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Supercritical CO_2 Extraction of Tomato Seed Oil

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Abstract: Supercritical CO_2 extraction (SC-CO_2) of oil from tomato seeds was carried out at varied extracting temperature, pressures, and time through single-factor and optimal experiments. The extracted oil was analyzed for the extracting yield, the fatty acid composition and the quality. The results showed that the order of the effects of the factors on the extracting yield were: extraction time > pressure > temperature. The optimum extracting conditions obtained were 30MPa for 2h at 50¡æ, which resulted in a yield of 96.34%. Determined by gas chromatography (GC), the fatty acid compositions and their contents in tomato seed oil after SC-CO_2 extraction were: C16:0, 13%; C18:0, 5.0%; C18:1, 22%; C18:2, 56%; and C20:0, 2.8%. Demonstrated by POV, A.V., S.V. and I.V., the qualities of the tomato seed oil extracted by SC-CO_2 were good as well. No significant effect of various extracting conditions was shown on such qualities of tomato seed oil.

Key words: Supercritical fluid extraction, Tomato seed oil, Extraction yield, Fatty acid composition

Introduction

Because tomato is rich in lycopene, natural vitamine E and carotenoids, tomato products including tomato sauce, tomato ketchup, tomato paste and tomato juice have become the favorites of many people due to their high contents of the nutritional components (Rozzi *et al.*, 2002; Turner and Mathiasson, 2000 and Barth *et al.*, 1995) with an increased consumption yearly.

In the recent years, the volume of trade in tomato products has been rising in the international markets, with a maximum of 1.2 million tons in 2002 (Technological information academe of Xinjiang, 2002). While in China, Xinjiang district occupies the predominant production of tomato products, with a maximum of 500 thousand tons per year.

As the byproducts of tomato production, the yield of tomato seed and skin has also increased. However, currently, because of fewer applications, most of tomato seeds have to be piled in the factories, which have caused pollution, and only a part of these seeds are used as the feeds or fertilizers. Furthermore, due to a large amount of oil and fat in the tomato seed, especially the octadecadienoic acid (18:2), which is recently the focus of much interest due to its many attributed beneficial effects, such as anticarcinogenic activity, inhibition of development of atherosclerosis and ability to reduce the catabolic effects of immune stimulation (Lawless *et al.*, 1999), tomato seed oil is regarded as a nutritional oil. Direct rejection of tomato seeds, therefore, causes significant losses.

The traditional oil extraction by solvents often requires multiple steps, is time-consuming, utilizes a lot of organic solvents (Barth *et al.*, 1995), and may not result in quantitative recovery. Concerning about the hazardous nature of many commonly used solvents, the costs and environmental dangers of waste solvent disposal (Luque de Castro & jimeenez-Carmona, 2000), and the residual solvent in the oil, supercritical CO_2 extraction (SC-CO_2) technique is an ideal method on the ground of the remarkable increment of the solubility and mass transfer capability of the supercritical fluid (Kalampoukas and Dervakos, 1996). Compared with traditional extraction method, SC-CO_2 extraction not only is environmentally benign, but also has significant properties as follows: no organic residual solvents, mild extraction conditions, good selectivity, nontoxic and simple procedures, low energy cost, and re-circulation of solvents (Turner *et al.*,2002; Tayler, 1996 and Reverchon, 1997). On account of these advantages, SC-CO_2 extraction has come to be one of the most effective methods for extraction of some nutritive components, flavors and thermally labile compounds (King, 2000 and Lang and Wai, 2001).

The goal of this research was to determine the effects of extraction temperature, pressure and time on the extraction yield and quality of oil, then to optimize conditions for extraction.

Materials and Methods

Sample Preparation: Tomato seeds were obtained from Xinjiang Tunhe Group (Xinjiang, China). One batch (27.95% oil content on dry weight basis) was stored at room temperature for one year, while another batch (25.13% oil content on dry weight basis) did not undergo any storage. Both batches were dried till water content was below 3% (Taylor, King & List, 2003), because water is only 0.3% soluble in supercritical CO_2 fluid, but can play an important role in the extraction, if excess water remained in the extraction vessel, highly water-soluble solutes would prefer to partition into the aqueous phase and, therefore, the SC-CO_2 extraction recovery would be low (Lang and Wai, 2001).

SC-CO_2 Extraction: SC-CO_2 extraction was performed with Huali 1L/50MPa IIBX equipment (Hangzhou Huali Bump Inc.).The procedures of extraction were illustrated in Fig. 1.

About 100g tomato seed powders were put into the 1L extracting chamber. According to the designs of the single-factor, orthogonal and optimal experiments, extractions were carried out by changing varied extracting conditions. Other factors assessed were as follows: the frequency of the syringe pump for CO_2 was 30Hz; the pressure of the first and second separation chambers were 6.5~7.2MPa and 4.0~5.0MPa respectively; the flow rate of CO_2 fluid was about 5kg/h.

Calculation: the extraction yield (% w/w) = the extracted mass of tomato seed oil / the original oil content of tomato seed ¡× 100%

Analysis of variance (ANOVA) was used to analyse the statistical significance of the effects of extraction temperature, time and pressure.

Gas Chromatography (GC): Sample preparation (Xiao, Sun & Cui, 1998): The extracts were saponified, and fatty acid methyl esters (FAME) were prepared by esterification using boron trifluoride (BF_3) in methanol (25%, v/v).

Conditions of GC: An Agilent 6890 series GC system (Agilent technologies Inc.) equipped with a flame ionization detector (FID) was used for the measurements. The chromatograph was fitted with a 30.0m¡× 0.25mm column BPX70. Nitrogen was used as carrier gas with a flow rate set to 0.8mL/min. FAMEs were detected by FID with hydrogen at a flow rate of 30mL/min and air at 110mL/min. The temperature of the injector and detector was 260¡æ. The sample of 0.2|ìL was injected with a split ratio of 60:1. The temperature program started with an isothermal period of 1min at 140¡æ, then was raised to 220¡æ at a rate of 8¡æ/min and kept there for 18min.

Determination of the Oil Quality: POV (Huang, 1989): About 2.0g oil sample was placed in darkness for 3min with 30mL $CHCL_3$-CH_3COOH (2:3,v/v) and 1mL saturated KI solution, then 100mL boiled distilled water was added and was titrated by 0.002N $Na_2S_2O_3$.

POV (meq/mg) = 78.8¡×N¡×(V_1-V_2)100¡×0.1269/W

A. V. (Huang, 1989): About 5.0g oil sample was dissolved in 50mL aether-ethanol (2:1,v/v), and titrated by 0.1N KOH.

A.V. = N¡×(V_1-V_2)¡× 56.1/W

I.V.: About 0.1g oil sample was dissolved in 15mL CCL_4. Then 25mL Wijs reagent confected by ICL was added to the sample. It was kept in darkness for 2h with slight vibration, 20mL KI was added, and was titrated by 0.1N $Na_2S_2O_3$ till the blue color faded.

I.V. = N¡×(V_1-V_2)¡×0.1269¡×100/W

S.V.: About 2.0g oil sample in 25mL 0.5N KOH-ethanol was heated in a water bath at 85¡æ for 1h till the reaction was completed. 10mL neutral ethanol was added and the sample was titrated by 0.5N HCL till the red color faded.

S.V. = N¡×(V_1-V_2)¡×56.1/W

Results and Discussion

Effect of Granularity of Tomato Seed Powder on the Extraction Yield: The first batch of tomato seed was smashed to two granularities and extracted at the same conditions. The effect of granularity of tomato seed powder on the extraction yield was presented in Table 1.

The data revealed that when granularity was less than 0.6mm, the yield of extraction was twice that of 0.6-1.34mm. So the granularity of 0.6mm was preferred in this extraction test.

The result was similar to the requirements of granularity for other supercritical fluid extractions of seed oils, such as 0.5mm for pumpkin seed oil extraction (Li, Yan & Wang, 2002), 0.9mm for walnut oil extraction (Liu, Zhang & Cui, 2002) and 0.45mm for grape seed oil extraction (Dong, Wan & Li, 2002), all of which are less than 1.0mm.

Single-Factor Experiments: Tomato seed oil was extracted from the first batch of tomato seeds under the following conditions: temperature of the extraction chamber (40,50, and 60¡æ), pressure of the extracting fluid (20,25 MPa), and the extraction time (1,2 h).

It can be observed from Table 2 that the extraction yield of 25MPa and 2h was much more than that of 20MPa and 1h respectively, and that when extraction temperature was over 60¡æ, the extraction yield reduced greatly.

Orthogonal Experiments: Tomato seed oil was extracted from the second batch of tomato seeds.

From the results of the single-factor experiments, the orthogonal experiments of three factors and three levels were designed (see Table 3). Nine experiments were conducted to assess the effects of the interaction of extraction time (0.5,1,2h) , pressure (20,25,30MPa) and temperature (30,40,50¡æ) on extraction yield.

The order of the effects of the factors on the extraction yield were: extraction time > pressure > temperature. The extraction time had a significant effect at $P<0.05$.

Optimal Experiments: Tomato seed oil was extracted from the second batch of tomato seeds.
By reason of the significant effect of extraction time on the extraction yield, which could conceal the effects of extraction temperature and pressure, for further study, the extraction time was fixed on the best level, namely 2h, so as to investigate the effects of extraction temperature and pressure.
The best condition obtained from intuitional observation was 50¡æ and 30MPa, at which the yield was 96.34%. Extraction pressure affected the yield at $P<0.025$, while extraction temperature affected at $P<0.1$.

Effect of Extraction Conditions on the Extraction Yield

Extraction Time: Longer extraction time ensures sufficient contact between tomato seed and the supercritical fluid, leading to higher extraction yields (Sun & Ding, 1998). Since the extraction yield had exceeded 95% when the extraction lasted 2h (Table 2), it was not expected to increase further; the extraction time was then limited to 2h.

Extraction Pressure: Generally, at any given temperature, elevated pressure increases the fluid density and the interaction between the solute and the fluid; it also reduces the diffusivity and increases the viscosity of the fluid, thereby raising the transporting velocity of the solute. In addition, elevated pressure also compresses the solute, which limits the diffusion of the fluid into the solute. All of these results in the increment of the extractability of the fluid (Rozzi *et al.*, 2002; Turner *et al.*, 2002) can be seen in Table 2 and Table 4.
Theoretically, at $T_R=1.0\sim1.2$ there is a dramatic increase in the fluid density, then in extraction yield, with only a small increase in pressure. On the contrary, the same change in pressure at $T_R>1.5$ hardly changes the fluid density and extraction yield (Turner *et al.*, 2002). In this study, the yield was enhanced by nearly 30% (from 65.91% to 95.66%) when the pressure was raised from 20MPa to 30MPa at 40¡æ; while only about 15% enhancement of the yield (from 81.27% to 96.34%) was achieved over the same change in pressure at 50¡æ (Table 4).

Extraction Temperature: Extraction temperature influences the yield in two ways. On the one hand, an increase in the temperature causes enhancements in the diffusivity and volatility of the solute (Rozzi *et al.*, 2002) and an amplification in the interaction of the solvent and solute (Barth *et al.*, 1995), which can increase the extractability of the fluid. On the other hand, an increase in the temperature also has a negative effect on the extractability of the fluid due to attenuating the fluid density.
In this study, when extracting temperature was raised from 30¡æ to 50¡æ, the former played the dominant part so that the extractability increased with the elevated temperature (Table 4). However, at 60¡æ, the extraction yield declined with the reduced fluid density (Table 2).

The Flow Rate of CO_2 Fluid: In most cases, the elevation of the flow rate of the fluid will bring on the enhancement of the extraction yield. However, this rule does not work when the flow rate is too high because it makes the fluid pass over the sample surface but not diffuse into the inner part of the sample (Rozzi *et al.*, 2002).
In our research, considering that the maximum flow rate of our equipment was 10kg/h, we kept the flow rate at about 5kg/h in all of our experiments.

Selection of Separating Conditions: All of the tomato seed oils extracted were collected in the first separating chamber at 6.5-7.2 Mpa. Theoretically, no matter what the separation temperature is, when separation pressure is below the critical point, the extractability of CO_2 fluid will diminish rapidly. In our study, because the room temperature was high (>35¡æ), and lack of the cooling device, we were unable to reduce the separation temperature below the critical point. Meanwhile, the solubility of the fluid will correspondingly decrease with the elevated temperature, so we set the separation temperature 10¡æ higher than the extraction temperature in the single-factor experiments. Whereas in the orthogonal experiments (room temperature was about 30¡æ), in order to keep the separation temperatures accordant and save the heating energy of separation chamber, we fixed them all at 30¡æ.

Table 1: Effect of granularity of tomato seed powder on the extraction yield

Number	Granularity (mm)	Pressure £¨MPa£©	Temperature £¨¡æ£©	Time £¨h£©	Yield £¨%£©
1	0.6-1.34	25	40	2	40.01
2	<0.6	25	40	2	95.28

Table 2: Results of single-factor experiments

Number	Pressure£¨MPa£©	Temperature£¨¡æ£©	Time £¨h£©	Yield £¨%£©	Appearance
1	20	40	1	69.28	Clear
2	25	40	1	83.37	Clear
3	25	50	1	89.84	Clear
4	25	60	1	51.57	Clear but sour
5	25	40	2	95.28	Clear

Table 3: Results of orthogonal experiments

Factors Number	Temperature A £¨¡æ£©	Time B (h)	Pressure C (MPa)	Yield £¨%£©
1	1(30)	1(0.5)	1(20)	28.01
2	1(30)	2(1)	2(25)	62.33
3	1(30)	3(2)	3(30)	88.12
4	2(40)	1(0.5)	2(25)	28.93
5	2(40)	2(1)	3(30)	72.10
6	2(40)	3(2)	1(20)	65.12
7	3(50)	1(0.5)	3(30)	33.96
8	3(50)	2(1)	1(20)	55.06
9	3(50)	3(2)	2(25)	91.75

Table 4: Results of optimal experiments

Factors Number	Temperature A £¨¡æ£©	Pressure B (MPa)	Yield £¨%£©
1	1(30)	1(20)	77.55
2	1(30)	2(25)	77.73
3	1(30)	3(30)	89.72
4	2(40)	1(20)	65.91
5	2(40)	2(25)	82.93
6	2(40)	3(30)	95.66
7	3(50)	1(20)	81.27
8	3(50)	2(25)	93.93
9	3(50)	3(30)	96.34

Table 5: Fatty acid compositions in tomato seed oil (%)

Fatty acid Tomato seed oil	Palmitic acid C16:0	Stearic acid C18:0	Oleic acid C18:1	octadecadienoic acid C18:2	Arachidic acid C20:0
3	13.21	5.39	22.00	56.60	2.80
5	13.48	4.99	21.80	56.96	2.77
6	13.02	5.92	22.16	56.14	2.76
7	13.30	5.64	22.47	55.77	2.82
8	13.21	5.45	22.06	56.52	2.76
9	13.36	5.57	21.88	56.43	2.76

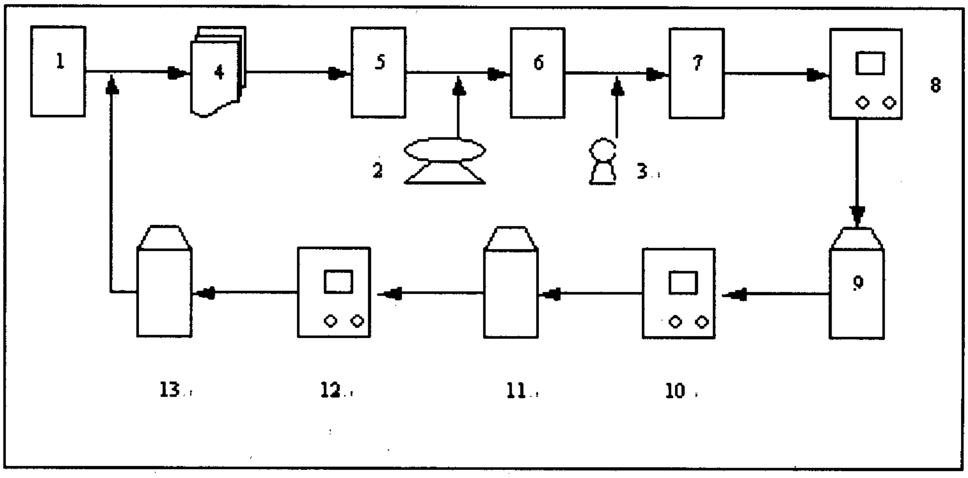

Fig. 1: The procedures of extraction: 1 CO_2 cylinder; 2 refrigeration; 3 syringe pump for CO_2; 4 equilibration coil; 5 purifying utensil; 6 evaporation; 7 blend; 8,10,12 heater; 9 extracting chamber; 11,13 separating chamber.

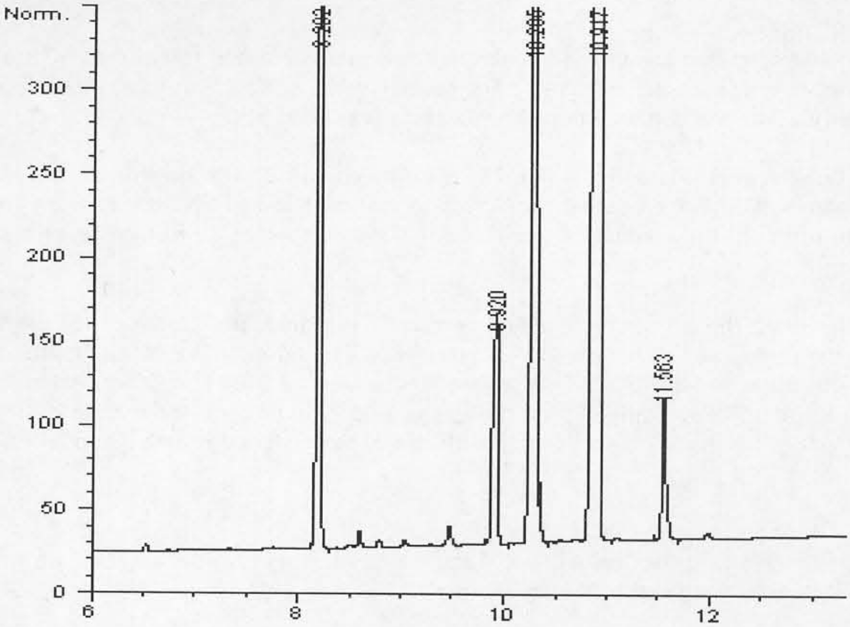

Fig. 2: Chromatogram of fatty acid profile of tomato seed oil extracted at 30MPa, 50¡æ for 2h

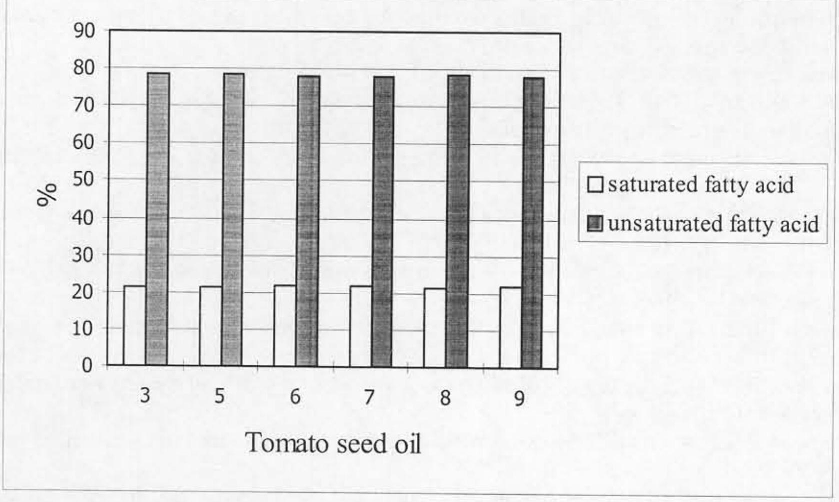

Fig. 3: Proportions of fatty acid in tomato seed oil

Table 6: Determination of the quality of tomato seed oil

Indexes Tomato seed oil	POD£¨meq/kg£©	A.V. £¨mg/g£©	I.V. £¨g/100g£©	S.V. £¨mg/g£©
3	0.1330	3.3518	190.52	185.90
5	0.1468	3.1175	190.04	190.85
6	0.2184	3.4977	186.12	184.59
7	0.2304	3.5387	187.60	186.96
8	0.1929	3.6765	192.91	188.73
9	0.1362	3.8120	192.06	191.53

The differences in the yields between the single-factor and orthogonal experiments may be rationalized by the distinct separation temperatures and dissimilarity of materials.

Analysis of Fatty Acid Composition in Tomato Seed Oil: Extracts whose extraction yields were above 80% in the optimal experiments, were analyzed by GC.

Fig. 2 showed that the fatty acid compositions in tomato seed oil extracted by SC-CO_2 were C16:0, C18:0, C18:

1, C18: 2 and C20:0.

Table 5 and Fig. 3 demonstrated that tomato seed oils were rich in unsaturated fatty acid (>78%); the content of C18:2 was the highest followed by that of C18:1. The relative value of C18:2 to C18:1 was more than 2 and the difference of the fatty acid composition among the extracts was not too big.

Determination of the Quality of Tomato Seed Oil: The determinations of the quality of the extracts whose extraction yields were above 80% in the optimal experiments were carried out. The results from Table 6 showed there was no significant effect of the various extracting conditions on the quality of tomato seed oils.

Conclusion

In light of these principles, both the extraction time and pressure had significant effects on the extraction yield of tomato seed oil, while the temperature had less significant effect. The optimum extraction conditions of tomato seed oil obtained were 2h, 50¡æ,and 30MPa, which resulted in the yield of 96.34%. Other better conditions were 2h-40¡æ-30MPa or 2h-50¡æ-25MPa. Tomato seed oil was a kind of nutritional oil due to its high content of unsaturated fatty acid (>78%). There were no significant effects of the various extracting conditions on the quality of tomato seed oils.

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