JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

J. Agric. Food Chem. **2003,** *51,* 7921−7925

Supercritical Carbon Dioxide Extraction of Oil and Squalene from *Amaranthus* Grain

HAN-PING HE,† HAROLD CORKE,*,† AND JIAN-GUO CAI‡

Department of Botany, The University of Hong Kong, Pokfulam Road,
Hong Kong, People's Republic of China, and College of Chemical Engineering,
East China University of Science and Technology, 130 Meilong Road,
Shanghai 200237, People's Republic of China

Supercritical carbon dioxide (SC CO_2) was used for the extraction of oil and squalene from *Amaranthus* grain. Very small amounts of oil could be extracted by SC CO_2 from undisrupted grains, although SC CO_2 possesses higher diffusivity. Grinding increased the extraction rate and oil yield, and smaller particle size gave higher extraction rate. The oil yield and initial extraction rate increased linearly with the increasing SC CO_2 flow rate from 1 to 2 L/min. Increasing the flow rate of SC CO_2 above 2 L/min resulted in only a slight increase of oil yield and extraction rate. In the pressure range of 150−250 bar, extraction decreased with increasing temperature at a constant pressure, whereas at a pressure of 300 bar, the extraction yield increased with increasing temperature. Possible reasons for this are discussed. Effects of temperature and pressure on squalene yield were different from those on oil yield. A good oil yield (4.77 g of oil/100 g of grain) was obtained at 40 °C and 250 bar. The highest squalene yield (0.31 g of squalene/100 g of grain) and concentration (15.3% in extract) were obtained at 50 °C and 200 bar, although the oil yield under this condition was low (2.07 g of oil/100 g of grain). The moisture content within 0−10% had little influence on yields of oil and squalene at 40 °C and 250 bar. Finally, the oil yield and the squalene concentration in the extracts by SC CO_2 were compared to those by solvent extraction.

KEYWORDS: ***Amaranthus*; carbon dioxide; oil; squalene; supercritical fluid extraction**

INTRODUCTION

Oil from *Amaranthus* grain has been reported to contain higher levels (up to 8%) of squalene than other vegetable oils (*1*), although amaranth is not considered to be a typical oilseed crop. Squalene is an important ingredient in skin cosmetics and a good lubricant for computer disks (*1*). Squalene has been reported to have important direct or indirect beneficial effects on health, such as decreasing the risk for various cancers and reducing serum cholesterol levels (*2*, *3*). Amaranth is a potential alternative to shark and whale as a source of squalene (*1*, *4*, *5*), which are currently the main sources of squalene and are limited in availability due to the protection of marine animals.

Supercritical fluid extraction (SFE) has received attention as an attractive alternative to conventional extraction using organic solvents. A supercritical fluid (SCF) possesses physical properties intermediate between those of a gas and a liquid. A liquid-like density, leading to high loadings of solutes, coupled with the pressure-dependent solvating ability of SCF makes it an excellent solvent for separations and reactions. The low viscosity and high molecular diffusivity like those of a gas, combined with low surface tension, make SCF an ideal candidate for mass transfer solvents, allowing better penetration into the sample matrix than liquid solvents. Carbon dioxide is the most often used fluid because of its nontoxicity, nonflammability, lack of chemical residue problem, and low critical temperature. There has been rapid development of SFE applications over the past two decades, for example, decaffeination of green coffee with supercritical (SC) CO_2 (*6*) and the extraction of hops (*7*), spices (*8*), fruit aromas (*9*), cholesterol from edible animal fats (*10*), perfumes and flavors from natural products (*11*), and unsaturated fatty acids from fish oil (*12*).

As for squalene separation by SFE, several works on the extraction and fractionation of squalene from shark liver oil, in which the squalene was present at high concentration (40−75% by mass), have been reported (*13*, *14*). In a previous work, extraction of the oil from *Amaranthus* grain was accomplished by using hexane via a process of four cycles of immersion, draining, and desolventizing (*15*). A vacuum distillation process was examined to fractionate squalene from the extracted amaranth oil (*1*). Those processes were tedious and were associated with high operational temperatures. The squalene concentration and recovery were not satisfactory. This investigation examines extraction of oil and squalene by SC CO_2 from

---

* Corresponding author (fax +852 2858 3477; telephone +852 2299 0314; e-mail hcorke@yahoo.com).
† The University of Hong Kong.
‡ East China University of Science and Technology.

10.1021/jf030488y CCC: $25.00

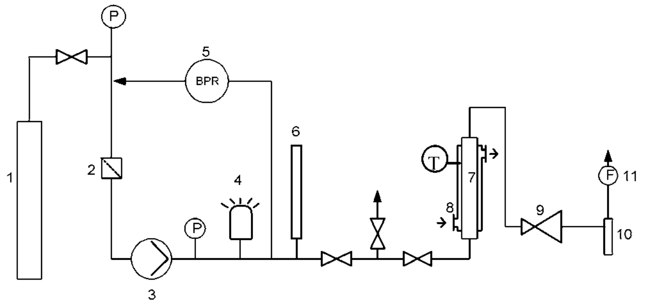

Figure 1. Schematic diagram of supercritical extraction apparatus: (1) CO_2 cylinder; (2) filter; (3) compressor; (4) safety valve; (5) back-pressure regulator; (6) compressed CO_2 tank; (7) extractor; (8) jacket water bath; (9) throttle valve; (10) collection vessel; (11) wet flow meter; P, pressure gauge; T, temperature gauge; F, flow rate meter.

amaranth grain, a potential alternative resource containing relatively low contents of oil (~5%) and squalene (~6% in oil). Effects of particle size, extraction time, flow rate of carbon dioxide, temperature, pressure, and moisture on oil extraction and on the composition of extracted oil were investigated.

EXPERIMENTAL PROCEDURES

Materials. Grain of *Amaranthus cruentus* var. K112 was planted at the experimental farm of Hubei Academy of Agricultural Science, Wuhan, China. Harvested grain was bulked for use in these experiments. Both intact and ground powder samples were used for SFE. The grain was ground by a laboratory mill (Kenwood) and was dried at 50 °C for 24 h (moisture content < 2.0%) before being used for extraction. The size distribution of powdered particles were measured by sieve analysis. Squalene (99.9% purity) standard was purchased from Sigma Chemical Co. (St. Louis, MO). All solvents used were of high performance liquid chromatography grade.

SFE: Apparatus and Operating Procedures. The schematic flow diagram of a semicontinuous supercritical extraction apparatus is shown in **Figure 1**. Liquid carbon dioxide (purity > 99%) from a cylinder was passed through a filter and was then compressed to operating pressure (150−350 bar) by a diaphragm pump. The pressure was controlled by a back-pressure regulator. Compressed carbon dioxide was introduced upward into a vertically mounted extraction vessel with an inner diameter of 25 mm and a length of 200 mm. Each extraction was run on 60 g powdered samples, placed in the vessel between two layers of glass wool to avoid loss of small particles of sample, and the remaining void in the cell was filled with defatted hygienic cotton. The temperature of the extraction vessel containing the sample to be extracted was controlled by a surrounding jacket water bath, the temperature being detected by a thermocouple in the water bath and regulated by a digital controller within an accuracy of ±0.1 °C. After the pressure and temperature reached the desired values, carbon dioxide was passed through the extractor to start the extraction. The oil-laden fluid from the extractor was depressurized to atmospheric pressure by passing through a throttle valve, and the extracted oil was collected in a cooled vessel. The volume of CO_2 consumed was measured using a wet gas meter. The experimental temperatures were 40, 50, 60, and 70 °C, and pressures were 150 ± 5, 200 ± 5, 250 ± 8, and 300 ± 10 bar, respectively. Except where specified, each extraction lasted for 2 h with a flow rate of 2.0 standard liters (at the standard state) per minute (2.0 SL/min).

Solvent Extraction. To compare with solvent extraction, the powdered samples were also extracted for oils by an extraction/desolventizing unit, Soxtec System HT6 (Tecator, Sweden), with petroleum ether (boiling range 40−60 °C) containing 0.01% butylated hydroxytoluene as an antioxidant to avoid the possible deterioration of unstable oil components (*16*). The moisture content was measured with the American Association of Cereal Chemistry (AACC) official method (*17*). Solvent extraction and moisture content determination were done in triplicate.

Table 1. Particle Size Distribution of the Samples Used for SC CO_2 Extraction

	>850 μm	500–850 μm	297–500 μm	<297 μm
undisrupted grain	~1 mm in diameter			
particle 1 (%)	66.7	19.5	4.7	9.1
particle 2 (%)	17.0	43.2	11.6	28.2
particle 3 (%)	8.9	43.0	16.2	31.9

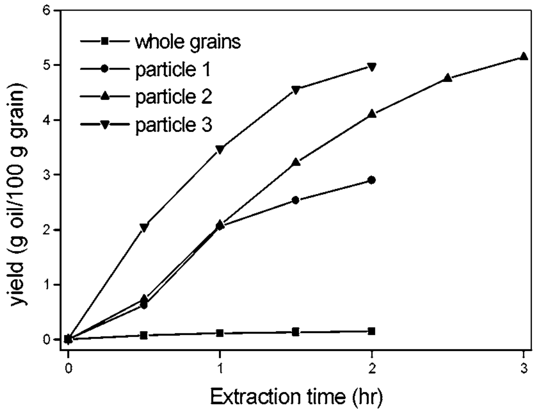

Figure 2. Effect of particle size on oil yield at 40 °C and 250 bar. (Particle size distribution is shown in **Table 1**.)

HPLC Analysis. Squalene contents in extracted oil samples were determined using a high-performance liquid chromatography system (Hewlett-Packard 1100) under the conditions described before (*16*).

RESULTS AND DISCUSSION

The size of amaranth grain is small, typically ~1 mm in diameter, and lenticular shaped. The grains were ground to powder and dried before being used for SFE. The effect of particle size on oil extraction was investigated at 40 °C and 250 bar with a flow rate 2 SL/min during an extraction time of 2 h. The particle size distributions of various samples are shown in **Table 1**. As with extraction by organic solvent (*16*), very little oil was extracted by SFE from undisrupted grains (**Figure 2**). Although SC CO_2 possesses higher diffusivity than the organic solvent, it is still difficult for SC CO_2 to penetrate the grain coat to access the inner embryonic tissue, containing lipid and lipid complex (*18*), for lipid extraction. The oil yield (grams of oil per 100 g of dry grain) by SC CO_2 extraction of the ground powder is obviously higher than from intact grain. The grinding process disrupts the grain coat and increases the specific surface area of particles, reducing mass transfer resistance and leaving the oil more accessible to the solvent, consequently increasing the extraction rate and the oil yield. The oil yield increased with decreasing average particle size because the intraparticle diffusion resistance is smaller for smaller particles due to the shorter diffusion path.

The effect of flow rate and extraction time on extraction of oil from the grain was examined at 40 °C and 250 bar, at flow rates of carbon dioxide of 1−5 SL/min. The extract was collected continuously every 30 min for 3−4 h in each operation. From the results (**Figure 3**), it was obvious that oil yield and initial extraction rate, obtained from the slope of the initial linear portion of the curves, increased with increasing SC CO_2 flow rate from 1 to 2 L/min. It seemed that a linear relationship existed between oil yield and flow rate of SC CO_2 when flow rate was below 2 SL/min during the first 2 h of extraction. Increasing flow rates of SC CO_2 above 2 L/min resulted in only a slight increase of extraction rate and oil yield (**Figure 3**),

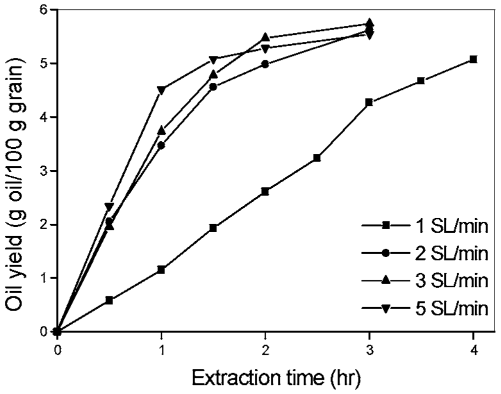

Figure 3. Oil yield at various flow rates of SC CO_2 (40 °C, 250 bar) as a function of extraction time.

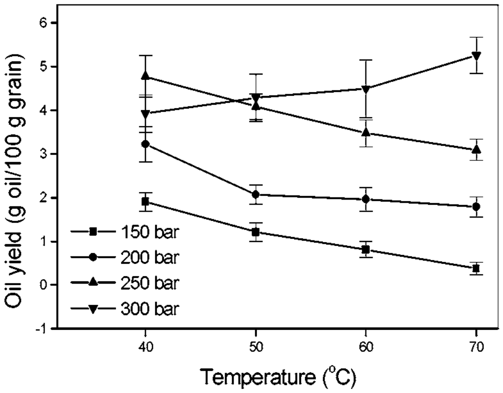

Figure 4. Oil yield at various temperature and pressure conditions for a 2 h extraction with a flow rate of 2 SL/min.

which indicated that for higher flow rate the carbon dioxide leaving the extractor is less saturated and the effect of increasing flow rate on extraction process is very small. After 1 h of extraction, the extraction rate reduced markedly and only a small amount of oil in the amaranth grain was extracted. In this portion of the extraction curve, mass transfer is determined by the diffusional resistance in the solid matrix. The difference of oil yield between 2 and 5 SL/min was not significant. Therefore, a flow rate of 2 SL/min was used in the following experiments to optimize other factors affecting extraction for its economic solute loading.

The influence of temperature and pressure on SFE carried out for 2 h of extraction at a constant CO_2 flow rate of 2.0 SL/min was studied. Oil yield increased markedly with increasing pressure over the range 150−250 bar at a constant temperature (**Figure 4**) because the high pressure resulted in an increase in the solubility of the oil. Temperature had an adverse effect on extraction yield at constant pressure, within the pressure range of 150−250 bar, and the extraction yield decreased with increasing temperature, reflecting the reduction in density of the solvent. Tsuda et al. (*19*) found that the extraction yield of antioxidative components from tamarind grain coat by SC CO_2 at 40 °C was higher than at 60 and 80 °C at a pressure of 300 bar. The extraction yield increase at higher pressure and lower temperature suggests that increased solvent density results in increased solubility of the oil in the solvent. However, it was observed in this study that the oil yield increased with increasing temperature at a pressure of 300 bar, whereas the density of SC CO_2 decreases with increasing temperature. An increase of extraction with increasing temperature was also reported when oryzanol was extracted from rice bran by SC CO_2 at a pressure of 680 bar over the temperature range of 30−75 °C (*20*). A similar crossover effect of temperature on extraction at different pressures was also observed on oil extraction from soybean by SC CO_2; that is, extraction is favored at lower temperature, whereas at higher pressures (>350 bar), the extraction increased with temperature (*21*). It is known that the solvent density undergoes only minor reductions at high pressure, tending to decrease solute solubility with increasing temperature, because CO_2 is nearly incompressible at high pressure. Simultaneously, the increasing temperature induces a vapor pressure increase of solutes, leading to increased solute solubility. A possible reason for the crossover effect of temperature mentioned above is that the vapor pressure effect is more significant than the density effect with increasing temperature under high pressure and the net result is that a high temperature in SFE resulted in higher yields at high pressure. Another effect is that, under high pressure, supercritical carbon dioxide at higher temperature may alter the physical properties of the grain powder matrix and make it more penetrable by extraction fluid (*20*). Although extraction of the oil at 70 °C under a pressure of 300 bar was slightly more than that obtained at 40 °C and 250 bar, the latter is preferable because it has a lower pressure and milder temperature condition for extraction, which can avoid possible degradation of some thermally labile compounds.

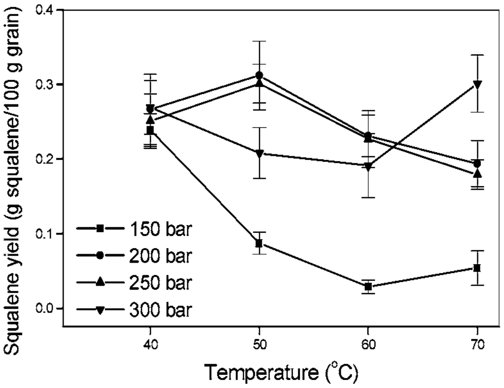

Figure 5. Squalene yield at various temperature and pressure conditions for a 2 h extraction with a flow rate of 2 SL/min.

The squalene yield in extracts prepared under various temperatures and pressures by SC CO_2 is shown in **Figure 5**. The squalene yields at 40 °C under different pressures were very close, ranging from 0.24 to 0.27 g of squalene/100 g of grain, although the oil yield varied significantly at this temperature. This indicates that squalene is easily extractable by SC CO_2 at 40 °C, and the lower oil yields at this temperature were due to the low solubility of oil components other than squalene. The squalene yields at 200 and 250 bar were similar, and at 50 °C both pressures gave maximum squalene yields. Squalene concentrations in the extracted oils under various temperatures and pressures are compared in **Figure 6**. The concentrations of squalene in extracts at a pressure of 200 bar are higher than at 250 and 300 bar. The polarity of squalene is weak, and thus it can be dissolved easily in SC CO_2. Therefore, the increase in solubility of other oil components with relatively stronger polarity, with increasing pressure, was higher than for squalene. As a result, the squalene concentration in extracts at pressures of 250 and 300 bar is less than at 200 bar. The fluctuation of squalene concentration in extracts at 150 bar might be caused by the lower moisture content (<2%) in the samples, which can significantly influence the phase behavior of oil components with SC CO_2 at low pressure.

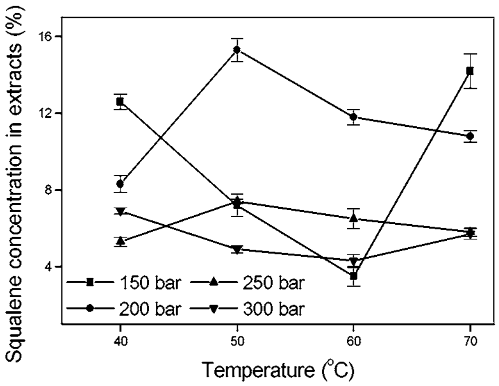

Figure 6. Squalene concentration in extracts at various temperature and pressure conditions for a 2 h extraction with a flow rate of 2 SL/min.

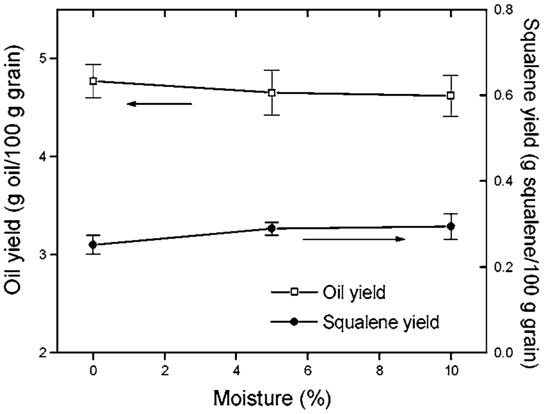

Figure 7. Effect of moisture content on oil yield and squalene yield at 40 °C and 250 bar.

Table 2. Comparison of Oil from *Amaranthus* Grain by SFE with Solvent Extraction

extraction method	oil yield (g of oil/100 g of grain)	squalen content in extracts (%)	squalene yield (g of squalene/ 100 g of grain)
solvent extraction[a]	4.98 ± 0.10	6.01 ± 0.11	0.30 ± 0.01
SC CO_2[b]	5.25 ± 0.41	5.73 ± 0.28	0.30 ± 0.02
SC CO_2[c]	4.77 ± 0.38	5.27 ± 0.23	0.25 ± 0.03
SC CO_2[d]	2.07 ± 0.22	15.3 ± 0.6	0.31 ± 0.05
SC CO_2[e]	0.38 ± 0.06	14.2 ± 0.9	0.05 ± 0.01

[a] Extraction with petroleum ether by Soxtec extraction. [b] Extraction at 70 °C under 300 bar with 2.0 SL/min for 2 h. [c] Extraction at 40 °C under 250 bar with 2.0 SL/min for 2 h. [d] Extraction at 50 °C under 200 bar with 2.0 SL/min for 2 h. [e] Extraction at 70 °C under 150 bar with 2.0 SL/min for 2 h.

The effect of moisture on oil and squalene extraction by SFE was examined at 40 °C and 250 bar. The moisture content did not exert any apparent influence on the extraction of oil and squalene from the grain by SFE at 0, 5, and 10% moisture (**Figure 7**). The influence of moisture content is thus negligible up to 10% moisture under the optimized operating conditions. The maximum moisture content of harvested grains is ~10%; thus, a drying process is not necessary when grain is used for oil extraction by SC CO_2.

The oil yield and squalene concentration in extracts by SFE were compared with those by solvent extraction (**Table 2**). The oil yields by SFE at 70 °C and 300 bar and at 40 °C and 250 bar were close to those by solvent extraction. Although the oil yield by SFE at 50 °C and 200 bar was low, it had a high squalene yield under the operation conditions employed, and the squalene concentration in the extract was much higher than others.

CONCLUSIONS

Oil and squalene extraction from *Amaranthus* grain by supercritical carbon dioxide was investigated. The operation conditions, including particle size, extraction time, flow rate of carbon dioxide, temperature, pressure, and moisture, were optimized. Oil and squalene could be successfully extracted by supercritical carbon dioxide, an efficient and environmentally friendly method, without consuming large amounts of solvent, time, and complex purification processing that are usually associated with solvent extraction. A high squalene yield (0.31 g of squalene/100 g of grain) and concentration (15.3% in extract) was obtained at 50 °C and 200 bar. The squalene concentration in extract obtained by solvent extraction was only 6 wt %. This result suggests that, by selecting suitable extraction conditions, we can selectively extract some compounds of interest by SFE that could not be achieved by solvent extraction.

LITERATURE CITED

(1) Sun, H.; Wiesenborn, D.; Tostenson, K.; Gillespie, J.; Rayas-Duarte, P. Fractionation of squalene from amaranth seed oil. *J. Am. Oil Chem. Soc.* **1997**, *74*, 413.

(2) Rao, C. V.; Newmark, H. L. Chemopreventive effect of squalene on colon cancer. *Carcinogenesis* **1998**, *19*, 287.

(3) Miettinen, T. A.; Vanhanen, H. Serum concentration and metabolism of cholesterol during rapeseed oil and squalene feeding. *Am. J. Clin. Nutr.* **1994**, *59*, 356.

(4) Marcone, M. F. First report of the characterization of the threatened plant species *Amaranthus pumilus* (seabeach amaranth). *J. Agric. Food Chem.* **2000**, *48*, 378.

(5) Bruni, R.; Medici, A.; Guerrini, A.; Scalia, S.; Poli, F.; Muzzoli, M.; Sacchetti, G. Wild *Amaranthus* caudatus seed oil, a nutraceutical resource from Ecuadorian flora. *J. Agric. Food Chem.* **2001**, *49*, 5455.

(6) Zosel, K. Process for the decaffeination of coffee. U.S. Patent 4,247,580, 1981.

(7) Vollbrecht, R. Extraction of hops with supercritical CO_2. *Chem. Ind.* **1982**, *19*, 397.

(8) Hubert, P.; Vitzthum, O. G. Fluid extraction of hops, spices, and tobacco, with supercritical gases. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 710.

(9) Schultz, W. G.; Randall, J. M. Liquid carbon dioxide for selective aroma extraction. *Food Technol.* **1970**, *24*, 94.

(10) Chao, R. R. Supercritical CO_2 extraction of meat products and edible animal fats for cholesterol reduction. In *Supercritical Fluid Technology in Oil and Lipid Chemistry*; King, J. W., List, G. R., Eds.; American Oil Chemists' Society: Champaign, IL, 1996; pp 230−246.

(11) Coenan, H.; Hagen, R.; Knuth, M. Method for obtaining aromatics and dyestuffs from bell peppers. U.S. Patent 4,400,398, 1983.

(12) Nilsson, W. B. Supercritical fluid extraction and fractionation of fish oil. In *Supercritical Fluid Technology in Oil and Lipid Chemistry*; King, J. W., List, G. R., Eds.; American Oil Chemists' Society: Champaign, IL, 1996; pp 180−212.

(13) Catchpole, O. J.; von Kamp, J. C.; Grey, J. B. Extraction of squalene from shark liver oil in a packed column using supercritical carbon dioxide. *Ind. Eng. Chem. Res.* **1997**, *36*, 4318.

(14) Catchpole, O. J.; Simoes, P.; Grey, J. B.; Nogueiro, E. M. M.; Carmelo, P. J.; da Ponte, M. N. Fractionation of lipids in a static mixer and packed column using supercritical carbon dioxide. *Ind. Eng. Chem. Res.* **2000**, *39*, 4820.

(15) Sun, H.; Wiesenborn, D.; Rayas-Duarte, P.; Mohamed, A.; Hagen, K. Bench-scale processing of amaranth seed for oil. *J. Am. Oil Chem. Soc.* **1995**, *72*, 1551.

(16) He, H. P.; Cai, Y. Z.; Sun, M.; Corke, H. Extraction and purification of squalene from *Amaranthus* grain. *J. Agric. Food Chem.* **2002**, *50*, 368.

(17) AACC. Moisture-air-oven (aluminum plate) method 44-16. In *Approved Methods of the American Association of Cereal Chemists*, 10th ed.; AACC: St. Paul, MN, 2000.

(18) Becker, R. Amaranth oil: composition, processing, and nutritional qualities. In *Amaranth Biology, Chemistry, and Technology*; Paredes-Lopez, O., Ed.; CRC Press: Boca Raton, FL, 1994; pp 133−144.

(19) Tsuda, T.; Mizuno, K.; Ohshima, K.; Kawakishi, S.; Osawa, T. Supercritical carbon-dioxide extraction of antioxidative components from tamarind (*Tamarindus indica* L) seed coat. *J. Agric. Food Chem.* **1995**, *43*, 2803.

(20) Xu, Z. M.; Godber, J. S. Comparison of supercritical fluid and solvent extraction methods in extracting γ-oryzanol from rice bran. *J. Am. Oil Chem. Soc.* **2000**, *77*, 547.

(21) Friedrich, J. P.; Pryde, E. H. Supercritical CO_2 extraction of lipid-bearing materials and characterization of the products. *J. Am. Oil Chem. Soc.* **1984**, *61*, 223.

Received for review July 7, 2003. Revised manuscript received October 21, 2003. Accepted October 22, 2003.

JF030488Y