Determination of Physicochemical Properties of Linseed Oil and Its Comparison with Sesame Oil

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Abstract
In this study, the physical and chemical properties of linseed oil and sesame seed oil were measured and compared. The results showed that the nitrogen content, total protein and total amino acids in linseed were 3.27%, 20.39%, 92158.22 μg/g FW, respectively, and 2.79%, 17.43%, 92246.59 μg/g FW in sesame seeds correspondingly. The content of unsaturated fatty acids such as linolenic acid and linoleic acid in linseed was 290.57 μg/g, 0.55 μg/g, and 3326.69 μg/g, 0.24 μg/g in sesame seed oil. In addition, the contents of palmitic acid, palmitoleic acid, arachidic acid and heptadecanoic acid in sesame were significantly higher than those in linseed. The contents of ergosterol, β-sitosterol and stigmasterol in linseed were 6.57 μg/g, 972.66 μg/g, and 123.27 μg/g, and 5.62 μg/g, 930.57 μg/g, and 145.67 μg in sesame, which were significantly different. The measurement results of metal elements showed that the metal content of linseed and sesame seed were both Mg>Ca>Fe>Zn, the content of Fe, Mg and Zn in linseed were both higher than that in sesame seed, the Ca element in sesame seeds was higher than that in linseed. It can be seen that linseed oil and sesame seed oil have their own advantages and have different nutrition and health functions.

Keywords Flaxseed; Sesame; Composition

Background
Flax (Linum usitatissimum), whose fiber is mainly used for textiles, is also an important oil crop. The oil content of linseed is about 35%~45% (An et al., 2020), and linseed is being obtained in the world food supply significant progress is expected to increase demand in the food market. Flaxseed has potential health benefits due to its rich α-linolenic acid, and has been the focus of increasing attention of nutritionists, and medical researchers. In addition, flax protein helps prevent or treat heart disease and supports the immune system (Goyal et al., 2014). Sesame (Sesamum indicum) has been eaten in Europe and Asia since ancient times and is one of the oldest oil crops in the world. China is the world's largest consumer of sesame. In 2014, China actually consumed about 1.3 million tons of sesame, accounting for about 30% of the world's total output. Sesame is rich in many trace active substances that are beneficial to human health. The World Health Organization listed sesame oil as the best edible oil at the “113th meeting of the World Health Organization” held in 2011.

The flax variety "Huaya 3" and the sesame variety "Zhongzhi 11" used in this experiment. "Huaya No. 3" was an excellent mutant single plant selected from the germplasm resource Pekinense imported from Poland by the Heilongjiang Academy of Agricultural Sciences with the goal of both fiber and oil, double high yield and strong stress resistance. It was an economic crop of the Heilongjiang Academy of Agricultural Sciences from Qinghua Kang Researcher of the Institute. "Zhongzhi 11" was China's first successful application of sesame seeds through aerial breeding. It integrates high-yield, high-oil, lodging resistance, disease resistance and other excellent traits. It was provided by the National Sesame Germplasm Bank. In this study, the physical and chemical properties of linseed oil and sesame seed oil were measured and compared, aiming to understand the contents of total amino acids, unsaturated fatty acids and metal elements in the two seed oils that were beneficial to human health.
1 Results and Analysis

1.1 Comparison of nitrogen content and total protein content of flaxseed and sesame seed

The nitrogen content and total protein of two varieties, "Huaya 3" and "Zhongzhi 11" were determined (Table 1). The data showed that the total protein content of "Huaya 3" seeds was 20.39%, and the crude protein content of "Zhongzhi 11" seeds was 17.43%. Through the analysis of variance, the nitrogen content and total protein in "Huaya 3" were higher than "Zhongzhi 11", and reached a very significant level of difference (Table 1).

| Type               | Huaya No.3 | Zhongzhi No.11 |
|--------------------|------------|----------------|
| Nitrogen content (%)| 3.27 ± 0.01A | 2.79 ± 0.0B |
| Total protein (%)   | 20.39 ± 0.13A | 17.43 ± 0.02B |

Note: Each value in the table is "mean ± standard deviation". Different lowercase letters in the same industry indicated significant differences at the level of $p<0.05$, and different uppercase letters indicated significant differences at the level of $p<0.01$, the same below

1.2 Comparison of amino acid content of flaxseed and sesame

Flaxseed protein is rich in arginine, aspartic acid, glutamic acid, and the limiting amino acids are lysine, methionine, and cysteine (Chung et al., 2005). Sesame was rich in protein and had a complete variety of amino acids, among which glutamic acid and arginine were the highest. There were 17 common amino acids in flaxseed and sesame. By comparison, the amino acids with the highest content in flaxseed and sesame were glutamic acid and arginine, and the content in flaxseed was 15895.28 μg/g FW, 8713.43 μg/g FW; the content in sesame was 17561.12 μg/g FW, 11296.13 μg/g FW, and the content in sesame was significantly higher than that in flax (Table 2). And from the perspective of total amino acid, the content of sesame was slightly higher than that of flaxseed. For restricted amino acids, the content in flaxseed was lysine value was 1824.56 μg/g FW, methionine value was 2283.36 μg/g FW; In sesame, the lysine value was 1953.50 μg/g FW, methionine value was 3039.85 μg/g FW. In terms of the total amount of restrictive amino acids, the content of sesame was higher than that of flaxseed.

| Type | Huaya No.3 | Zhongzhi No.11 |
|------|------------|----------------|
| Asp  | 8208.27 ± 12.36A | 7 558.67 ± 37.22B |
| Glu  | 15 895.28 ± 48.37B | 17 561.12 ± 33.31A |
| Ser  | 6 046.84 ± 48.47A | 5 731.16 ± 54.78b |
| Gly  | 8 360.22 ± 93.14A | 6 522.15 ± 84.68B |
| His  | 1 797.86 ± 74.04B | 3 011.60 ± 63.04A |
| Arg  | 8 713.43 ± 23.50B | 11 296.13 ± 198.04A |
| Thr  | 4 142.07 ± 17.43a | 3 622.24 ± 68.74b |
| Ala  | 4 503.56 ± 141.11a | 4 872.50 ± 123.05a |
| Pro  | 7 358.12 ± 162.98a | 5 276.62 ± 143.13b |
| Tyr  | 3108.96 ± 18.04b | 3927.69 ± 115.57a |
| Val  | 3382.04 ± 23.44a | 2964.19 ± 64.39b |
| Met  | 2 283.36 ± 2.56b | 3 039.85 ± 170.78a |
| Cys  | 377.40 ± 35.19A | 13.02 ± 0.91B |
| Ile  | 3 083.08 ± 19.06a | 2 646.28 ± 61.50b |
| Leu  | 6 624.58 ± 61.31a | 7 078.75 ± 118.36a |
| Phe  | 6 448.65 ± 52.37a | 5 171.12 ± 122.29b |
| Lys  | 1 824.56 ± 12.57b | 1 953.50 ± 21.39a |

Note: Numbers with different letters (a, b, A, B) within a column under the same parameter were significantly different and numbers with the same letter were not significantly different by Duncan’s multiple range test (a or b, $p<0.05$; A or B, $p<0.01$)

1.3 Comparison of fatty acid content of flaxseed and sesame seed

Fatty acid content was related to the quality of edible oil. The saturated fatty acids in vegetable oils include
palmitic acid, arachidic acid, palmitic acid, stearic acid, etc. Excessive intake of saturated fatty acids will increase the risk of coronary heart disease. Among them, linoleic acid and linoleic acid were representative of polyunsaturated fatty acids and essential fatty acids (German and Dillard., 2010). Consumption of linoleic acid reduced postprandial glucose response and had nutritional benefits for humans (Cunnane et al., 1993), while linoleic acid had a positive effect on the prevention of diabetes (Hodge et al., 2007). Flaxseed had a high content of unsaturated fatty acids (Qiang et al., 2019). There were a large number of unsaturated fatty acids in sesame oil, mainly oleic acid and linoleic acid. As shown in Table 3, the contents of linolenic acid, palmitic acid, palmitoleic acid, arachidic acid, heptadecanoic acid, and methyl cis-11,14-eicosapentaenoate acid in "Zhongzhi 11" are all significantly higher than "Huaya 3", especially the content of linolenic acid, is 3326.69 μg/g in Zhongzhi 11, which is 11 times that of "Huaya 3". The content of linoleic acid, myristic acid, behenic acid, and methyl cis-5,8,11,14,17-eicosapentaenoate acid in "Huaya 3" is higher than that of "Zhongzhi 11". The content of oleic acid in "Huaya 3" is more than twice that in "Zhongzhi 11".

### Table 3 Comparison of fatty acid content of flaxseed and sesame

| Fatty acid                              | Huaya No. 3          | Zhongzhi No. 11         |
|-----------------------------------------|----------------------|-------------------------|
| Linolenic acid (μg/g)                   | 290.57 ± 3.56B       | 3326.69 ± 25.11A        |
| Linoleic acid (μg/g)                    | 0.55 ± 0.02A         | 0.24 ± 0.01B            |
| Palmitic acid (mg/g)                    | 44.81 ± 0.96B        | 71.19 ± 0.62A           |
| Palmitoleic acid                        | 146.713 ± 2.65B      | 353.269 ± 0.81A         |
| Myristic acid                           | 77.940 ± 0.73A       | 47.006 ± 0.37B          |
| Arachidic acid                          | 303.067 ± 9.92B      | 1107.434 ± 4.11A        |
| Behenic acid                            | 315.338 ± 47.44a     | 214.739 ± 70.28B        |
| Heptadecanoic acid                      | 82.719 ± 2.93B       | 156.936 ± 45.34A        |
| Methyl cis, cis-11,14-eicosapentaenoate acid | 45.817 ± 1.15B   | 71.179 ± 8.29A          |
| Methyl cis-5,8,11,14,17-eicosapentaenoate acid | 260.989 ± 21.56A | 131.214 ± 7.24B         |

Note: Numbers with different letters (a, b, A, B) within a column under the same parameter were significantly different and numbers with the same letter were not significantly different by Duncan’s multiple range test (a or b, p<0.05; A or B, p<0.01)

### 1.4 Comparison of phytosterol content in flaxseed and sesame

Phytosterol is a plant metabolite of the triterpene family, an essential human health biomolecule that must be obtained from food, and its structure is similar to cholesterol. At the same time, plant sterols have antioxidant effects, can prevent cardiovascular diseases and reduce serum cholesterol levels, and are also ideal food additives. Phytosterols are the most abundant compound in the unsaponifiable part of linseed oil, and are also abundant in sesame oil.

The content of ergosterol in "Huaya 3" and "Zhongzhi 11" was not much different. The stigmasterol in "Zhongzhi 11" was significantly higher than that in "Huaya 3"; in "Huaya 3" The content of β-sitosterol was higher than "Zhongzhi No. 11". The content of free cholesterol and total cholesterol in "Huaya 3" was extremely significantly higher than that in "Zhongzhi 11" (Table 4).

### Table 4 Comparison of phytosterol content in flaxseed and sesame

| Phytosterol (μg/g) | Huaya No. 3 | Zhongzhi No. 11 |
|--------------------|-------------|-----------------|
| Ergosterol         | 6.57 ± 0.29a| 5.62 ± 0.36a    |
| β-sitosterol       | 972.66 ± 2.36a| 930.57 ± 4.75b |
| Stigmasterol       | 123.27 ± 2.92b| 145.67 ± 0.91a |
| Free cholesterol   | 99.03 ± 0.99A | 53.02 ± 1.22B   |
| Total cholesterol  | 410.98 ± 2.26A| 193.91 ± 4.19B |

Note: Numbers with different letters (a, b, A, B) within a column under the same parameter were significantly different and numbers with the same letter were not significantly different by Duncan’s multiple range test (a or b, p<0.05; A or B, p<0.01)
1.5 Comparison of metal element content in flaxseed and sesame seed

The metal content in flaxseed and sesame was Mg>Ca>Fe>Zn. Ca and Mg were essential nutrients for the human body, and they were commonly found in edible oil (Table 5). Fe and Zn were trace elements that cannot be synthesized by the human body. They need to be obtained from the outside world and were present in most edible oils. Fe element participated in the transportation and storage of human oxygen and was a component of hemoglobin and myoglobin. Zinc played an important role in regulating human immunity. Magnesium could be used as an enzyme activator. 99% of calcium was present in teeth and bones. Both had a variety of biological functions to maintain human health. The content of Fe, Mg, and Zn in "Huaya 3" was higher than that in "Zhongzhi No. 11". Among them, the content of Fe and Zn was extremely significant. The content of Fe in "Huaya 3" was more than twice that in "Zhongzhi 11". The Ca content in "Zhongzhi 11" was extremely significantly higher than that in "Huaya 3".

| Metal Elements | Huaya No. 3 | Zhongzhi No. 11 |
|----------------|-------------|-----------------|
| Fe (mg/kg)     | 562.60 ± 0.06A | 225.73 ± 3.19B |
| Mg (g/kg)      | 4.61 ± 0.05a | 4.42 ± 0.11a   |
| Zn (mg/kg)     | 38.97 ± 0.02A | 30.82 ± 0.59B  |
| Ca (g/kg)      | 1.04 ± 0.02B | 2.40 ± 0.07A   |

Note: Numbers with different letters (a, b, A, B) within a column under the same parameter were significantly different and numbers with the same letter were not significantly different by Duncan’s multiple range test (a or b, \( p < 0.05 \); A or B, \( p < 0.01 \)).

2 Discussion

This article uses the same extraction and measurement methods to analyze the composition of flaxseed and sesame seeds, including measurement and comparative analysis of total protein, amino acids, fatty acids, plant sterols and metal elements. The results showed that both sesame and linseed were rich in amino acids. The content of amino acids in sesame was higher than that of linseed; the content of iron, magnesium and zinc in linseed was higher than that of sesame; the content of calcium was lower than that of sesame, and both were higher than peony "fenyu" Calcium content in "Nu" and peony "Fengdanbai" (Ma et al., 2017). In this study, the content of linolenic acid in "Zhongzhi 11" in sesame seeds was higher than that in "Huaya 3" in flax seeds, and both were higher than those in rapeseed "Zheyouza 108" (An et al., 2020); The content of saturated fatty acids was higher than that of flaxseed, while the content of phytosterols was slightly lower than that of flaxseed. It could be seen that linseed oil and sesame seed oil have their own advantages. They had different nutritional and health functions in terms of ingredients, which are beneficial to human health. Both have research and edible value. As specialty edible oils, they could meet the different needs of consumers. The Chinese people consume large amounts of soybean oil, peanut oil and rapeseed oil, and there was a general lack of linolenic acid intake. Therefore, further development of linseed oil and sesame seed oil can enrich the Chinese edible oil market.

3 Materials and Methods

3.1 Varieties tested

The test base was located in the base of the Cotton and Linen Research Institute in Xiaoshan District, Hangzhou (30.4'13'' N, 120.13'27'' E). The tested flax variety was "Huaya 3", provided by Heilongjiang Academy of Agricultural Sciences. The sesame variety is "Zhongzhi No. 11", which was provided by the National Sesame Germplasm Bank.

3.2 Main reagents

Amino acid, linolenic acid, linoleic acid, palmitic acid, palmitoleic acid, myristic acid, arachidic acid, behenic acid, heptadecanoic acid, methyl cis, cis-11,14-eicosapentaenoate acid, methyl cis-5,8,11,14,17-eicosapentaenoate acid, stigmasterol, ergosterol, \( \beta \)-sitosterol, free cholesterol, total cholesterol standards, concentrated nitric acid (GR).
3.3 Main instruments and equipment

Rigol L3000 high performance liquid chromatograph, Kromasil C18 reversed phase chromatographic column (250 mm*4.6 mm, 5 μm), Kjeldahl nitrogen analyzer, flame photometer, mettler ML204 one-tenth balance, American thermoelectric ICP Inductively coupled plasma emission spectrometer ICAP6300, constant temperature drying oven, electric heating plate, inductively coupled plasma mass spectrometer X-series.

3.4 Determination of total protein and amino acid content

The total protein content was determined by Kjeldahl method. Amino acid content determination method: Weigh approximately 0.1 g of the experimental material, then add a volume of 1.5 mL of 0.1% phenol 6 mol/L hydrochloric acid aqueous solution, mix it evenly, grind it into a slurry, and transfer it to a sterile EP tube. Put it in an oven at 100°C for hydrolysis for about 20 h. After cooling for a period of time, took the volume of the hydrolysate about 0.5 mL, blow it to near dryness with a nitrogen blower, added 1 mL of 0.1 mol/L dilute hydrochloric acid to dissolve, and filter the membrane to be derivatized. Amino acid derivatization: (1) Took the above-mentioned clear solution to be derivatized with a volume of about 200 uL and an amino acid standard solution with a volume of about 200 uL, and added them to a 1.5 mL sterile EP tube; (2) accurately added the norleucine internal standard solution volume to each centrifuge tube about 20 uL; (3) the volume of the triethylamine acetonitrile solution was about 200 uL (special attention should be paid to ensure that PH>7), and the volume of the phenyl isothiocyanate acetonitrile solution was about 100 uL. After mixing well, let it stand at 25°C for about 1 h; (4) Then add n-hexane with a volume of about 400 μL to the centrifuge tube, shake it gently and let it stand for about 10 min; (5) Aspirate the lower layer solution and filter it with a 0.45 μm needle filter. Mobile phase A: Weigh 7.6 g of anhydrous sodium acetate, add 925 mL of water, adjust the pH to 6.5 with glacial acetic acid after fully dissolving, then add 70 mL of acetonitrile, mix well, and filter with a 0.45 um filter membrane; Mobile phase B: 80% acetonitrile aqueous solution; respectively set the injection volume to 10 μL, the flow rate to 1.0 mL/min, the column temperature to 40°C, and the sampling time to 45 min.

3.5 Determination of fatty acids

(1) Determination of linolenic acid and linoleic acid

Weigh about 0.5 g of the experimental sample, fully grind it, added 1.5 mL of pre-cooled petroleum ether, ultrasonically extract for about 60 min, centrifuge at 10,000 g for about 10 min, extract the supernatant, and set the volume to 0.5 mL with nitrogen. HPLC liquid phase conditions: the mobile phase was acetonitrile: 0.2% phosphoric acid water (80:20). The injection volume is about 10 μL, the flow rate is 1 mL/min, the column temperature is 30°C, the sampling time is 40 min, and the UV wavelength is set to 204 nm.

(2) Determination of palmitic acid content

Dry about 5 g of the experimental sample in an oven at 60°C overnight, weigh about 0.2 g of the dried sample, fully grind it, and transfer it to a sterile centrifuge tube with a volume of 15 mL, and use a volume of 5 Rinse the mortar with mL of n-hexane, and transfer the rinsed solution to a centrifuge tube. The ultrasonic treatment time at 50°C is about 30 min. Centrifuge at a speed of 8 000 r/min for about 10 min, and transfer the supernatant to a 15 mL sterile centrifuge tube. Add about 5 mL of n-hexane to the residue, repeat the above extraction steps twice, and combine the supernatants obtained each time. Add an appropriate amount of anhydrous sodium sulfate to the obtained supernatant, shake and mix thoroughly, and centrifuge. Then transfer the supernatant to a 2 mL sterile EP tube, and use a nitrogen blowing method until the solvent is completely evaporated. Add 0.8 mL of 0.5 mol/L KOH-CH3OH solution to a sterile EP tube, shake it enough to dissolve, and use light-proof treatment for about 1 h. Add a volume of 0.8 mL n-hexane, shake and mix thoroughly for 1 min, stand still for separation, transfer the upper layer of n-hexane to a 2 mL sterile EP tube, extract 3 times, and combine the n-hexane phase. After drying by nitrogen blowing method, add a volume of 15 mL of n-hexane, shake and mix well to dissolve, and filter an appropriate amount of the solution into a sample bottle with a lined tube to be tested using a syringe filter. Gas chromatography settings: adjust the pressure of the nitrogen cylinder pressure valve to 0.4 MPa, set the column box temperature to 200°C, the front detector temperature to 250°C, and the rear inlet temperature to 220°C. After the column temperature rises to 100°C, it will ignite. Monitor the baseline. After the baseline stabilizes, start adding samples.
3.6 Determination of phytosterol content

(1) Determination of β-sitosterol and ergosterol
Weigh about 0.5 g of the sample, grind and add 1 mL of pre-cooled ethanol, ultrasonically extract for 60 min, centrifuge at 10,000 g for 10 min, and extract the supernatant. HPLC liquid phase conditions: the mobile phase is pure methanol. The injection volume is about 10 μL, the flow rate is 0.7 mL/min, the column temperature is 30°C, the sampling time is 40 min, and the UV wavelength is set to 210 nm (β-sitosterol) and 282 nm (ergosterol).

(2) Measurement of stigmasterol and free cholesterol
Weigh about 0.1 g sample, add 1 mL of pre-cooled ether after grinding, ultrasonic extraction time is 60 min, centrifuge at 10,000 g for 10 min, extract the supernatant, and blow dry by nitrogen blowing. Use methanol to make the volume to 0.5 mL. HPLC liquid phase conditions: the mobile phase is pure methanol. The injection volume is about 10 μL, the flow rate is 1 mL/min, the column temperature is 30°C, the sampling time is 40 min, and the UV wavelength is set to 210 nm.

(3) Measurement of total cholesterol
Weigh about 0.1 g of the sample, add 1 mL of pre-chilled ether after grind, ultrasonically extract for 60 min, centrifuge at 10,000 g for 10 min, extract the supernatant, use potassium hydroxide-absolute ethanol solution at 50°C The reaction time for the lower saponification is about 1 h. Petroleum ether is used for extraction with nitrogen blowing, and the volume is adjusted to 0.5 mL with methanol. HPLC liquid phase conditions: the mobile phase is pure methanol. The injection volume is about 10 μL, the flow rate is 1 mL/min, the column temperature is 30°C, the sampling time is 40 min, and the UV wavelength is set to 210 nm.

3.7 Determination of metal elements
Digestion with nitric acid and ICP-AES/MS are used to determine the content of iron, zinc and magnesium in plants. Refer to GB 5009.268-2016 National Food Safety Standard "Determination of Multiple Elements in Food". The total calcium content of plants is determined by high temperature digestion of plant samples with concentrated sulfuric acid and measured by flame photometer.

3.8 Statistical analysis
The data was sorted through Excel 2016, and SPSS was used for analysis of variance and significance testing.

Authors' contributions
Xia An was the executor of the experimental design and experimental research of this study, completing data analysis and writing the draft of the paper; Yaning Bao and Guanrong Jin participated in the experimental design; Xia An was the project conceiver and person in charge, guiding experimental design, data analysis, and thesis Writing and revising. All authors read and approved the final manuscript.

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