Supercritical Carbon Dioxide Extraction of *Spirulina platensis* Component and Removing the Stench

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The chemical component of spirulina was determined by supercritical CO_2 extraction. The protein and essential amino acid contents of spirulina powder were not significantly decreased through supercritical CO_2 extraction, but the contents of total amino acid and lipids were reduced. The spirulina powder had a stench smell before, but not after, supercritical CO_2 extractions. The highest yield rate of lipids was obtained at an extraction pressure of 35 MPa and an extraction time of 4 h. The lipids could be used as additives of health foods containing γ-linolenic acid.

Keywords: *Spirulina; supercritical carbon dioxide; extract; stench smell; γ-linolenic acid*

INTRODUCTION

Spirulina is rich in protein and many kinds of active components, such as γ-linolenic acid, β-carotene, and polysaccharides. Spirulina, both as a food supplement and as a medicine, has attracted extensive attention all over the world (Hu et al., 1997; Zhen and Geng, 1996; Li, 1993). In China, the exploitation of spirulina was developed very quickly in recent years. At present, we chiefly export spirulina powder or put roughly processed products into the market. The special stench smell of spirulina is a major obstacle to marketing and acceptance of this product, and this is a problem that should be solved immediately. Supercritical CO_2 extraction is a new technique of extraction and separation in the food industry. It has special characteristics of having high efficiency of extraction and no toxic solvents, preserving the heat labile active component (Mucunjin, 1985; Li, 1996; Roughen, 1989; John, 1997; Wei, 1995). The author used a supercritical CO_2 extraction technique to separate and purify the active component of spirulina and get rid of its stench smell and, thus, developed the deep process of spirulina.

MATERIALS AND METHODS

Spirulina Powder. Experimental spirulina powder was bought from The Soil and Fertilizer Station, Jiangsu Agriculture and Forestry Office.

Supercritical CO_2 Extraction Equipment. The supercritical CO_2 extraction equipment was a HA121-50-02, produced by Nantong Hua'an Supercritical Extraction Ltd. The maximum working pressure is 50 MPa, and the working temperature is from 0 to 90 °C.

Technological Process. Spirulina powder is placed into the extraction vat, and CO_2 gas, compressed to a supercritical fluid on freezing, is added to the extraction vat. Thus, the extraction has begun. After the fluid that has extraction substances dissolved in it enters the separation machine to reduce the pressure, the extraction substance is separated from the liquid CO_2, as CO_2 is converted to gas. The extracted spirulina ingredient is removed from the separation machine.

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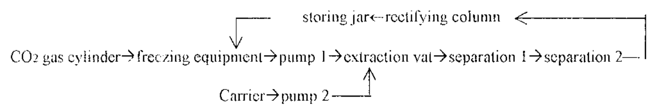

Figure 1. Technological process of supercritical CO_2 extraction.

Design of Experiment. Extraction pressure, extraction temperature, extraction time, and volume of flow of CO_2 are chosen as the experimental factors. Extraction pressure and time are assigned to be the main influential factors by preparatory experiments. The adopted technology parameters are as follows: pressure, 30, 35, and 40 MPa; extraction time, 2, 3, and 4 h; extraction temperature, 40 °C; volume of flow of CO_2, 24 kg/h.

Chemical Analyses of Spirulina's Ingredients. Protein, lipid, moisture, and amino acid contents of samples were determined by Kjeldahl method, Soxhlet extraction method, common pressure drying, and amino acid automatic analysis (Hitachi-835), respectively. The fatty acid composition of spirulina lipids was determined by gas chromatography [GC-9A (Shimadzu)] under the following conditions: packing column, 20% (w/w) PEGS/101 coated on Chromasorb W.AW.D-MCS, 60−80 mesh; detector, FID; column temp, 180 °C; injector temp, 250 °C; detector temp, 250 °C; carrier gas, (N_2) 40 mL/min, (H_2) 50 mL/min, (air) 40 mL/min.

RESULTS AND DISCUSSION

Effect of Condition of Supercritical CO_2 Extraction on Lipid Rate. Extraction pressure is one of the most important technological parameters of supercritical CO_2 extraction. When the temperature is fixed, the higher the pressure increases, the more the thickness of the supercritical CO_2 increases, so the capacity of its dissolution increases, although they are not in linear relation. When the pressure has increased to some degree, the capacity of dissolution increases slowly. Therefore, the higher pressure is not the more effective extraction rate.

The results of Table 1 revealed that when the extraction time was fixed eternally and the pressure increased from 30 to 35 MPa, the lipid rate increased, but when the pressure was increased from 35 to 40 MPa, the rate reduced a little, so 35 MPa was the best extraction pressure for extracting lipids. When the extraction pressure was fixed, as the extraction time

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Table 1. Effect of Condition of Supercritical CO_2 Extraction on Lipid Rate

extraction time (h)	2			3			4		
extraction pressure (MPa)	30	35	40	30	35	40	30	35	40
lipid rate (%)	4.1	5.9	6.5	6.1	6.9	6.7	6.3	7.2	7.0

Table 2. Effect of Supercritical CO_2 Extraction on Composition and Content of Fatty Acid[a]

	% by weight of lipids				
pressure (MPa)	oleic acid C18:1	linolic acid C18:2	α-linolenic acid C18:3	γ-linolenic acid C18:3	GLA (% of dry matter)
20	35.20	29.10	5.76	18.5	0.12
30	15.20	30.72	8.38	18.97	0.16
35	16.91	36.51	9.16	19.68	0.18
40	12.31	36.10	15.0	23.18	0.29

[a] Extraction temperature, 40 °C; extraction time, 4 h; CO_2 flow, 24 kg/h (the same as in Table 3).

lengthened, the lipid rate rose, so the industrial production should control time in 4 h.

Effect of Supercritical CO_2 Extraction on Composition and Content of Fatty Acid. The results of Table 2 showed that when the extraction pressure increased, the percentage content of oleic acid in lipids was decreased but the percentage content of linoleic acid increased. The extraction rate of γ-linolenic was especially increased with the pressure's rising. The lipids extracted from spirulina by supercritical CO_2 had very high contents of γ-linolenic acid, an essential fatty acid potentially of considerable importance in human nutrition. Spirulina is a kind of important natural food resource. The lipids that were obtained by supercritical CO_2 extraction can be utilized as additives in health food rich in γ-linolenic acid.

Supercritical CO_2 Extraction Getting Rid of Spirulina's Stench Smell and Effect on Nutrients of Spirulina. I found beyond expectation that after the spirulina powder was extracted by supercritical CO_2, the stench smell had been removed. I therefore determined the change in the main constituent of the spirulina before and after the extraction. Table 3 reveals that after the spirulina powder was extracted by supercritical CO_2, the protein content did not change markedly, but lipids and total amino acids were reduced 2.32 and 3.12%, respectively, and total essential amino acids were reduced 0.95%. Therefore, the nutritive value of the spirulina changed little, so supercritical CO_2 extraction has proven to be an effective method to remove the stench smell of spirulina. Compared with other methods, supercritical CO_2 extraction of spirulina has the advantage of preserving heat labile components, producing a solvent-free product. The original quality of spirulina is preserved, and the stench smell is removed. The composition of spirulina's stench smell and of its physical and chemical essence is unknown and presents a challenge for the future.

Table 3. Effect of Supercritical CO_2 Extraction on Constituent of Spirulina Powder (Percent)

constituent	check	treatment	constituent	check	treatment
protein	69.75	69.79	moisture	6.57	6.68
lipids	10.38	8.06	free amino	0.94	0.69
Asp	6.66	6.43	Thr	3.45	3.34
Ser	3.27	3.17	Glu	9.92	9.16
Gly	3.52	3.38	Ala	5.44	5.07
Cys	0.88	0.76	Val	5.03	4.69
Met	0.88	0.83	Ile	3.82	3.62
Leu	6.19	5.93	Tyr	2.85	2.56
Phe	2.90	2.91	Lys	3.32	3.18
His	1.15	1.16	Arg	4.29	3.99
total essential amino acids	24.67	23.72	total amino acids	66.28	63.16

LITERATURE CITED

Hu, Qiuhui; Jiang, Mei; Chen, Xiaohong. Analysis on spirulina's nutrients and its function of nutrition and health. *Chin. Livestock Prod. Food* **1997**, *4* (2), 69−70.

John, E. K. *Advances in Food and Nutrition Research*; Academic Press: New York, 1997; Vol. 35, pp 111−113.

Li, Dingmei. *Spirulina*; Chinese Agricultural Science Publisher: Beijing, China, 1993.

Li, Shufeng. The quality research of supercritical CO_2 extracting natural product. National research supercritical fluid technique and practice symposium. *Shi Jiazhuang* **1996**, *10*, 30−35.

Mucunjin, Guihetianguangnan. *Adv. Food Process. Technol.* **1985**, *10*, 41−64.

Roughen, P. G. Spirulina. A Source of Dietary γ-Linolenic acid. *J. Sci Food Agric.* **1989**, *47*, 85−93.

Wei, Xiyao. Determination of flavonoid compounds in *Gingko biloba* leaves supercritical fluid extraction and high performance Liquid Chromatography (SFE-HPLC). *Chin. Chem. Lett.* **1995**, *6* (7), 589−592.

Zhen, Jianxian; Geng, Liping. The basic material of functional food−r-linolenic acid. *Food Ferment. Ind.* **1996**, *1*, 49−54.

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