2.5 Response Surface Methodology for Extraction of Seabuckthorn Seed Oil

There are three factors and three levels in this experiment. See Table 3 for factor level and Table 4 for experimental table.

Table 3. Response Surface Experiment Selection Conditions

Level	Factor		
	Seabuckthorn seed quality (g)	Crushing time (s)	Soaking time (min)
1	1	9	20
2	2	11	30
3	3	13	40

Table 4. Response Surface Experimental Table

Serial number	Seabuckthorn seed quality (g)	Crushing time (s)	Soaking time (min)
1	2	11	30
2	1	13	30
3	2	11	30
4	1	11	40
5	2	9	40
6	3	11	20
7	2	13	40
8	1	9	30
9	2	11	30
10	3	11	40
11	2	11	30
12	2	13	20
13	1	11	20
14	2	9	20
15	3	13	30
16	2	11	30
17	3	9	30

2.6 Determination of Extraction Rate of Seabuckthorn Seed Oil

Weighing of seabuckthorn seed oil: Weigh the original quality of the extraction cup and the quality of the extraction cup after constant weight, and the twice quality difference is the extraction amount of seabuckthorn seed oil (Zhao & Liu, 2019).

In the equation: m_1 is the mass of seabuckthorn seed powder taken at the beginning of the experiment, g; m_2 is the quality of seabuckthorn seed oil obtained by extraction, g.

2.7 Determination of Total Fatty Acid Composition in Seabuckthorn Seed Oil

Take 5 μL seabuckthorn seed oil into a 10 mL gland centrifuge tube, add 1 mL chromatographic pure n-hexane, 200 μL potassium hydroxide methanol solution (2 mol/L) and swirl for 1 min, then add 1 g sodium bisulfate, and swirl until no sodium bisulfate is attached to the centrifuge tube wall. After vortexing, let stand, take 1 mL supernatant, transfer it to the injection brown vial, and perform GC analysis.

Parameters of GC analysis instrument are: Agilent 7820A gas chromatograph; FID detector; Chromatographic column DB-23(30m \times 0.250mm); Carrier gas: nitrogen. Programmed temperature rise: keep the temperature of the column box at 50°C for 2 min, raise it to 180°C at a rate of 10°C/min, keep it for 5 min, and then raise it to 230°C at a rate of 5°C/min, and keep it for 5 min. The temperature of the rear inlet is 250°C; 12.97kpa pressure; The split ratio is 15: 1; The flow rate is 12ml/min; Total flow rate is 14.4ml/min; Septum sweeping 1.2; Carrier gas saving: off. The post detector temperature is 280°C. The flow rate of hydrogen is 30ml/min; The air flow rate is 300ml/min; Exhaust gas (nitrogen) 25ml/min; Sample injection needle size 10 μL ; Sample injection volume is 2 μL . Mark the sequence table, start the analysis after the instrument is stable, mark each fatty acid in the running chart, and calculate the average value and standard deviation between the two experimental groups.

2.8 Determination of sn-2 Fatty Acid Composition in Seabuckthorn Seed Oil

Add 50 μL seabuckthorn seed oil into a 10 mL centrifuge tube with stopper, add 0.5 mL analytically pure n-hexane, 2 mL Tris-HCl buffer (C=1 mol/L,PH=8), 0.5 mL sodium cholate solution (C=1 g/L), 0.2 mL calcium chloride solution (C=220 g/L), and then add 20 mg sodium cholate solution. Seal the periphery of the cover with a sealing film, put it into a 40°C thermostatic magnetic stirring bath for heating in water bath, shake it up and down by hand for 10 min, finally add 1 mL hydrogen chloride solution (C=6 mol/L), add 0.8 mL anhydrous ether after shaking it evenly, then put it into a centrifuge for 5 min at the speed of 3500 r/min, after centrifugation, transfer in a fume hood, take out the supernatant, evaporate to 0.5 mL, and use a 10 μL pipette gun to spot samples (Bo & Qin, 2008; Zeng, Deng, & Yu, 2020).

Adding about 1 cm of chromatographic solution (n-hexane analytical grade: ether: formic acid = 130: 70: 3) into the chromatographic cylinder, and standing for 30-40 min to homogenize the chromatographic solution. After homogenization, the plated boards (triplicate boards with the size of 10 cm*10 cm) were placed in a chromatography cylinder, and the chromatography plate was taken out to dry in a fume hood when the chromatography liquid ran to 1-2 cm from the top of the chromatography plate; If the separation of triester and fatty acid was not obvious during observation, the isochromatographic solution ran to the top and waited for 3-5 min to separate it, the chromatographic plate was taken out to dry, stained with staining agent (anhydrous ethanol: 2', 7'-dichlorofluorescein C=4 g/L), and observed by ultraviolet light after staining and drying.

The chromatographic plate was observed by ultraviolet light. The silica gel on the monoglyceride strip was scraped off and placed in a 10 mL gland-covered centrifuge tube. Then 0.7 mL chromatographic

pure normal hexane and 200 μ L potassium hydroxide methanol solution (2 mol/L) were successively added and vortexed for 1 min. Then 1 g sodium hydrogen sulfate was added and vortexed until no sodium hydrogen sulfate was attached to the wall of the centrifuge tube. After vortexing, allow to stand and transfer 1 mL of the supernatant to a brown vial for GC analysis.

Parameters of GC analysis instrument refer to those during determination of total fatty acids.

3. Results and Analysis

3.1 Single Factor Experimental Results and Analysis of Extracting Seabuckthorn Seed Oil by Soxhlet Extraction

3.1.1 Effect of Seabuckthorn Seed Quality on Extraction Rate of Seabuckthorn Seed Oil

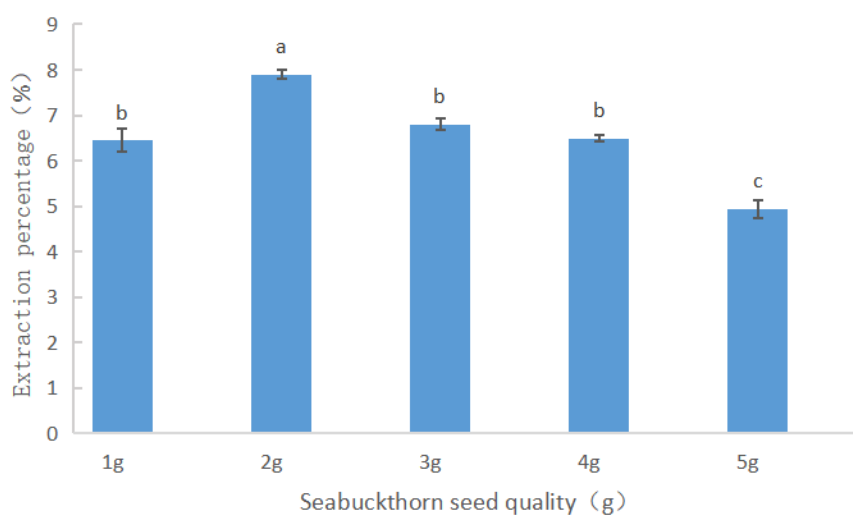


Figure 1. Effect of Seabuckthorn Seed Quality on Extract Rate of Seabuckthorn Seed Oil

It can be seen from Figure 1 that with the increase of seabuckthorn seed quality, the extraction of seabuckthorn seed oil first increased and then decreased. When the mass of seabuckthorn seeds is 2 g, the extraction rate of seabuckthorn seed oil is the highest, which is 7.89%. When the mass is 3 g, the extraction rate tends to decrease obviously. The SPSS software was used to analyze its significance. There was a significant difference between the extraction rate of seabuckthorn seed oil when it was 2 g and when the seabuckthorn seed quality was 1 g, 3 g, 4 g and 5 g ($p < 0.05$), so the seabuckthorn seed quality was finally determined to be 2 g for experiment.

3.1.2 Effect of Crushing Time on Extraction Rate of Seabuckthorn Seed Oil

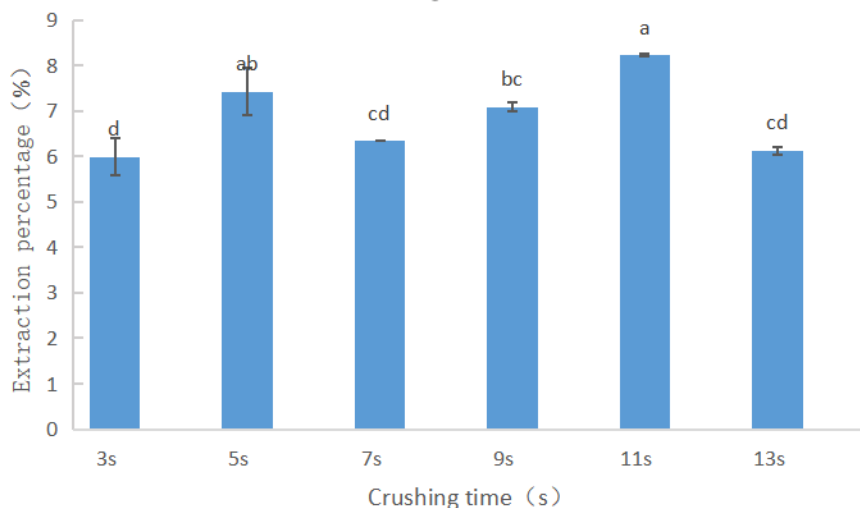


Figure 2. Effect of Broken Time on Extract Rate of Seabuckthorn Seed Oil

It can be seen from fig. 2 that the extraction rate of seabuckthorn seed oil tends to increase slowly with the increase of the breaking time of seabuckthorn seeds. When the crushing time of seabuckthorn seeds is 11 s, the extraction rate of seabuckthorn seed oil is higher. The extraction rate was 8.22% in 11 s. By SPSS software, the extraction rate of seabuckthorn seed oil was significantly different between the crushing time of 11 s and the crushing time of 3 s ($p < 0.05$), but there was no significant difference among other crushing times. Therefore, when extracting seabuckthorn seed oil, the crushing time of seabuckthorn seeds was 11 s.

3.1.3 Effect of Reagent Types on Extraction Rate of Seabuckthorn Seed Oil

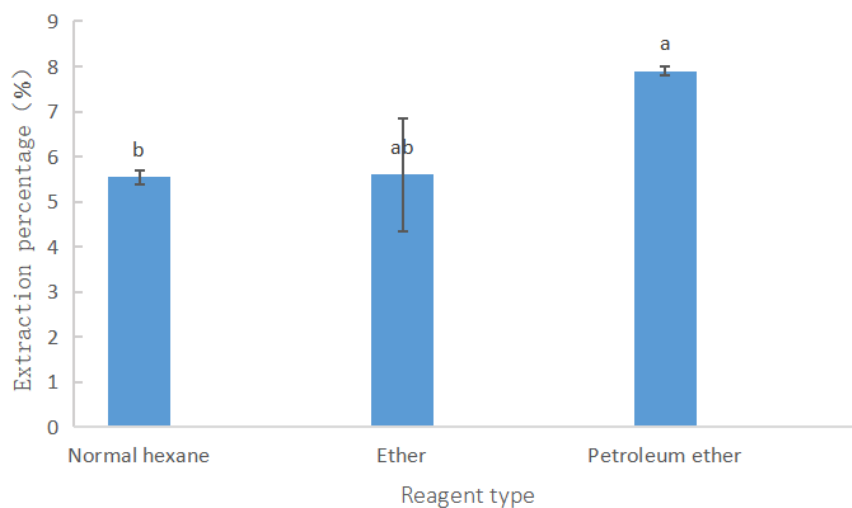


Figure 3. Effect of Reagent Types on Extract Rate of Seabuckthorn Seed Oil

As shown in Figure 3, when petroleum ether was used as the extraction solvent, the extraction rate of seabuckthorn seed oil was the highest, reaching 7.89%. Statistical analysis was performed using SPSS software for statistical significance. When petroleum ether and n-hexane were used, there was a

significant difference in the extraction rates of seabuckthorn seed oil ($p < 0.05$). Therefore, when seabuckthorn seed oil was extracted, petroleum ether was selected as the extraction solvent for the experiment.

3.1.4 Effect of Soaking Time on Extraction Rate of Seabuckthorn Seed Oil

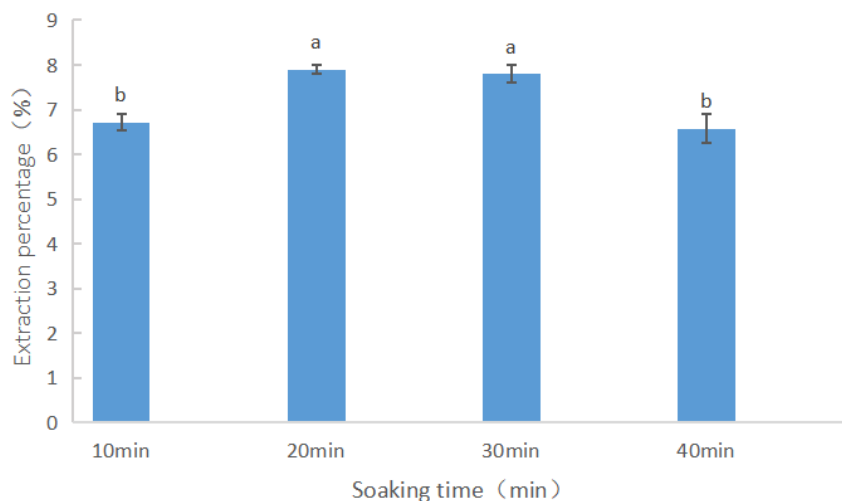


Figure 4. Effect of Soaking Time on Extract Rate of Seabuckthorn Seed Oil

As shown in Figure 4, SPSS software was used for significance analysis. The results showed that there was a significant difference in extraction rates of seabuckthorn seed oil when the soaking time was 10 min, and when the soaking time was 20 min or 30 min ($P < 0.05$). There was significant difference in extraction rates of seabuckthorn seed oil when the soaking time was 40 min, and when the soaking time was 20 min or 30 min ($P < 0.05$). When the soaking time was 20 min and 30 min, there was no significant difference in the extraction rates of seabuckthorn seed oil. When the soaking time was 20 min, the extraction rate of seabuckthorn seed oil increased significantly, and the extraction rate was 7.79%. The change of extraction rate was gentle and almost unchanged from 20 to 30 min. The extraction rate of seabuckthorn seed oil decreased significantly when the soaking time was 40 min. Therefore, when seabuckthorn seed oil was extracted, the soaking time of 30 min was taken for the experiment.

3.2 Determination of the Best Conditions for Extracting Seabuckthorn Seed Oil by Soxhlet Extraction

Through single factor experiment, Design-Expert 8.06 was used to design response surface methodology, and the three factors that greatly affected the extraction rate of seabuckthorn seed oil, including seed quality, crushing time and soaking time, were selected as Box-Behnken design. With the extraction rate of seabuckthorn seed oil as the response value, the design and results are shown in the following table.

Table 5. Response Surface Experiment Data

Serial number	A	B	C	Extraction
	Seabuckthorn seed quality g	Crushing time s	Soaking time min	percentage %
1	2	11	30	6.042
2	1	13	30	5.474
3	2	11	30	5.941
4	1	11	40	5.08
5	2	9	40	3.493
6	3	11	20	4
7	2	13	40	5.897
8	1	9	30	4.786
9	2	11	30	6.645
10	3	11	40	4.494
11	2	11	30	6.838
12	2	13	20	5.15
13	1	11	20	6.993
14	2	9	20	4.65
15	3	13	30	5.53
16	2	11	30	6.691
17	3	9	30	3.228

The analysis of variance of the regression model is shown in Table 6.

Table 6. Regression Model Variance Analysis

Source of variance	Sum of squares	Freedom	Variance	F value	P value	
Model	19.65	9	2.18	11.43	0.0020	Significance
A	3.23	1	3.23	16.88	0.0045	
B	4.34	1	4.34	22.72	0.0020	
C	0.42	1	0.42	2.19	0.1826	
AB	0.65	1	0.65	3.41	0.1074	
AC	1.45	1	1.45	7.58	0.0284	
BC	0.91	1	0.91	4.74	0.0659	
A2	1.87	1	1.87	9.78	0.0167	
B2	4.30	1	4.30	22.50	0.0021	
C2	1.64	1	1.64	8.56	0.0222	

Residual	1.34	7	0.19			
Missing item	0.67	3	0.22	1.33	0.3828	Insignificance
Pure error	0.67	4	0.17			
Total	20.99	16				
R2			0.9363			
C.V.%			8.17			

It can be seen from ANOVA of regression model in Table 6 that the P value of the model selected in the experiment was 0.0020, which was extremely significant ($P < 0.05$ was reasonable in theory), indicating that the linear relationship between each factor described in the regression equation and the response value was significant, and also indicating the reliability of this test method. The missing term $P = 0.3828 > 0.05$ was not significant, indicating that the equation had good fitting condition for the experiment and small experimental error. The method could be used to predict the extraction rate of seabuckthorn seed oil under different reaction conditions.

By analyzing the p-values of the primary, interactive and quadratic terms, we can find that the quadratic term, the primary term and the interactive term have the most significant effects on the equation. Through fitting, the quadratic polynomial regression equation of seabuckthorn seed oil extraction rate (R) on seabuckthorn seed quality (A), crushing time (B) and soaking time (C) was obtained:

$$R = 6.43 - 0.64A + 0.74B - 0.23C + 0.40AB + 0.60AC + 0.48BC - 0.67A^2 - 1.01B^2 - 0.62C^2$$

A closer R2 to 1 indicates that this model is more predictive of its response values. $R^2 = 0.9363$ in this experiment, that is, 93.63% of the changes in response value was derived from the selected variables, indicating that the equation fitted well.

The value of coefficient of variation (C.V.%) indicated the problem of accuracy. The experimental reliability was negatively correlated with the coefficient of variation. The coefficient of variation of this experiment was 8.17, indicating that the experiment was reliable.

The surface diagram of the three-dimensional space composed of response values for each test factor A, B and C is the response surface diagram, which vividly presents the interaction between the optimal parameters and each parameter. When the eigenvalues are both positive and negative, the figure is a saddle-shaped surface, and there is no polar value. When the characteristic value is negative, the figure is a hill surface and there is a maximum; When all the eigenvalues are positive, the graph is a valley-shaped surface with minimal value. The following is the response surface analysis diagram of different factors made according to the regression equation.

Figures 5 and 6 showed the interactive effects of crushing time and quality on the extraction rate of seabuckthorn seed oil when the soaking time was 30 min. The strength of interaction could be reflected by the shape of contour line: if the interaction was significant, the graph presented as saddle-shaped or elliptical; if the interaction was not significant, the graph presented as circular.

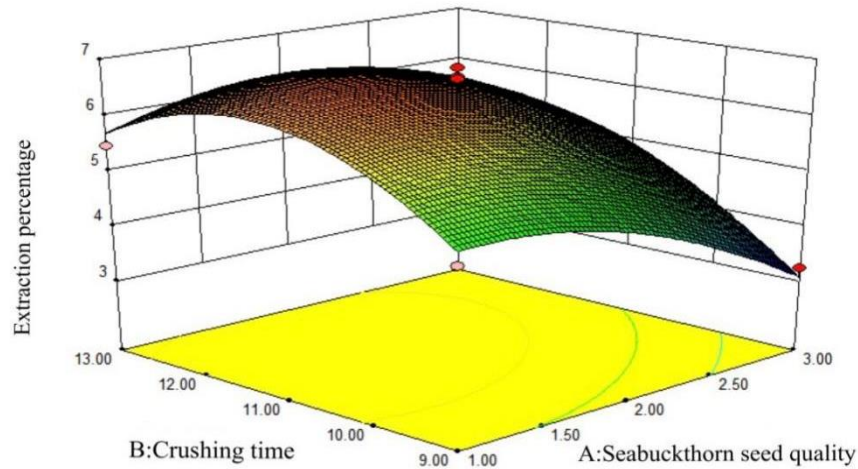


Figure 5. Response Surface Diagram of Crushing Time and Seabuckthorn Seed Quality

As shown in Figure 5, the crushing time had little effect on the extraction rate of seabuckthorn seed oil, and the curve was smooth, while the quality of seabuckthorn seed was significantly affected, and the curve was steep.

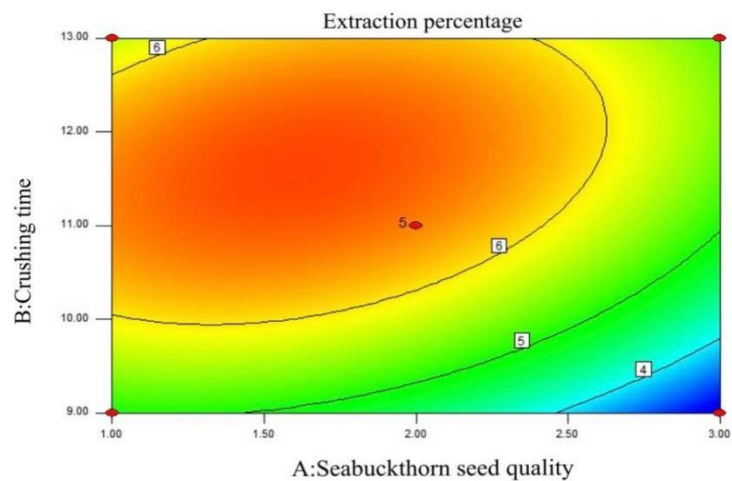


Figure 6. Contour Lines of Breaking Time and Seabuckthorn Seed Quality

As shown in Figure 6, when the crushing time was 11 s, the contour line was elliptical, indicating that the interactive effect of crushing time and seed quality on the extraction rate of seabuckthorn seed was significant.

Figures 7 and 8 showed the interactive effects of soaking time and seed quality on the extraction rate of seabuckthorn seed oil when the crushing time was 11 s.

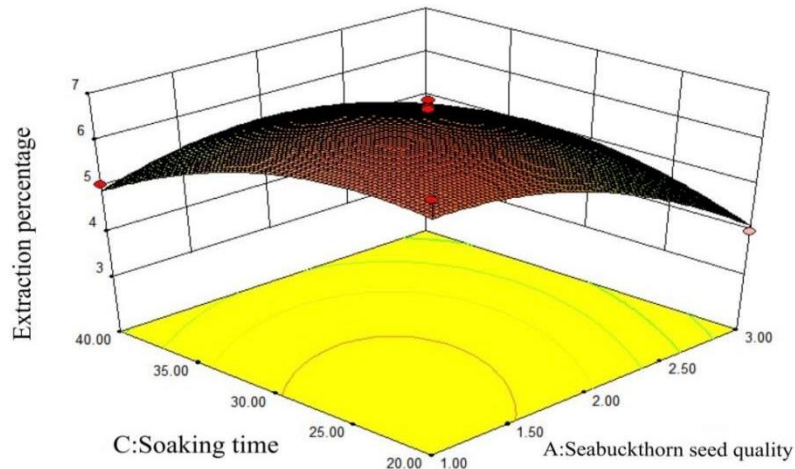


Figure 7. Response Surface Plot of Soaking Time and Seabuckthorn Seed Quality to the Extraction Rate of Seabuckthorn Seed Oil

As shown in Figure 7, the image presented a saddle shape, indicating that the effects of soaking time and seed quality on the extraction rate of seabuckthorn seed oil were similar.

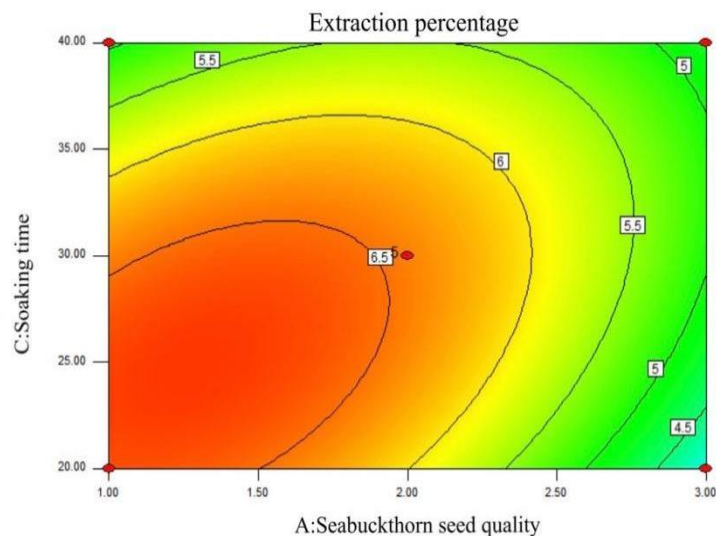


Figure 8. Contour Lines of Soaking Time and Seabuckthorn Seed Quality

As shown in Figure 8, compared with the effect of soaking time and the quality of seabuckthorn seed on the extraction rate of seabuckthorn seed oil, the effect of the quality of seabuckthorn seed was more significant, and the contour line was closer to the quality of seabuckthorn seed.

Figures 9 and 10 showed the interaction between soaking time and crushing time on the extraction rate of seabuckthorn seed oil when the quality of seabuckthorn seed was 2 g.

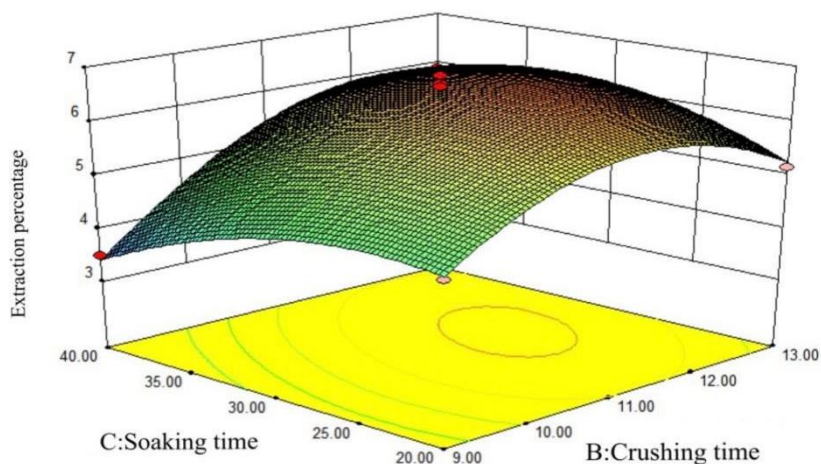


Figure 9. Response Surface Plot of Soaking Time and Crushing Time to the Extraction Rate of Seabuckthorn Seed Oil

As shown in Figure 9, soaking time had little effect on the extraction rate of seabuckthorn seed oil, manifested as a smooth curve, while crushing time had greater effect, manifested as a steep curve.

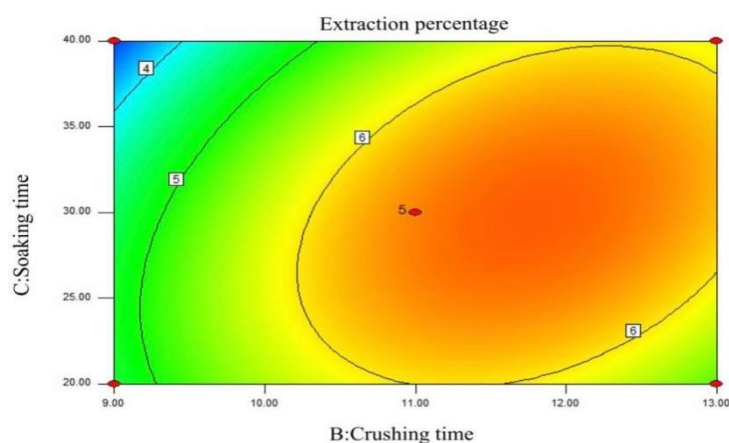


Figure 10. Contours of Soaking Time and Crushing Time

As shown in Figure 10, compared with the effect of soaking time and crushing time on the extraction rate of seabuckthorn seed oil, the effect of crushing time was more significant, and the contour line was closer to the crushing time.

3.3 Verification Test

The theoretical optimum extraction conditions and response values were obtained by optimizing the extraction conditions with the experimental assistant software Design Expert as follows: seabuckthorn seed mass is 1.37 g, crushing time is 11.27 s, soaking time is 25.62 min, and the extraction rate of seabuckthorn seed oil is 6.73%.

Verification of the model: considering and analyzing the good operability of the experiment, the experiment was carried out under the conditions of 1.40 g seabuckthorn seed mass, 11 s crushing time

and 26 min soaking time. The extraction rate of seabuckthorn seed oil calculated by the extraction rate calculation method is 6.56%, and the relative error is 2.82%, less than 10%. It is proved that it is feasible to optimize the extraction rate of seabuckthorn seed oil by response surface methodology.

3.4 Fatty Acid Composition Analysis of Seabuckthorn Seed Oil

The gas chromatographic diagram of total fatty acids in seabuckthorn seed oil is shown in Figure 11.

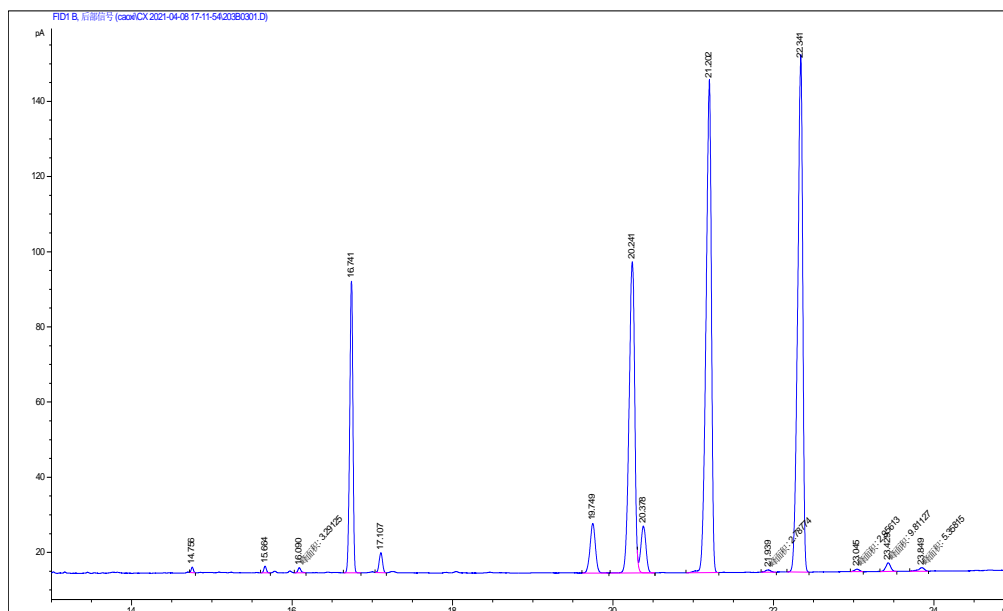


Figure 11. Chromatogram of Fatty Acid Composition of Seabuckthorn Seed Oil

Chromatograms of *sn*-2 fatty acid in seabuckthorn seed oil are shown in Figure 12.

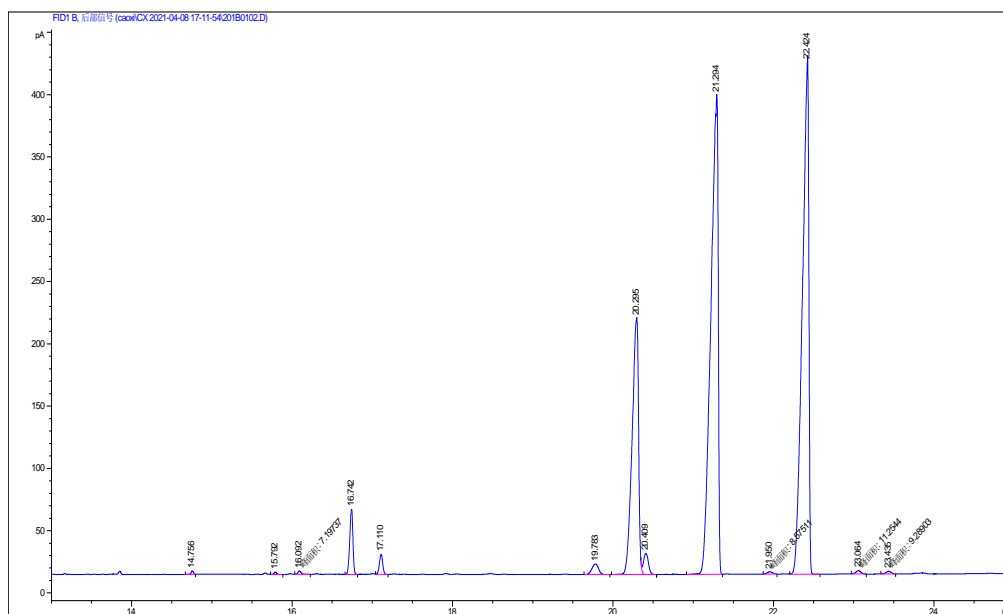


Figure 12. Chromatogram of Fatty Acid Composition at *sn*-2 Position of Seabuckthorn Seed Oil

Table 7. Fatty Acid Composition Content

Fatty acid species	Total content(%)	<i>Sn</i> -2 position content(%)
C14:0	0.14 ±0.00	0.08 ±0.02
C14:1	0.19 ±0.00	0.07 ±0.00
C15:0	0.12 ±0.07	0.14 ±0.03
C16:0	10.77 ±0.03	2.11 ±0.27
C16:1	0.79 ±0.02	0.71 ±0.03
C18:0	3.09 ±0.02	0.71 ±0.09
C18:1n9	20.21 ±0.22	18.44 ±0.01
C18:1n7	2.73 ±0.01	1.08 ±0.08
C18:2n6	30.17 ±0.23	39.31 ±0.18
C18:3n6	0.13 ±0.01	0.13 ±0.01
C18:3n3	30.37 ±0.03	36.98 ±0.36
C20:0	0.56 ±0.58	0.12 ±0.08
C20:1	0.49 ±0.01	0.15 ±0.00
C20:2	0.27 ±0.01	ND

Note. ND indicates that the result is not detected

The results in Table 7 showed that the seed oil of *Hippophae rhamnoides* contained more linoleic acid, linolenic acid and oleic acid, 30.17%, 30.37% and 20.21% respectively, which were also the main *sn*-2 fatty acids, with the contents of 39.31%, 36.98% and 18.44%, respectively.

We can calculate the proportion of *sn*-1 or *sn*-3 by using the formula: $(3x-y)/2=z$ through Table 1 and the 39.31% linoleic acid at *sn*-2. X: linoleic acid in total acid; Y: linoleic acid% ratio in *sn*-2 position; Z: linoleic acid at position *sn*-1 or *sn*-3. The calculated linoleic acid content at the *sn*-1 or *sn*-3 position was 25.6%. Therefore, we could know that the linoleic acid in seabuckthorn seed oil was mainly present at the *sn*-2 position, while the linoleic acid at the *sn*-1, *sn*-3 positions was less (Zhao & Liu, 2019).

From 18.44% oleic acid at the *sn*-2 position, we can calculate an oleic acid content of 21.10% at the *sn*-1 or *sn*-3 position. Therefore, we could know that the oleic acid in seabuckthorn seed oil was mainly present at the *sn*-1 and *sn*-3 positions, while the oleic acid at the *sn*-2 position was less.

We can calculate the linolenic acid content at *sn*-1 or *sn*-3 as 27.07% from 36.98% at *sn*-2. Therefore, we could know that the linolenic acid in seabuckthorn seed oil was mainly present at the *sn*-2 position, while the linolenic acid at the *sn*-1, *sn*-3 positions was less.

4. Conclusion

4.1 Single Factor Experimental Conclusion

Through single factor test, the experimental conditions of high extraction rate of seabuckthorn seed oil were determined as follows: seabuckthorn seed mass 2.00 g, crushing time 11 s and soaking time 30 min. Subsequently, the experimental conditions were optimized by response surface methodology and the optimal experimental conditions were obtained.

4.2 Response Surface Experimental Conclusion

The optimum extraction conditions of seabuckthorn seed oil were determined by Box—Behnken response surface methodology: the weight of seabuckthorn seed was 1.40 g, the crushing time was 11 s and the soaking time was 26 min. Under these conditions, the extraction rate of seabuckthorn seed oil reached 6.56%.

4.3 Fatty Acid Composition Analysis of Seabuckthorn Seed Oil

The seed oil of *Hippophae rhamnoides* contains more linoleic acid, linolenic acid and oleic acid, 30.17%, 30.37% and 20.21% respectively, which are also the main *sn*-2 fatty acids, with the contents of 39.31%, 36.98% and 18.44% respectively.

The results showed that linoleic acid in seabuckthorn seed oil mainly existed at *sn*-2 position; The oleic acid in seabuckthorn seed oil mainly existed at *sn*-1 and *sn*-3 positions. Linolenic acid in seabuckthorn seed oil mainly existed at *sn*-2 position.

5. Summary and Experience

From the middle of December, my experimental subject was determined as extraction of seabuckthorn seed oil and analysis of its fatty acid composition. Now I have successfully completed the experiment. In these two short months, I have learned many things that I cannot learn in class or from books. I have made great progress in both theoretical knowledge and practical ability. More importantly, I have learned how to use some instruments and equipment. I have benefited a lot.

After I determine the design topic, under the guidance of teacher Cao Xi I access to a large number of papers related to the subject, translation of literature, in the process of my research content and subject related research progress also have more understanding, let me from the beginning at a loss to the back slowly in-depth understanding, ideas gradually clear.

In these two months, we have paid a lot, but we have also gained a lot. Through this graduation project, I have been further familiar with and studied the correct use of basic instruments in the laboratory and the relevant precautions, and deepened the understanding and experience of the knowledge learned in the four years of college. The process of experiment is a process of continuous exploration, finding new problems and solving problems in the exploration. In this process, I learned a lot of knowledge by going to the library to look for materials and reading books. I expanded my knowledge and, more importantly, improved my ability to think, analyze and solve problems. In the experiment, sometimes to get a set of data and a conclusion, we need to constantly repeat it. After several failures, we can get a

better result. This makes me deeply realize that the experiment needs scientific analysis, rigorous method and correct operation. It also needs a persistent and persistent spirit.

Through this experiment, I have deeply realized that “learning from paper is like learning from experience, and you never know how to practice it”. I thought I knew enough about the experimental operation by consulting the literature, but when I conducted the experiment, I found that these were far from enough. Only by putting the theory into practice can the theory be tested. Faced with difficulties and challenges, to actively face and solve, active learning, exercise their ability to find and solve problems. However, at the same time, “knowing it is knowing it, and not knowing it is not knowing it.” When facing problems that one cannot solve, one should communicate with the teacher in time to sort out the thinking, instead of relying on imagination alone, which wastes time and is useless. This intuitive and practical operation, face-to-face communication and discussion with my mentor, helped me consolidate and deepen my knowledge learned in the four years of college. When I finally completed the experiment and finished writing the experimental records, what I felt was the satisfaction and pride of my hard work and achievements. In this period of hard but substantial time, it was the mentor’s patient character and rigorous scientific attitude that helped and encouraged me to complete my graduation experiment and let me learn how to be a human being.

I believe that this graduation project process will benefit me for my whole life.

Acknowledgements

First of all, I would like to express my sincere thanks to my supervisor, teacher Cao Xi, for her careful guidance and the teacher elder sister Pan Yayu from the laboratory for her warm-hearted help. Thank you from the bottom of my heart!

In these two months of experiments, I have encountered many difficulties and doubts. Fortunately, you are here, not only to help me, to reassure me, but also to encourage me and support me. Cao teacher meticulous style affects me, make me in the analysis of the problem, more rigorous when dealing with problems; The experimental operation is more careful. In the whole process of graduation design, each set of data is the result of my strict operation, repeated many times and careful verification. At the same time, teacher Cao’s persuasion also makes my understanding of the problem more clear and profound, and strive to do extrapolate, which also let me benefit a lot. When I encounter doubts and problems in the process of experiment, there is always the teacher elder sister’s enthusiastic help; When I encountered difficulties and setbacks in the process of experiment, there was always their encouragement and support. All of these are the power source that inspired me to persist in the meticulous and rigorous completion of the experiment and complete the graduation project successfully.

Finally, I would also like to thank all the teachers for their teaching and all the students for their care and help in the past four years. Thank you!

References

- Bo, H. B., & Qin, R. (2008). Comparative study on fatty acid composition of seabuckthorn fruit oil and seabuckthorn seed oil. *Food Science*, 29(5), 378-381.
- Chen, Y. D., & Jiang, Z. R. (1990). Study on chemical composition and properties of seabuckthorn fruit and its oil. *Forest Products Chemistry and Industry*, 10, 163-257.
- Dubey, S., Ramana, M. V., & Mishra, A. (2018). Seabuckthorn (*Hippophae rhamnoides* and *Hippophae salicifolia*) Seed Oil in Combating Inflammation: A Mechanistic Approach. *Pharmacognosy Research*, 10(4), 404-407. https://doi.org/10.4103/pr.pr_71_18
- Hao, W., He, Z., & Zhu, H. (2019). Sea buckthorn seed oil reduces blood cholesterol and modulates gut microbiota. *Food & Function*, 10(9), 5669-5681. <https://doi.org/10.1039/C9FO01232J>
- Hou, D. Y., Hui, R. H., & LiH T. C. et al. (2002). Research progress of *Hippophae rhamnoides*. *Journal of Anshan Normal University*, 4(1), 49-53.
- Huang, N. K. Matthan, N. R., & Galluccio, J. M. (2010). Supplementation with Seabuckthorn Oil Augmented in 16:1n-7t Increases Serum Trans-Palmitoleic Acid in Metabolically Healthy Adults: A Randomized Crossover Dose-Escalation Study. *Journal of Nutrition*, 150(6), 1388-1396. <https://doi.org/10.1093/jn/nxaa060>
- Liu, H. B., Wen, N., Zhou, M. K. et al. (2016). Experimental study on microwave extraction of seabuckthorn seed oil. *World Traditional Chinese Medicine*, 11(10), 2134-2137.
- Shi, J. Wang, L., & Lu, Y. (2017). Protective effects of seabuckthorn pulp and seed oils against radiation-induced acute intestinal injury. *Journal of Radiation Research*, 58(1), 24-32. <https://doi.org/10.1093/jrr/rrw069>
- Vashishtha, V., Barhwal, K., & Kumar. A. (2017). Effect of seabuckthorn seed oil in reducing cardiovascular risk factors: A longitudinal controlled trial on hypertensive subjects. *Clinical Nutrition*, 36(5), 1231-1238. <https://doi.org/10.1016/j.clnu.2016.07.013>
- Wu, Y., & Wang, B. W. (2000). Effect of seabuckthorn oil on microcirculation. *Pharmacology and clinical of traditional Chinese medicine*, 16(6), 18-19.
- Zang, Q. Q., Deng, G. C., & Cong, R. H. et al. (2015). Research progress on the effective components and pharmacological functions of seabuckthorn oil. *Chinese Grease*, 40(05), 76-81.
- ZEB, A. (2004). Chemical and nutritional constituents of sea buck thorn juice. *Pakistan J Nutr*, 3(2), 99-106.
- Zeng, F. Z., Deng, G. C., & Yu, X. (2020). Effects of processing parts and extraction process on the quality characteristics of seabuckthorn oil and oil phase migration of major lipid companions. *China Grease*, 351(05), 100-106. <https://doi.org/10.3923/pjn.2004.99.106>
- Zhang, A. L., & Ni, R. H. (2001). Plant chemistry and medical care function of *Hippophae*. *Western Cereal and Oil Science and Technology*, 06, 32-35.
- Zhang, D., & Chen, B. H. (2010). Analysis on the current situation and development of seabuckthorn extraction industry in China. *Qinghai Science and Technology*, 17(05), 12-15.

Zhang, G. H., Jin, R. Y., Zhang, S. A. et al. (2018). Optimization of extraction process and antioxidant activity of seabuckthorn seed oil. *International Journal of Traditional Chinese Medicine*, 40(11), 1075-1078.

Zhao, H. P., & Liu, J. A. (2019). Optimization of microwave-assisted extraction of seabuckthorn seed oil by response surface methodology. *Journal of Jilin Institute of Technology*, 36(11), 17-21.

Author's brief introduction:

Ren Guangling (1998-), female, Han, Chengdu, Sichuan Province, Undergraduate, Research direction: Food quality and safety.