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"The Impact of the USCT technology on active ingredients and efficacy of Chinese Specialty Plant Oils"

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1. Introduction

Specialty plant oils are rich in unique fatty acids and active ingredients. Perilla seed oil is a plant oil extracted from the seeds of the Lamiaceae plant Perilla, with an oil content of 20.24% to 53.71%, mainly unsaturated fatty acids, including α-linolenic acid, oleic acid and linoleic acid, among which α-linolenic acid has the highest content, reaching 39.10% to 73.06%[1].Camellia Oil, also known as tea seed oil or tea oil, is a vegetable oil extracted from the seeds of Camellia oleifera Abel, a plant of the Theaceae family. It is rich in various bioactive ingredients, such as tocopherol, tea polyphenols, sterols, tea saponins and squalene.Almond, the kernel of the mature seed of the apricot tree of the Rosaceae family, contains about 50% oil, mainly unsaturated fatty acids with a high content of oleic acid. It also contains active ingredients such as phytosterols, tocopherol and squalene. More than 90% of the vitamins in almond oil are tocopherol.Among unsaturated fatty acids, α-linolenic acid and oleic acid are beneficial fatty acids. α-linolenic acid can improve symptoms related to skin inflammation, protect against light damage caused by UV rays, and reduce skin pigmentation caused by UV rays[2].Oleic Acid Oleic acid strengthens the lipid structure of the skin barrier and helps lock in moisture while promoting the regeneration and repair of skin cells.Linoleic acid is a harmful fatty acid. Excessive intake will cause it to transform into arachidonic acid (AA), which is a typical inflammatory factor for chronic skin inflammation.Among the active ingredients, squalene and tocopherol both exhibit strong antioxidant properties. Squalene protects the skin from oxidative stress caused by external factors such as temperature changes and ultraviolet rays.In the field of skin physiology, squalene not only acts as an antioxidant and moisturizer, but is also used to treat skin problems such as seborrheic dermatitis and acne, and helps prevent skin aging[3].Tocopherol has the ability to neutralize free radicals, which can reduce the damage to cells caused by oxidative stress and thus slow down the aging process.Perilla seed oil, camellia seed oil and almond oil, these

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plant oils have been widely used in the cosmetics industry due to their unique fatty acids and active ingredients, as well as their antioxidant, anti-inflammatory and moisturizing properties.

2. Materials and Methods

2.1 USCT and cold pressing extraction process of specialty vegetable oils

Perilla seeds, camellia seeds and almonds are selected and firstly washed and dried to ensure the purity and stability of the raw materials. Then USCT technology and cold pressing technology are used to extract the three kinds of plant oils respectively.In USCT technology, the oil is effectively separated from the raw material by precisely controlling the temperature and pressure conditions and taking advantage of the difference in solubility of substances in different solvents. At the same time, cold pressing technology is used as a control group to extract the oil through mechanical pressure without heating.The extracted oils were then subjected to a double-blind randomized comparative test, with 21 people with different skin types comparing and scoring each group. They scored the oils based on smell, skin feel, and absorbency to ensure the objectivity and accuracy of the evaluation results.

2.2 Detection of fatty acid content by gas chromatography-mass spectrometry

The fatty acid content of the special vegetable oils extracted by USCT technology and cold pressing technology was tested by gas chromatography-mass spectrometry.Pretreatment of the sample to be tested: Take a certain amount of oil sample, add 5 mL of n-hexane to dissolve, add 300μL of potassium hydroxide methanol solution, shake vigorously for 1 min, and let it stand to clarify. Add 1.0 g of sodium bicarbonate, shake, and neutralize potassium hydroxide. Let it stand to clarify, absorb the supernatant, and put it on the machine. The test conditions are as follows: DB-FFAP capillary column (30m × 0.25μm × 0.25μm); injection port temperature 260℃; injection volume 1.0μL; carrier gas flow rate 1 mL/min; ion source temperature 230℃; temperature program is 70℃ for 5 min, then increase to 200℃ at 20℃ · min^{-1}, then increase to 240℃ at 2℃ · min^{-1}, and hold for 10 min; split mode; split ratio 50:1. Finally, the content is detected.

2.3 Detection of squalene content by gas chromatography-mass spectrometry

The characteristic plant oils extracted by USCT technology and cold pressing technology were used to detect the squalene content by gas chromatography-mass spectrometry. Sample processing: Weigh the samples with accurate mass (**Table 1**), add 1ml of 0.5mol/L potassium hydroxide/methanol solution, vortex evenly, then add 2ml of n-hexane, vortex, centrifuge at 4000rpm for 5min, absorb the supernatant, and put it on the machine. The detection method is as follows: chromatographic column: DB-5MS (30m×0.25mm×0.25μm); injection temperature: 270℃; split ratio: 5:1; helium (99.999%) flow rate: 1mL/min; column temperature: 100℃ for 2min, increase to 300℃ at 8℃/min, and maintain for 10min; interface temperature: 280℃; ion source temperature: 250℃;

ionization mode: EI+, 70ev; scanning mode: full scan; mass range: 33-550. Finally, the content was detected.

Table 1. Sampling mass of squalene content test samples

Sample Name	Quality（g）
Almond oil - cold pressed	0.2259
Almond oil-USCT	0.2606
Perilla seed oil-cold pressed	0.0198
Perilla Seed Oil-USCT	0.2311
Camellia Seed Oil - Cold Pressed	0.0561
Camellia Seed Oil-USCT	0.1543

2.4 Detection of tocopherol content by gas chromatography-mass spectrometry

The tocopherol content of the characteristic vegetable oils extracted by USCT technology and cold pressing technology was detected by gas chromatography-mass spectrometry. Sample processing: Weigh the samples with accurate mass (**Table 2**), add 10ml methanol, vortex evenly, centrifuge at 8000rpm for 3min, absorb the supernatant, and put it on the machine. The detection method is as follows: chromatographic column: DB-5MS (30m×0.25mm×0.25μm); injection temperature: 300℃; split ratio: no split; helium (99.999%) flow rate: 1mL/min; column temperature: initial temperature 60℃, increase to 300℃ at 40℃/min, maintain 8min; interface temperature: 280℃; ion source temperature: 230℃; ionization mode: EI+, home mass 70ev; scanning mode; SIM word mass number: 151, 191, 416, 165, 205, 430. Finally, the content was detected.

Table 2. Sampling quality of tocopherol content test samples

Sample Name	Quality（g）
Almond oil - cold pressed	79.1
Almond oil-USCT	72.4
Perilla seed oil-cold pressed	83.2
Perilla Seed Oil-USCT	80.9
Camellia Seed Oil - Cold Pressed	84.5
Camellia Seed Oil-USCT	77.0

2.5 Detection of inflammatory factor IL-6 content

The ELISA kit method was used to complete the cytotoxicity CCK8 test based on mouse mononuclear macrophage RAW 264.7. After determining the specific cytotoxicity of the characteristic vegetable oil, the following tests were completed: (1) Cell inoculation: After the cells were revived and passed to the third generation (or after the cell state was stable), the cells were inoculated into a 24-well plate (1 mL per well) at 1×105/mL after the cells filled the culture bottle with one layer (70%-80%), and cultured in a CO_2 incubator (37°C, 5% CO2) for 24 hours. (2) Cell administration: The ELISA kit method was used to detect the IL-6 inflammatory factor index in the mouse mononuclear macrophage RAW 264.7 detection model according to the blank control group without administration, the control group administered LPS (20μg/mL) and the experimental group LPS (20μg/mL) + 1%, LPS (20μg/mL) + 2%. (3) Transfer the cell supernatant to a clean, sterile 1.5 mL EP tube and store it at -80°C for long-term storage for subsequent ELISA kit detection and analysis.

2.6 Data analysis

The results of the inflammatory factor detection experiment were statistically analyzed using Graphpad. The dependent variable was the sample concentration, and $p < 0.05$ was considered statistically significant.

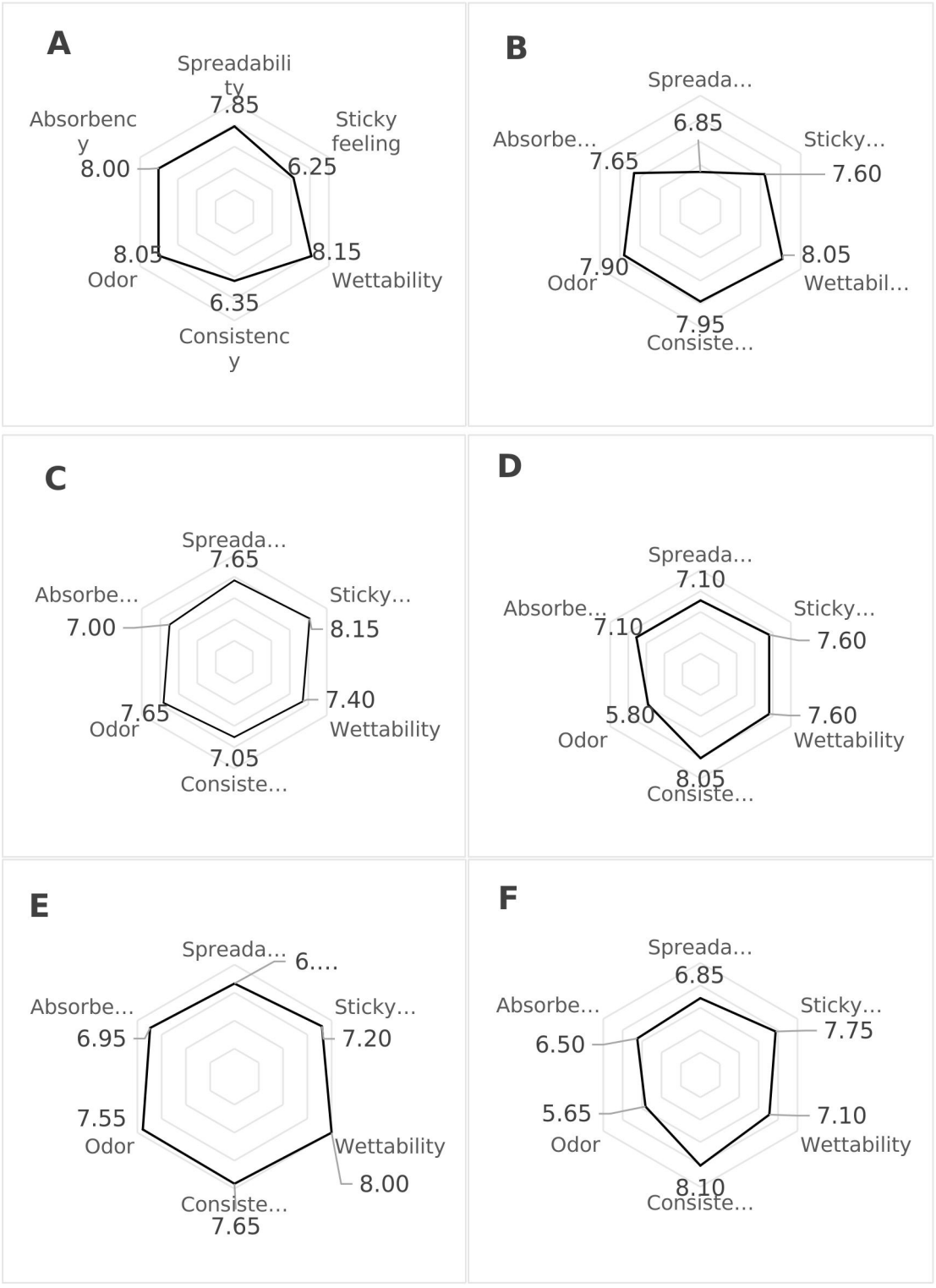
3. Results

3.1 Evaluation of odor, skin feel and absorbency

Table 3. Differences in the extraction of characteristic vegetable oils by USCT and cold pressing

	USCT Method	Cold Pressing
Active ingredient content	High	Low
Odor	Restore the fruity aroma	Characteristic smell
Skin feel	Lightweight	Thick
Absorbance	High	Low

The oil products extracted by USCT method are lighter, more absorbent, and restore the unique fragrance of the fruit. In addition, seven combinations of special plant mixed oils with different proportions were made to observe which formula is the best(**Table 3**).


A
Spreadability
7.85
Sticky feeling
6.25
Wettability
8.15
Consistency
6.35
Odor
8.05
Absorbency
8.00
B
Spreada...
6.85
Sticky...
7.60
Wettabil...
8.05
Consiste...
7.95
Odor
7.90
Absorbe...
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Wettability
7.40
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Odor
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Wettability
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Wettability
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Spreada...
6.85
Sticky...
7.75
Wettability
7.10
Consiste...
8.10
Odor
5.65
Absorbe...
6.50

Figure 1. Skin feel evaluation of 7 different ratios of special plant mixed oils

After comparing seven special plant oils with different proportions, it was found that Group A has a better sensory experience: less sticky and faster absorption; light skin feel and high moisturizing degree; soft smell, with a light plant fragrance(**Figure 1**).

3.2 Contents of fatty acids, tocopherol and squalene in specialty vegetable oils extracted by USCT and cold pressing

Table 4. Contents of oleic acid, linoleic acid, α-linolenic acid, tocopherol and squalene in characteristic vegetable oils extracted by different methods

	Tocopherol (mg/g)	Squalene (mg/g)	Oleic acid (mg/mg)	Linoleic acid (mg/mg)	α -linolenic acid (mg/mg)
Almond Oil - Cold Pressed	0.5855	0.0988	0.1993	0.0332	0.0221
Almond Oil-USCT	0.9059	0.1404	0.3491	0.0167	0.0013
Perilla seed oil-cold pressed	0.4893	0.0198	0.1057	0.0192	0.2353
Perilla Seed Oil-USCT	1.1711	0.2311	0.1009	0.0110	0.2838
Camellia Seed Oil - Cold Pressed	0.6003	0.0561	0.2612	0.0340	0.0269
Camellia Seed Oil-USCT	0.4334	0.1543	0.4290	0.0097	0.0043

The USCT method can greatly increase the content of effective substances and reduce the content of linoleic acid. The linoleic acid content in almond oil, camellia seed oil, and perilla seed oil obtained by the USCT method was reduced by 49.70%, 71.47%, and 37.50%, respectively. The contents of oleic acid and α-linolenic acid were increased. In addition, the content of tocopherol and squalene in the characteristic plant oils extracted by the USCT method was also significantly higher than that of the cold pressing method. The tocopherol content in almond oil and perilla seed oil increased by 54.72% and 139.37%, respectively, and the squalene content in almond oil, perilla seed oil, and camellia seed oil increased by 42.11%, 1067.17%, and 175.04%, respectively.

3.3 Anti-inflammatory effects of special plant oils extracted by USCT and cold pressing

Table 5. IL-6 inflammatory factor content and inhibition rate of special vegetable oils extracted by different methods

	Control	50ng/ml LPS	Perilla seed oil-cold pressed	Camellia Seed Oil - Cold Pressed	Almond Oil - Cold Pressed	Perilla Seed Oil-USCT	Camellia Seed Oil-USCT	Almond Oil-USCT
IL-6 inflammatory factor content	15.6342593	526.708333	393.642323	465.574861	437.2146775	15.2407407 4	311.185185 2	108.9166667
IL-6 inhibition rate	/	/	25.26%	11.61%	16.99%	97.11%	40.92%	79.32%

Table 6. IL-6 test results of different ratios of special plant mixed oils

Sample name	IL-6			
	Mean（pg/mL）	SD	P-value（vs LPS）	Inhibition rate（%）
Control	31.28	3.289	<0.0001	\
LPS	552	7.344	\	\
LPS+1%	403.6	18.58	<0.0001	26.88%

LPS+2%	32.21	5.329	<0.0001	94.16%

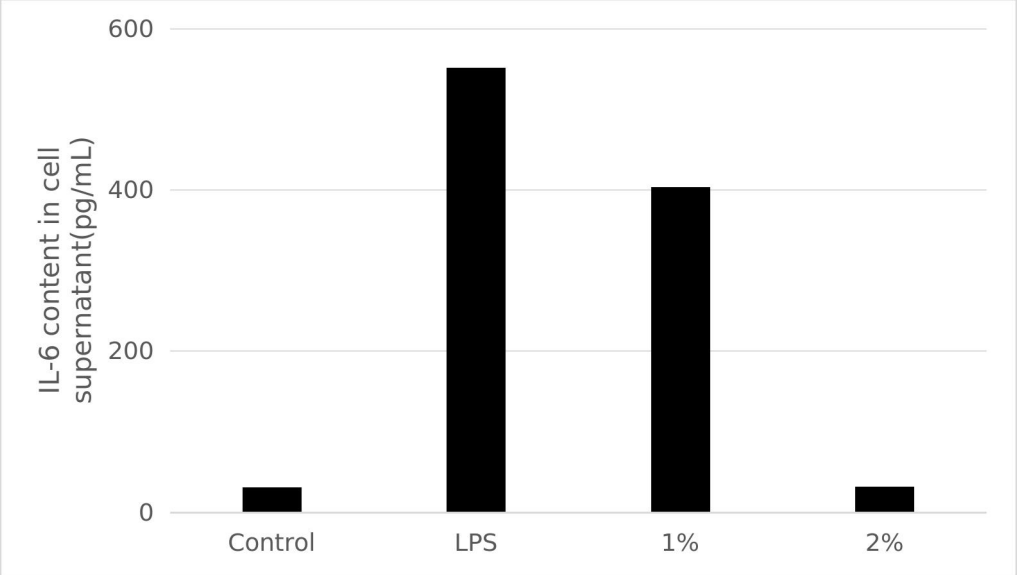

Figure 2. Experimental results of the effects of different ratios of special plant mixed oils on cell supernatant IL-6 (The values are expressed as mean±SD, experimental data n≥3)

By comparing the inhibition rate of IL-6 by USCT technology and cold pressing method, it was found that the inhibition rate of IL-6 by the special vegetable oil obtained by USCT technology was significantly higher than that by the cold pressing method. According to the experimental test data in Table 6 and Figure 2, when the concentration of the special vegetable mixed oil was 1%, compared with the LPS group, the inhibition rate of the inflammatory factor IL-6 in the cell supernatant was 26.28%, which was significantly different ($p<0.001$ or $p<0.0001$). When the concentration of the special vegetable mixed oil was 2%, compared with the LPS group, the inhibition rate of the inflammatory factor IL-6 in the cell supernatant was 94.16%, which was significantly different ($p<0.001$ or $p<0.0001$).

4. Discussion

This paper studies a new oil extraction technology, USCT technology, which uses water as a solvent and extracts perilla seed oil, camellia seed oil and almond oil efficiently under low temperature conditions based on the difference in solubility and material density. The results show that USCT technology is significantly superior to traditional cold pressing in many aspects: the oil extracted by USCT is lighter and easier to absorb, retains the fragrance of the fruit, and has a higher sensory evaluation score. Gas chromatography-mass spectrometry analysis shows that the USCT method greatly increases the content of beneficial ingredients, such as tocopherol and squalene (increased by 139.37% and 1067.17% in perilla seed oil, respectively), while reducing the content of harmful fatty acid linoleic acid (an

average decrease of 37.50% in the three oils). Cell experiments confirmed that the oil extracted by USCT significantly enhanced the inhibitory effect of the inflammatory factor IL-6. When the concentration of the special plant mixed oil was 1% and 2%, they showed a certain inhibitory effect on the inflammatory mediator IL-6 in the cell supernatant. In particular, when the concentration was increased to 2%, the inhibitory effect on IL-6 was particularly significant.

This study used USCT technology to not only improve the fatty acid composition of specialty plant oils, but also significantly increase the level of their bioactive ingredients, thereby enhancing their potential in promoting skin health. The extraction of oils using USCT technology increased the content of oleic acid and α-linolenic acid in the plant oils, while reducing the content of linoleic acid, and better inhibited the activity of the inflammatory factor IL-6, which can more effectively relieve skin sensitivity and reduce inflammatory responses. In addition, the content of active ingredients tocopherol and squalene in the oils was increased, which synergized with oleic acid and α-linolenic acid to strengthen the skin barrier function and reduce transepidermal water loss (TEWL), thereby providing a superior moisturizing and repairing effect. Moreover, the antioxidant properties of tocopherol and squalene enable the specialty plant oils to prevent oxidative damage caused by ultraviolet rays after use, protect the sebum membrane from oxidative damage, and neutralize free radicals, which helps to delay skin aging.

5. Conclusion

USCT technology has shown significant advantages in the field of oil extraction. Its low-temperature all-water phase extraction process avoids the destruction of effective ingredients, is highly efficient, and is green and environmentally friendly. This technology provides an innovative solution for extracting high-quality specialty plant oils, helps promote the efficient application of natural plant oils in skin care products, and meets consumers' demand for safe, healthy, and efficient skin care products. Therefore, USCT technology has broad application prospects in the field of oil extraction and deserves further promotion.

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Conflict of Interest Statement.

The authors declared that they have no conflicts of interest to this work.

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