Supercritical Carbon Dioxide Extraction of Antioxidative Components from Tamarind (*Tamarindus indica* L.) Seed Coat

Takanori Tsuda,*,† Kosuke Mizuno,‡ Katsumi Ohshima,† Shunro Kawakishi,§ and Toshihiko Osawa§

Food Research Institute, Aichi Prefectural Government, 2-1-1 Shinpukuji-cho, Nishi-ku, Nagoya 451, Japan, Department of Agricultural Chemistry, Meijo University, Tenpaku-ku, Nagoya 468, Japan, and Department of Applied Biological Sciences, Nagoya University, Nagoya 464-01, Japan

Supercritical carbon dioxide (SC-CO_2) extraction was investigated for extraction of antioxidants from tamarind (*Tamarindus indica* L.) seed coat. Different combinations of pressure and temperature were used with and without ethanol as modifier. As temperature and pressure were increased, more antioxidants were extracted. Using ethanol as the modifier gave more extraction yield of antioxidant compounds. The SC-CO_2 extract with ethanol (30 MPa, 80 °C) was also a strong antioxidant for lard as well as linoleic acid autoxidation. These results suggest that the antioxidants included in tamarind seed coat can be extracted using SC-CO_2 and that they may be used for increasing the shelf life of foods by preventing lipid peroxidation.

Keywords: *Supercritical fluid extraction; antioxidant; tamarind; Tamarindus indica L.; seed coat*

Recently, much attention in food industries has been focused on supercritical fluid extraction (SFE). Carbon dioxide (CO_2) has been employed as a supercritical fluid because it has a low critical temperature (31.1 °C) and pressure (7.28 MPa), which make it an ideal solvent for extracting thermally sensitive materials. CO_2 is nontoxic, nonflammable, low cost, and preferred over organic solvents for utilization by food industries. Further, oxygen is cut off under extraction by using CO_2. Therefore, supercritical CO_2 (SC-CO_2) extraction is used commercially in the decaffeination of coffee and tea (Zosel, 1978) and extraction of hops (Sharpe and Crabb, 1980). Reduction of milk and egg yolk fat and cholesterol (Bhaskar et al., 1993; Froning, 1990; Bradley, 1989), extraction of lipids from meat (J. King et al., 1993; Merkle and Larick, 1993), extraction of β-carotene from sweetpotato (Spanos et al., 1993), and purification of oil from seed flakes (Fattori et al., 1987; List et al., 1993) have been experimentally investigated. Furthermore, essential oils production (Reverchon and Senatore, 1994) and application to flavors and aromas (M. King et al., 1993; Kerrola and Kallio, 1993) have been also reported. In addition, the application to herbicides, pesticides, and mycotoxin residue analysis has been demonstrated in recent years in various samples, such as soil (Locke, 1993; Lopez-Avila and Dodhiwala, 1993), grain (Taylor et al., 1993), meat (Nam and King, 1994), and vegetables and fruits (Aharonson et al., 1994; Wigfield and Lanouette, 1993).

Tamarind (*Tamarindus indica* L.) belongs to Leguminosae, grows naturally now in tropical and subtropical regions, and is one of the most important plant resources for food materials (Duke, 1981). The germ obtained from the seeds is used for manufacturing tamarind gum, and it has been added to many kinds of foods in Japan to improve their viscosity. However, the seed coat as a byproduct of tamarind gum has hardly been used, and there have been no reports on the antioxidative activity of the seeds. Therefore, we have investigated the antioxidative activity of tamarind seeds, and the antioxidants contained in ethyl acetate extract prepared from the seed coat have been already isolated and identified (Tsuda et al., 1993, 1994). Organic solvents have limited use in the food industry; ethyl acetate, in particular, is a dangerous solvent for human health and is undesirable for use in extracting antioxidants for food. This stimulated our interest in using SC-CO_2 to extract antioxidative components from tamarind seed coat as a safe natural extract. The purpose of this study was to investigate SC-CO_2 extraction of antioxidative components from tamarind seed coat and to evaluate the composition and quantification of antioxidant levels contained in the SC-CO_2 extract.

MATERIALS AND METHODS

Chemicals. Tamarind seed coat was obtained from Yaegaki Zymotechnics, Inc., Japan. The seed coat was cultivated in India in 1993, cleaned, and stored at 4 °C until used. Linoleic acid and α-tocopherol were purchased from Wako Pure Chemical Industries, Ltd., Japan. Lard (no additives) was obtained from NOF Co., Japan. Mixed tocopherols (mixed isomers from vegetable oils, 86% as α-tocopherol) were obtained from Eisai Co. Ltd., Japan. 2-Hydroxy-3′,4′-dihydroxyacetophenone (HDA), methyl 3,4-dihydroxybenzoate (MDB), 3,4-dihydroxyphenyl acetate (DPA), and (−)-epicatechin (EC) were extracted via ethyl acetate and purified by using preparative high-performance liquid chromatography (HPLC). These structures and purities were confirmed by ^{1}H-NMR, ^{13}C-NMR, EI-MS, UV−vis, and IR spectra (Tsuda et al., 1994).

Extraction Apparatus. SC-CO_2 extraction was performed by using JASCO SUPER 200 supercritical extraction equipment (JASCO, Japan) with 10 or 50 mL stainless steel extraction vessels.

Organic Solvent Extraction. Tamarind seed coat (100 g) was ground by using a chemical grinder (NRK Inc., Japan), extracted three times with ethyl acetate (1000 mL), and then filtered. The extraction was performed without stirring at room temperature, and the extraction time was a period of 17 h for each of the three extraction steps. The filtrate was concentrated to dryness *in vacuo*.

SC-CO_2 Extraction. After ground tamarind seed coat (2 g) was packed in the extraction vessel, SC-CO_2 extraction was

* Author to whom correspondence should be addressed.
† Food Research Institute.
‡ Meijo University.
§ Nagoya University.

0021-8561/95/1443-2803$09.00/0

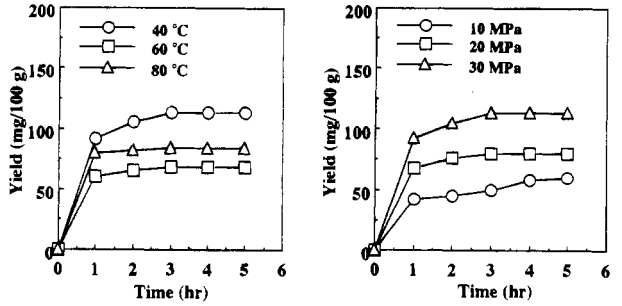

Figure 1. Effect of supercritical CO_2 extraction conditions on extractivity of tamarind seed coat extract without modifier: (A, left) influence on the yield when the extraction temperature was changed (40, 60, and 80 °C) and the pressure was kept constant at 30 MPa; (B, right) Influence on the yield when the extraction pressure was changed (10, 20, and 30 MPa) and the temperature was kept constant at 40 °C.

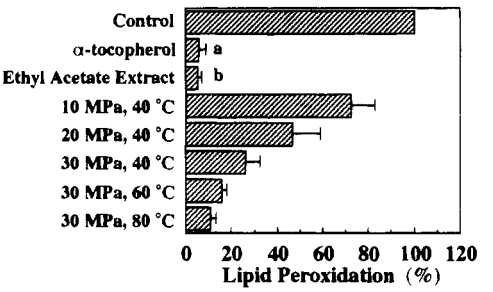

Figure 2. Antioxidative activity of supercritical CO_2 extracts prepared from tamarind seed coat as measured by the thiocyanate method. The extraction time of supercritical CO_2 extracted samples was 5 h under all conditions. Reported values are the mean ± SD (n = 3). A control containing no added samples or standards on its values represents 100% lipid peroxidation. (a, b) $P < 0.05$, compared to 30 MPa, 80 °C.

performed for 5 h. Liquid CO_2 (the purity of CO_2 was more than 99.9%) was compressed and then pumped through the extraction vessel. The flow rate was 5.0 mL/min, and the total volume of CO_2 passed through each sample was 1500 mL. The extraction temperature (40, 60, or 80 °C) was regulated with a controller (±0.1 °C). The pressures in both the extraction and separation vessels were controlled by means of back-pressure regulators. In all cases the extracts were trapped in ethanol and then evaporated to dryness *in vacuo*.

Extraction Modifier. Ethanol was used as extraction modifier. The modifier flow rate was 0.5 mL/min.

Antioxidative Assay of SC-CO_2 Extracts in Linoleic Acid System. Antioxidative assay was carried out by using a linoleic acid system (Osawa and Namiki, 1981). Each sample (200 μg) was added to a solution mixture of linoleic acid (0.13 mL), 99.0% distilled ethanol (10 mL), and 50 mM phosphate buffer (pH 7.0, 10 mL); the total volume was adjusted to 25 mL with distilled water. The solution was incubated at 40 °C, and the degree of oxidation was measured according to the thiocyanate method (Mitsuda et al., 1966) for measuring peroxides by reading the absorbance at 500 nm after coloring with $FeCl_2$ and ammonium thiocyanate and by the thiobarbituric acid (TBA) method (Ottolenghi, 1959). α-Tocopherol (200 μg) was used as the standard antioxidant sample.

Measurement of Antioxidative Components in SC-CO_2 Extracts. Measurement of the antioxidative components (HDA, MDB, DPA, and EC) in the SC-CO_2 extracts was performed by HPLC. HPLC was carried out on a Develosil ODS-5 column (Nomura Chemical Co. Ltd., Japan, 4.6 mm × 250 mm), with a UV spectrophotometric detector (Shimadzu Works Co. Ltd., Japan, LC-10AV, 280 nm), and 17% acetonitrile in water containing 0.1% trifluoroacetic acid (TFA) as a solvent at a flow rate of 1.0 mL/min. The extraction time of measured SC-CO_2 extracted samples was 5 h under all conditions.

Effect of SC-CO_2 Extracts on Oxidative Stability in Edible Oil. The effect of SC-CO_2 extracts on peroxide value (POV) level in edible oil was determined by using an iodiometric method following the standard method of the Japan Oil Chemists' Society (JOCS, 1972). The SC-CO_2 extraction parameters were 30 MPa and 80 °C. The extraction was based on 5 h with ethanol as modifier, and the total volume of CO_2 passed through the sample was 1500 mL. Edible oil (lard) containing 0.1% of the SC-CO_2 extract or 0.1% of mixed tocopherols was prepared and incubated at 60 °C. POV (expressed as milliequivalents of peroxide per kilogram of lipid) was measured at intervals during the incubation.

Statistics. Statistical analysis was performed by using Student's t test.

RESULTS AND DISCUSSION

SC-CO_2 Extraction of Antioxidants without Extraction Modifier. The yield of SC-CO_2 extract from tamarind seed coat with neat CO_2 is shown in Figure 1. Figure 1A shows the yield when the extraction temperature was changed (40, 60, and 80 °C) while the pressure was kept constant at 30 MPa. The yield was greatest at 40 °C. Figure 1B shows the yield when the extraction pressure was changed (10, 20, and 30 MPa) while the temperature was kept constant at 40 °C. The yield increased as the pressure was increased. The yields were maximum at 3 h under all conditions. The total amount of extract obtained by ethyl acetate was 740 mg/100 g of seed coat, and yield from SC-CO_2 under these conditions was lower than that of the ethyl acetate extract.

Antioxidative Activity of Neat SC-CO_2 Extracts. The antioxidative activity of the SC-CO_2 extract as measured by the thiocyanate method is shown in Figure 2. The extraction conditions of 30 MPa and 80 °C exhibited the strongest antioxidative activity of the five extract conditions; however, conditions of 30 MPa and 40 °C gave more yield than did 30 MPa and 80 °C, since SC-CO_2 has a higher density at low temperature. The extent of activity of these SC-CO_2 extracts was lower than that of α-tocopherol and the ethyl acetate extract (significantly different, $P < 0.05$). The results of the TBA method showed the same tendency (data not shown).

Measurement of Antioxidants Level in Neat SC-CO_2 Extracts. The measurement of antioxidants contained in the neat SC-CO_2 extracts is shown in Table 1. The higher extraction pressure and temperature provided more antioxidants. Among the antioxidants, EC was obtained in the greatest yield under all conditions. Compared to the amount of antioxidants extracted with ethyl acetate extract, neat SC-CO_2 was a less effective extraction solvent; while milligram quantities of the antioxidant compounds were extracted by ethyl acetate, only microgram quantities were obtained with unmodified SC-CO_2.

Modified SC-CO_2 Extraction of Antioxidants. In general, nonpolar substances are easily extracted by neat SC-CO_2, but it is difficult to extract polar substances. Extraction modifiers have been used recently to improve the extractivity and solubility of polar substances (Hawthorne, 1990), but the solvents should be safe and permitted in food industries. Therefore, ethanol was chosen as the modifier in this study.

The yield of SC-CO_2 extract from tamarind seed coat using ethanol as modifier is shown in Figure 3. Figure 3A shows the results when the temperature was changed (40, 60, and 80 °C) while the pressure was kept constant at 30 MPa. The extracted amount increased as the temperature decreased. Figure 3B shows the extracted amount when the pressure was changed (10, 20, and

Table 1. Measurement of Antioxidants in Supercritical CO_2 Extracts at Various Conditions without Modifier

	μg/100 g of seed coat				
	HDA[a]	MDB[a]	DPA[a]	EC[a]	total
10 MPa, 40 °C	ND[b]	ND	2.4 ± 0.7	13.2 ± 1.3	15.9 ± 0.9
20 MPa, 40 °C	ND	ND	3.1 ± 0.2	98.4 ± 8.5	108.5 ± 7.2
30 MPa, 40 °C	ND	0.3 ± 0.2	4.4 ± 0.4	167.0 ± 12.1	173.5 ± 10.9
30 MPa, 60 °C	0.2 ± 0.1	2.4 ± 0.2	24.0 ± 0.3	278.6 ± 10.0	301.0 ± 12.1
30 MPa, 80 °C	0.2 ± 0.1	3.1 ± 0.1	34.6 ± 0.7	336.0 ± 9.8	377.8 ± 10.0
ethyl acetate extr	7.1 ± 0.3[c]	7.7 ± 0.3[c]	69.2 ± 2.9[c]	32.0 ± 1.2[c]	116.0 ± 3.2[c]

[a] HDA, 2-hydroxy-3′,4′-dihydroxyacetophenone; MDB, methyl 3,4-dihydroxybenzoate; DPA, 3,4-dihydroxyphenyl acetate; EC, (−)-epicatechin. Reported values are the mean ± SD (n = 3). [b] ND, none determined. [c] In mg/100 g of seed coat.

Table 2. Measurement of Antioxidants in Supercritical CO_2 Extracts at Various Conditions with Ethanol as Modifier

	mg/100 g of seed coat				
	HDA[a]	MDB[a]	DPA[a]	EC[a]	total
10 MPa, 40 °C	4.1 ± 0.2	5.1 ± 0.3	50.7 ± 1.0	21.4 ± 1.3	80.1 ± 3.4
20 MPa, 40 °C	3.7 ± 0.1	6.2 ± 0.4	82.2 ± 1.7	17.3 ± 2.2	107.3 ± 6.0
30 MPa, 40 °C	4.3 ± 0.2	6.8 ± 0.5	88.5 ± 2.8	16.4 ± 1.7	118.4 ± 5.4
30 MPa, 60 °C	1.7 ± 0.1	7.4 ± 0.3	105.6 ± 6.0	20.6 ± 1.9	137.9 ± 7.1
30 MPa, 80 °C	6.2 ± 0.2	10.1 ± 0.1	123.9 ± 7.1	26.1 ± 2.4	166.9 ± 7.9
ethyl acetate extr	7.1 ± 0.3	7.7 ± 0.3	69.2 ± 2.9	32.0 ± 1.2	116.0 ± 3.2

[a] HDA, 2-hydroxy-3′,4′-dihydroxyacetophenone; MDB, methyl 3,4-dihydroxybenzoate; DPA, 3,4-dihydroxyphenyl acetate; EC, (−)-epicatechin. Reported values are the mean ± SD (n = 3).

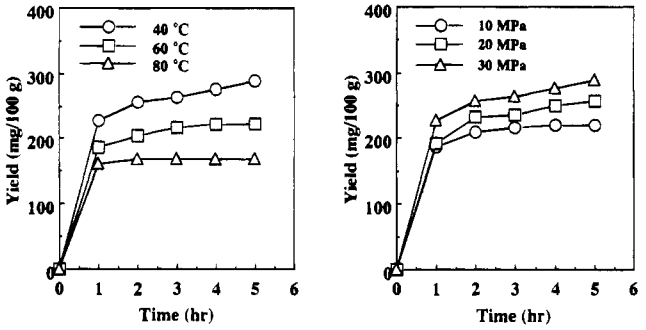

Figure 3. Effect of supercritical CO_2 extraction conditions on extractivity of tamarind seed coat extract with ethanol as modifier: (A, left) influence on the yield when the extraction temperature was changed (40, 60, and 80 °C) and the pressure was kept constant at 30 MPa; (B, right) influence on the yield when the extraction pressure was changed (10, 20, and 30 MPa) and the temperature was kept constant at 40 °C.

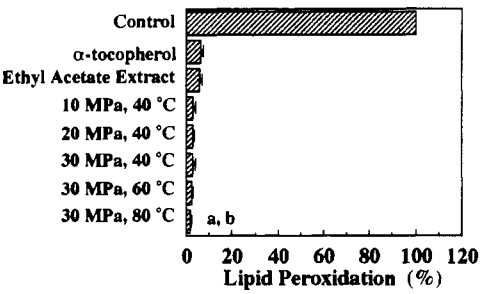

Figure 4. Antioxidative activity of ethanol-modified supercritical CO_2 extracts prepared from tamarind seed coat as measured by the thiocyanate method. The extraction time of ethanol-modified supercritical CO_2 extracted samples was 5 h under all conditions. Reported values are the mean ± SD (n = 3). A control containing no added samples or standards on its values represents 100% lipid peroxidation. (a) $P < 0.05$, compared to α-tocopherol; (b) $P < 0.05$, compared to the ethyl acetate extract.

30 MPa) while the temperature was kept constant at 40 °C. The yield increased as the pressure increased, just as for the case of neat CO_2 SFE. Using ethanol-modified CO_2 gave higher yields than did neat CO_2 extraction.

Antioxidative Activity of Ethanol-Modified SC-CO_2 Extracts. The antioxidative activity of the ethanol-modified SC-CO_2 extract as measured by the thiocyanate method is shown in Figure 4. All extracts had strong antioxidative activity. Extraction conditions of 30 MPa and 80 °C were the best for extraction of antioxidants from tamarind seed coat. The activity was higher than that of α-tocopherol and the ethyl acetate extract (significantly different, $P < 0.05$). The results of the TBA method showed the same tendency (data not shown). The ethanol-modified SC-CO_2 resulted in higher antioxidative activity than did neat CO_2.

Measurement of Antioxidant Level in Ethanol-Modified SC-CO_2 Extraction. The amounts of antioxidants contained in ethanol-modified SC-CO_2 extracts are shown in Table 2. Higher pressure combined with higher temperature provided a greater total yield of antioxidants. Among the antioxidants, DPA was obtained in the greatest yield under all conditions. While the total yield of the extract decreased as the temperature increased at constant pressure, the yield of antioxidant compounds actually increased. Therefore, the selectivity of the extraction for the antioxidant compounds was improved at high temperatures using ethanol as modifier. SFE performed at 30 MPa and 80 °C was more effective than ethyl acetate extraction of antioxidants from tamarind seed coat (significantly different, $P < 0.01$, in total amount of antioxidants).

Effect of Ethanol-Modified SC-CO_2 Extracts on POV in Edible Oil. Ethanol-modified SC-CO_2 extraction performed at 30 MPa and 80 °C provided a greater yield of antioxidants. Therefore, the SC-CO_2 extract prepared under the above conditions was added to edible oil (lard), after which the antioxidative activity was assayed by measuring POV. The effect of SC-CO_2 extract on POV in lard is shown in Figure 5. The SC-CO_2 extract strongly inhibited the production of peroxide. The activity was the same as that of mixed tocopherols (not significantly different) during incubation. These results suggest that ethanol-modified SC-CO_2 extracts are good for foods containing lipids.

Conclusion. Because of its nontoxicity and inert nature, SC-CO_2 extraction could be an effective tech-

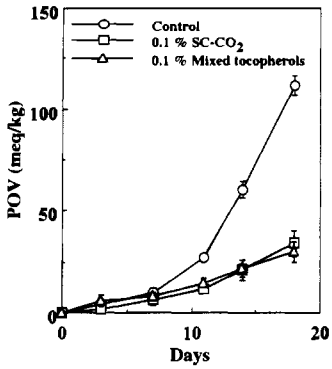

Figure 5. Antioxidative activity of the ethanol-modified supercritical CO_2 extract in lard.

nique for the food industries. We established SC-CO_2 extraction of antioxidants from tamarind seed coat by using an extraction modifier. This SC-CO_2 extract may be used for increasing the shelf life of foods by preventing lipid peroxidation. Application of the antioxidants to processed foods containing lipids is now under investigation.

ACKNOWLEDGMENT

We are grateful to Akira Yamamoto, Yaegaki Zymotechnics, Inc., Japan, for providing tamarind seed coat.

LITERATURE CITED

Aharonson, N.; Lehotay, S. J.; Ibrahim, M. A. Supercritical fluid extraction and HPLC analysis of benzimidazole fungicides in potato, apple, and banana. *J. Agric. Food Chem.* **1994**, *42*, 2817–2823.

Bhaskar, A. R.; Rizvi, S. S. H.; Sherbon, J. W. Anhydrous milk fat fractionation with continuous countercurrent supercritical carbon dioxide. *J. Food Sci.* **1993**, *58*, 748–752.

Bradley, R. L., Jr. Removal of cholesterol from milk fat using supercritical carbon dioxide. *J. Dairy Sci.* **1989**, *72*, 2834–2840.

Duke, J. A. *Handbook of Legumes of World Economic Importance*; Plenum Press: New York, 1981; pp 228–230.

Fattori, M.; Bulley, N. R.; Meisen, A. Fatty acid and phosphorus contents of canola seed extracts obtained with supercritical carbon dioxide. *J. Agric. Food Chem.* **1987**, *35*, 739–743.

Froning, G. W.; Wehling, R. L.; Cuppett, S. L.; Pierce, M. M.; Niemann, L.; Siekman, D. K. Extraction of cholesterol and other lipids from dried egg yolk using supercritical carbon dioxide. *J. Food Sci.* **1990**, *55*, 95–98.

Hawthorne, S. B. Analytical-scale supercritical fluid extraction. *Anal. Chem.* **1990**, *62*, 633A–642A.

JOCS. *Standard Methods for the Analysis of Oils, Fats and Derivatives*. Japan Oil Chemists' Society: Tokyo, 1972; p 2·4·12-71.

Kerrola, K.; Kallio, H. Volatile compounds and odor characteristics of carbon dioxide extracts of coriander (*Coriandrum sativum* L.) fruits. *J. Agric. Food Chem.* **1993**, *41*, 785–790.

King, J. W.; Johnson, J. H.; Orton, W. L.; McKeith, F. K.; O'Connor, P. L.; Novakofski, J.; Carr, T. R. Fat and cholesterol content of beef patties as affected by supercritical CO_2 extraction. *J. Food Sci.* **1993**, *58*, 950–952.

King, M. F.; Hamilton, B. L.; Matthews, M. A.; Rule, D. C.; Field, R. A. Isolation and identification of volatiles and condensable material in raw beef with supercritical carbon dioxide extraction. *J. Agric. Food Chem.* **1993**, *41*, 1974–1981.

List, G. R.; King, J. W.; Johnson, J. H.; Warner, K.; Mounts, T. L. Supercritical CO_2 degumming and physical refining of soybean oil. *J. Am. Oil Chem Soc.* **1993**, *70*, 473–476.

Locke, M. A. Supercritical CO_2 fluid extraction of fluometuron herbicide from soil. *J. Agric. Food Chem.* **1993**, *41*, 1081–1084.

Lopez-Avila, V.; Dodhiwala, N. S.; Beckert, W. F. Developments in the supercritical fluid extraction of chlorophenoxy acid herbicides from soil samples. *J. Agric. Food Chem.* **1993**, *41*, 2038–2044.

Merkle, J. A.; Larick, D. K. Triglyceride content of supercritical carbon dioxide extracted fractions of beef fat. *J. Food Sci.* **1993**, *58*, 1237–1240.

Mitsuda, H.; Yasumoto, K.; Iwami, K. Antioxidative action of indole compounds during the autoxidation of linoleic acid. *Eiyo to Shokuryo* **1966**, *19*, 210–214.

Nam, K. S.; King, J. W. Supercritical fluid extraction and enzyme immunoassay for pesticide detection in meat products *J. Agric. Food Chem.* **1994**, *42*, 1469–1474.

Osawa, T.; Namiki, M. A novel type of antioxidant isolated from leaf wax of *Eucalyptus* leaves. *Agric. Biol. Chem.* **1981**, *45*, 735–739.

Ottolenghi, A. Interaction of ascorbic acid and mitochondrial lipides. *Arch. Biochem. Biophys.* **1959**, *79*, 355–363.

Reverchon, E.; Senatore, F. Supercritical carbon dioxide extraction of chamomile essential oil and its analysis by gas chromatography-mass spectrometry. *J. Agric. Food Chem.* **1994**, *42*, 154–158.

Sharpe, F. R.; Crabb, D. Pilot plant extraction of hops with liquid carbon dioxide and the use of these extracts in pilot and production scale brewing. *J. Inst. Brew.* **1980**, *86*, 60.

Spanos, G. A.; Chen, H.; Schwartz, J. Supercritical CO_2 extraction of β-carotene from sweet potatoes. *J. Food Sci.* **1993**, *58*, 817–820.

Taylor, S. L.; King, J. W.; Richard, J. L.; Greer, J. I. Analytical-scale supercritical fluid extraction of aflatoxin B_1 from field-inoculated corn. *J. Agric. Food Chem.* **1993**, *41*, 910–913.

Tsuda, T.; Makino, Y.; Kato, H.; Osawa, T.; Kawakishi, S. Screening for antioxidative activity of edible pulses. *Biosci., Biotechnol., Biochem.* **1993**, *57*, 1606–1608.

Tsuda, T.; Watanabe, M.; Ohshima, K.; Yamamoto, A.; Kawakishi, S.; Osawa, T. Antioxidative components isolated from the seed of tamarind (*Tamarindus indica* L.). *J. Agric. Food Chem.* **1994**, *42*, 2671–2674.

Wigfield, Y. Y.; Lanouette, M. Supercritical fluid extraction of the fortified residues of fluazifop-P-butyl (fusilade II) and its major metabolite, fuazifop-P, in onions. *J. Agric. Food Chem.* **1993**, *41*, 84–88.

Zosel, K. Separation with supercritical gases: practical applications. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 702–709.

Received for review April 10, 1995. Revised manuscript received July 20, 1995. Accepted August 7, 1995.[⊗]

JF950216Y

[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1995.