Oxidative Stability of Sunflower Oil Extracted with Supercritical Carbon Dioxide

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Extraction of oilseeds with supercritical carbon dioxide ($SC\text{-}CO_2$) is a promising technique to obtain vegetable oils. However, instability of such oils has been associated in the past with $SC\text{-}CO_2$ extraction. The reasons underlying such instability were unclear. Results presented here suggest that oil instability may be related to the oxygen content of CO_2. In fact, oil stability decreases sharply when refined oil (additive-free) is re-extracted with $SC\text{-}CO_2$ and can be related to the oxygen content in the CO_2. Nevertheless, oil stability could be improved to the level of conventionally extracted oil by adding trace amounts of ascorbic acid.

KEY WORDS: Oil composition, oil stability, $SC\text{-}CO_2$ extraction.

Extraction of oil from soybeans, sunflower and rapeseed with supercritical carbon dioxide ($SC\text{-}CO_2$) has been described previously (1–4). $SC\text{-}CO_2$-extracted sunflower oil is reported to be similar to hexane-extracted oil in terms of triglyceride composition and organoleptic properties. However, it behaves poorly toward oxidative deterioration. Similar results have been shown for $SC\text{-}CO_2$-extracted oil from soybean (5) and rice bran (6).

In previous reports (7), poor oxidative stability has been attributed to the low content of phospholipids. However, results presented here suggest that low stability may be associated with the $SC\text{-}CO_2$ extraction step. Practical methods to prevent oxidation are described.

EXPERIMENTAL PROCEDURES

Sunflower seeds, finely chopped with a grinder, were used to obtain $SC\text{-}CO_2$-extracted oil and hexane-extracted oil. Refined oil, free from antioxidant additives, was purchased in a local market (Vallodolid, Spain). CO_2 used in the experiments was drawn as a liquid at 5.0 Mpa from pressurized steel cylinders, (Sociedad Española de Oxigeno, Valladolid, Spain). The impurities of three types of commercial CO_2 used in this study are summarized in Table 1.

$SC\text{-}CO_2$-extracted oil was obtained with apparatus consisting of a homemade extractor and sample collector in tandem with a commercial pump. A detailed description of the equipment has been previously provided (8). A weighed amount of chopped seed (about 50 g) was placed inside the extractor. Two stainless-steel filter meshes were placed vertically in the extractor to prevent small seed fragments from plugging pipes and valves. The extractor was then introduced into the temperature-controlled chamber, where the desired temperature for extraction is reached. Care was taken to ensure that air was purged prior to extraction.

Liquid CO_2 was pumped up to the extraction pressure and directed into the bottom of the extractor. Dissolved oil and CO_2 in the SC phase left the extractor and reached the valve, where the pressure was reduced to atmospheric conditions. Oil was collected in a test tube while CO_2 left through the flowmeter into the atmosphere. Hexane-extracted oil was obtained by Soxhlet extraction.

Triglycerides were determined by high-performance liquid chromatography (9). Tocopherol and phosphorous were analyzed according to the methods UNE 55 108 and UNE 55 073 (10), respectively.

Fluorescence spectra of $SC\text{-}CO_2$- and hexane-extracted oils were recorded in order to observe pure tocopherol and tocophenylquinone moieties. Tocopherols (α, β and γ) exhibit the same excitation and fluorescent spectra. However, tocophenylquinone, the oxidized form of the vitamin, is nonfluorescent.

Oxidative stability of the oils was determined by the Olcott and Einset Method (11). In order to study oil oxidative stability, several additives were used, including tocopherol (mixed isomers from vegetable oils, 670 mg/g; Sigma Chemical Co., St. Louis, MO), lecithin (phosphatidylcholine, 99%; Sigma) and ascorbic acid (>99.95%; Sigma) in the amounts specified in the Results and Discussion section.

TABLE 1

Impurities of Three Types of Commercial Carbon Dioxide (CO_2) (ppm)

Quality	H_2O	O_2	CO	C_nH_m	N_2	H_2
Industrial CO_2		$H_2 + O_2 + N_2 < 0.5\%$				
N40 CO_2	10	10		5		
N50 CO_2	3	2		2		0.5

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RESULTS AND DISCUSSION

Chemical and physical characteristics of $SC\text{-}CO_2$-extracted oil. The triglyceride content of $SC\text{-}CO_2$- and hexane-extracted crude oils are shown in Table 2. There were no appreciable differences in the triglyceride contents of $SC\text{-}CO_2$- and hexane-extracted oils, which agrees with previous studies (5,6).

With respect to tocopherol content, considered to be a natural antioxidant, $SC\text{-}CO_2$-extracted oil contained larger amounts of tocopherols than did crude hexane-extracted oil (Table 3). List and Friedrich (7) reported a similar trend for soybean oil, although Zhao *et al.* (6) found little difference in the tocopherol content of $SC\text{-}CO_2$-and crude hexane-extracted rice bran oil.

Refined hexane-extracted oil typically contains low phospholipid concentrations (less than 1 ppm of total phosphorus), which is similar to the amount found for crude $SC\text{-}CO_2$-extracted oil. Our findings are in agreement to those obtained for soybean (5), canola (12), rice bran (6) and corn germ oils (13).

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TABLE 2

Triglyceride Composition of Supercritical Carbon Dioxide ($SC-CO_2$)- and Hexane-Extracted Sunflower Oil

Triglycerides[a]	$SC-CO_2$-extracted (%)	Hexane-extracted (%)
LLL	34.9	37.9
OLL	24.8	25.1
PLL	12.2	11
OOL	5.2	5.7
POL + SLL	13.7	13.6
OOO	0.8	1.2
POO	3.4	3.6
SOO	0.6	1.2
SOS	0.4	0.7

[a]L, linoleic acid; O, oleic acid; P, palmitic acid; S, stearic acid.

TABLE 3

Tocopherol Content of Supercritical Carbon Dioxide ($SC-CO_2$)- and Hexane-Extracted Sunflower Oil (ppm)

	Tocopherol content (ppm)	
Extraction method	α-Tocopherol	γ-Tocopherol
$SC-CO_2$	1,157	62
Hexane	250	52

Stability of sunflower oil. $SC-CO_2$-extracted oil from sunflower seed was less stable than hexane-extracted oil (Fig. 1). Similar results have been reported for soybean (5) and rice bran oils (6).

The oxidative stability of $SC-CO_2$-extracted oil was independent of changes in pressure and temperature conditions in the range of 25.0–35.0 MPa and between 25 and 80°C, respectively. No differences were observed during the induction period or in the oxidation rate when successive fractions were tested.

Poor oxidative stability has been attributed to low phospholipid content (5). This hypothesis is supported by the fact that phospholipids may act as an oxygen barrier at the oil/air interface, and thus reduce the rate of oxygen uptake by the sample. Consequently, the poor oxidative stability of $SC-CO_2$-extracted oil could be explained by the low content of phosphorous as compared to crude hexane-extracted oil. However, the oxidative stability of $SC-CO_2$-extracted oil was not significantly improved by adding lecithin at 0.01 and 0.1% levels (Fig. 2a). This is in agreement with the fact that the phospholipid content in refined hexane-extracted oil (additive-free) is similar to that of $SC-CO_2$-extracted oil, although it has been shown to be more stable in the hexane-extracted oil.

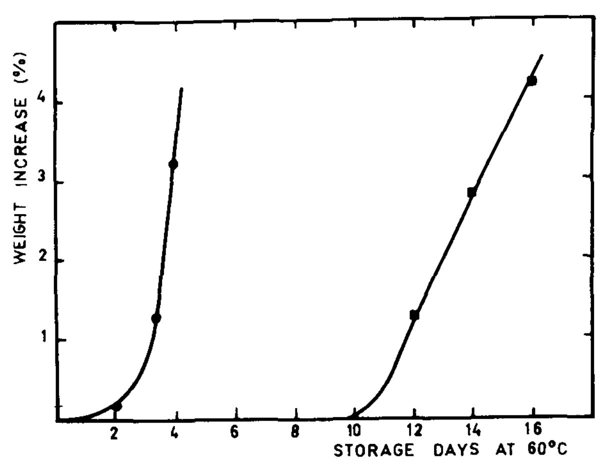


FIG. 1. Oxidative stability of supercritical carbon dioxide ($SC-CO_2$)- and hexane-extracted crude oils from sunflower seeds, under Schall oven conditions (●, N50 $SC-CO_2$-extracted oil; ■, hexane-extracted oil).

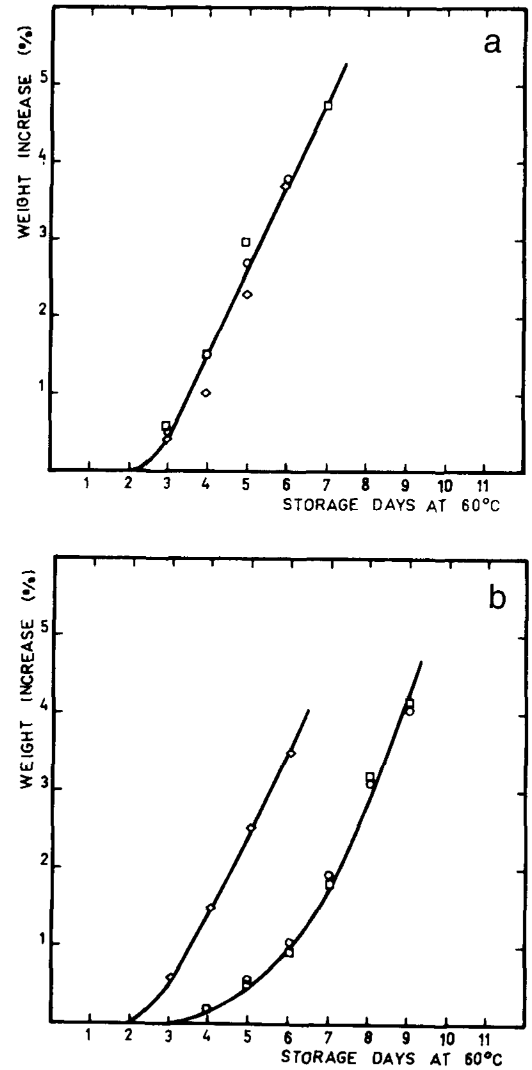
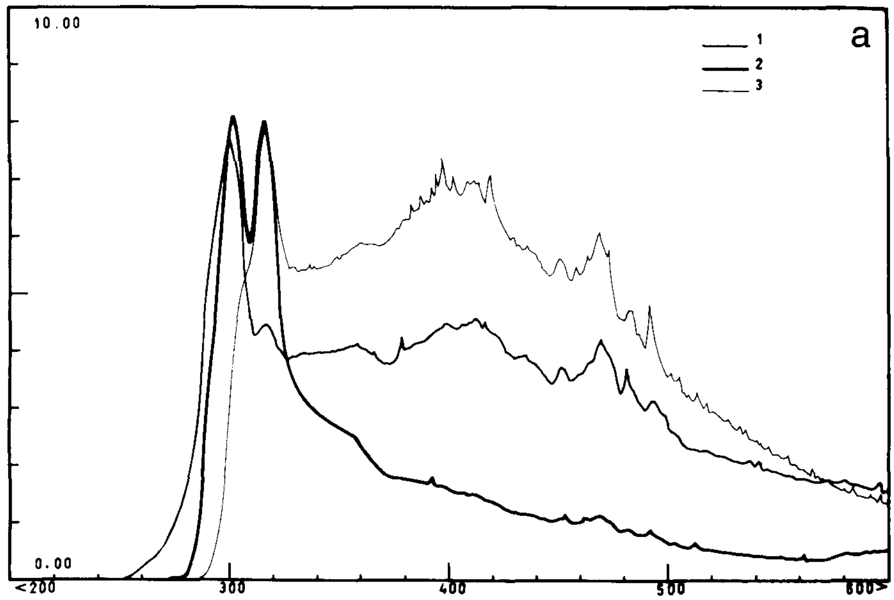


FIG. 2. (a) Effect of natural phospholipid addition upon the oxidation process of $SC-CO_2$-extracted oil (□, refined hexane oil re-extracted with $SC-CO_2$; ○, the same oil with 0.01%; and ◇, with 0.1% added lecithin). (b) Effect of antioxidant addition upon the oxidation process of $SC-CO_2$-extracted oil (◇, refined hexane oil reextracted with $SC-CO_2$; ○, the same oil with 0.01%; and □, with 0.1% added tocopherol). Abbreviation as in Figure 1.

Differences in stability cannot be attributed to tocopherol content either because it is present in higher amounts in $SC-CO_2$-extracted oil (Table 3), and the addition of tocopherol at 0.01 and 0.1% showed no effect upon oil stability (Fig. 2b). However, tocopherol seems to be partially affected by the extraction process. As shown in Figure 3a, it is produced as an intermediate compound



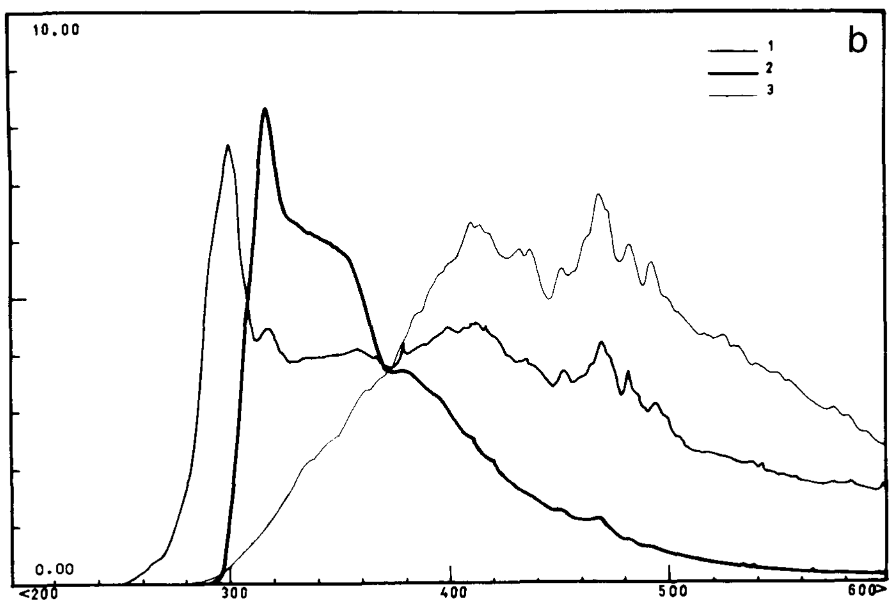


FIG. 3. (a) Excitation fluorescence spectra of three different oil samples: 1, Refined hexane-extracted oil; the maximum at *ca.* 300 μm corresponds to the reduced antioxidant tocopherol level; 2, refined hexane-extracted oil reextracted with SC-CO_2; 3, refined oil reextracted with SC-CO_2 after three days of storage at 60°C. (b) Excitation fluorescence spectra of three samples of refined hexane-extracted oil at different moments of their oxidation processes: 1, Refined hexane-extracted oil; 2, refined hexane-extracted oil after four days of storage at 60°C; 3, hexane-extracted oil after six days. Abbreviation as in Figure 1.

(maximum at 316 μm) and disappears as oxidation proceeds, producing tocophenylquinone, which is nonfluorescent. This effect is observed during oxidation of commercial oil (Fig. 2b).

Because differences in stability cannot be fully explained by differences in chemical composition, it appears that there is something intrinsic to the extraction procedure itself. This becomes evident when refined oil (additive-free, 496 ppm α-tocopherol and 2–8 ppm γ-tocopherol) is reextracted with SC-CO_2. As shown in Figure 4, oil stability decreases sharply after SC-CO_2 reextraction, and it can be related to the oxygen content of the CO_2. This effect can be prevented by antioxidant additives, such as ascorbic acid, when it is added into the extractor prior to the extraction (Fig. 5).

During oil deterioration, oxygen is added to double bonds of the triglycerides. The amount of oxygen uptake by the oil is increased during SC extraction by the oxygen present in the extraction solvent, CO_2. A large amount of total oxygen is passed through the seed bed to obtain the oil. Oil solubility at 30.0 mPa and 40°C is *ca.* 6.5 mg/g CO_2 (8); therefore, 154 g CO_2 are needed to obtain 1 g of oil. The oxygen concentration in top-quality CO_2 is 2 ppm. So, for each 1 g of oil, 0.32 mg O_2 is available,

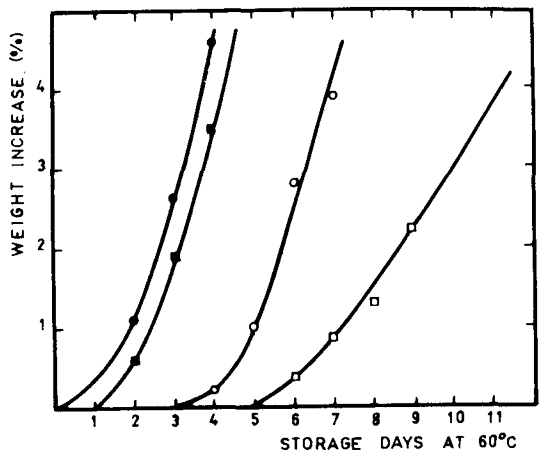


FIG. 4. Comparison of oxidative deterioration processes between refined hexane-extracted oil and the same oil reextracted with SC-CO_2 of three different qualities (□, refined hexane oil; ●, refined hexane oil reextracted with industrial CO_2; ■, reextracted with N40 CO_2; ○, reextracted with N50 CO_2). Abbreviation as in Figure 1.

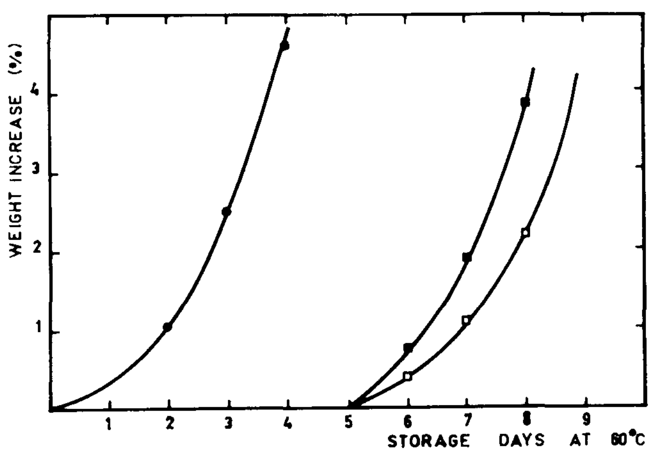


FIG. 5. Effect of ascorbic acid addition upon the oxidation process of SC-CO_2-extracted oil. (□, refined hexane-extracted oil; ●, the same oil reextracted with SC-CO_2; ■, SC-CO_2 reextracted oil but with 10 ppm of ascorbic acid added). Abbreviation as in Figure 1.

which means a ratio of 100 mol oil/mol O_2. For the second best-quality CO_2, this ratio becomes 20 mol oil/mol O_2. This quantity could be enough to initiate oxidation. Besides, this reaction takes place without being limited by the mass transfer of the gas into the oil. During extraction, the solvent (CO_2 and O_2) and oil are present in the same phase, and oxidation may occur as a homogeneous reaction. The more oxygen present in the CO_2, the more fatty acid molecules will be exposed to oxygen, and the faster the reaction will occur.

To summarize, oxidation of SC-CO_2-extracted oil appears to result from the presence of trace amounts of oxygen in the extraction solvent. The presence of oxygen and the absence of mass transfer limitations promote oxidation of oil triglycerides in the SC phase. Tocopherol, which is present in considerable quantity, seems to be ineffective in retarding autoxidation of the oil.

Oil stability can be improved greatly by purging oxygen or air from all pipes and valves and from the extractor, and by using top-quality CO_2, which is low in O_2. However, addition of ascorbic acid can be a reasonable solution when it is either impractical or expensive to eliminate oxygen from the CO_2. The stability of SC-CO_2-extracted oil treated with ascorbic acid is similar to or better than that of refined hexane-extracted oil.

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