

Deacidification of Black Cumin Seed Oil by Selective Supercritical Carbon Dioxide Extraction

Selma Türkay^{1,*}, Mark D. Burford, M. Kemal Sangün, Ekrem Ekinci¹,
Keith D. Bartle, and Anthony A. Clifford

School of Chemistry, Leeds University, Leeds, LS2, 9JT, United Kingdom

ABSTRACT: The deacidification of high-acidity oils from Black cumin seeds (*Nigella sativa*) was investigated with supercritical carbon dioxide at two temperatures (40 and 60°C), pressures (15 and 20 MPa) and polarities (pure CO₂ and CO₂/10% MeOH). For pure CO₂ at a relatively low pressure (15 MPa) and relatively high temperature (60°C), the deacidification of a high-acidity (37.7 wt% free fatty acid) oil to a low-acidity (7.8 wt% free fatty acid) oil was achieved. The free fatty acids were quantitatively (90 wt%) extracted from the oil and left the majority (77 wt%) of the valuable neutral oils in the seed to be recovered at a later stage by using a higher extraction pressure. By reducing the extraction temperature to 40°C, increasing the extraction pressure to 20 MPa, or increasing the polarity of the supercritical fluid via the addition of a methanol modifier, the selectivity of the extraction was significantly reduced; the amount of neutral oil that co-extracted with the free fatty acids was increased from 23 to 94 wt%.

JAOCs 73, 1265–1270 (1996).

KEY WORDS: Black cumin seed oil, deacidification, fractionation, free fatty acids, *Nigella sativa*, supercritical carbon dioxide extraction.

Black cumin (*Nigella sativa*) seeds are used exclusively for edible and medical purposes (1). The seeds contain 30–40 wt% oil, and the main fatty acids present are linoleic (58–62 wt%), oleic (22–24 wt%), and palmitic acid (13–15 wt%). The most important property of black cumin seeds is its high content of lipase enzymes (2). In dormant seeds, lipase enzymes are generally inactive, but when the seeds are ground to obtain the oil, the lipase and oil come into contact, and enzymatic hydrolysis reactions commence immediately. Thus, grinding the sample may increase the free fatty acid content (FFA wt%) of oil to 50% or higher, depending mainly on the time between grinding and extraction, the storage temperature of ground seeds, the moisture content of the seeds, and the relative humidity of the storage medium. This undesirable phenomenon can be observed in other oil-bearing materials that contain active lipase enzymes, such as olive, palm, and rice bran (3).

¹Present address: Istanbul Technical University, Chemical Engineering Department, 80626, Maslak, Istanbul, Turkey.

*To whom correspondence should be addressed at Istanbul Technical University, Chemical Engineering Department, 80626, Maslak, Istanbul, Turkey.

Deacidification of high-acidity oils can be accomplished either by miscella refining or by physical refining instead of conventional alkali neutralization processes. These latter methods normally cannot be applied to oils that contain more than 8–10 wt% FFA (4). Another deacidification method for high-acidity oils is liquid-liquid extraction based on different solubilities of fatty acids and triglycerides in various organic solvents, such as ethanol, methanol, and acetone (5,6).

A new alternative deacidification process is supercritical-fluid extraction (SFE) of oils or oil-bearing seeds that contain high-acidity oils with carbon dioxide as extraction solvent. Several researchers have demonstrated the suitability of supercritical carbon dioxide (SF-CO₂) as a solvent for the extraction of various seed oils for both industrial and analytical applications (7–16). The attractive features of SF-CO₂ over the presently used organic solvents, such as hexane, are its nontoxicity, safety, ease of separation, and cheapness (17,18). Another advantage of SFE as opposed to hexane extraction is that SFE can be made highly selective by controlling temperature and pressure. Solubility data of fatty acids and triglycerides in SF-CO₂ show that fatty acids are more soluble in CO₂ than the corresponding triglycerides at certain temperatures and pressures (19,20). Based on these solubility differences, Brunetti *et al.* (19) investigated the deacidification of high-acidity olive oil with SF-CO₂ at extraction pressures of 20 and 30 MPa, and extraction temperatures of 40 and 60°C. They reported that the solvent selectivity for fatty acids was higher at 20 MPa and 60°C and increased significantly as the free acid concentration of the oil decreased. Similarly, Bondioli *et al.* (21) determined the optimal operating parameters for deacidification of lampante olive oil (≤3.93 wt% FFA) in a SF-CO₂ extraction plant that was operated in a continuous countercurrent mode. Zhao *et al.* (22) combined the extraction and refining procedures by sequentially extracting rice bran oil (11.9 wt% FFA) with SF-CO₂ at 15 and 35 MPa at 40°C. With this two-step approach, the undesirable FFA, unsaponifiable matter, and tocopherols were extracted at low pressure, and the remaining decontaminated oil could then be readily recovered with SF-CO₂ at the higher pressure. Thus, the oil was refined by a simple process in which alkali refining as well as the degumming steps could be omitted.

In this study, selective extraction of Black cumin seeds

with SF-CO₂ was investigated to determine the variation in oil composition as a function of temperature, pressure, and modifier parameters.

EXPERIMENTAL PROCEDURES

Black cumin seeds were obtained from a market in Istanbul, Turkey. The seeds were ground in a coffee mill, and then sieved. Particles smaller than 425 μ m were used in the study. The oil (40.5 wt%) content of the seeds was determined by a six-hour Soxhlet extraction with hexane. The moisture (7.1 wt%) content was determined by oven-drying the seeds at 105°C. For SFE experiments, ground seeds were stored before use in a covered flask at room temperature so as to increase the FFA content of the oil by enzymatic action. To prevent enzymatic hydrolysis between two successive extractions, the ground seeds were stored in a refrigerator at -10°C.

To determine FFA wt% change in the seed oil by enzymatic action during storage, the ground seeds in the covered flask were stored in a drying cabinet at 33 \pm 1°C, and the FFA content of the oil obtained by Soxhlet extraction was determined by titration with a standard NaOH solution and phenolphthalein as the indicator (23).

SFE experiments were performed dynamically with a supercritical-fluid extractor, SFX 2-10 (Isco, Inc., Lincoln, NE). Ground seeds (1 g) were loaded into the extraction cell (10 mL), and glass wool plugs were used to retain the seeds in the cell. Carbon dioxide at the desired pressure was delivered to the temperature-controlled extractor with a 260 D syringe pump (Isco, Inc.). The oil-laden gas that left the extractor through the capillary restrictor (50 μ m i.d., 375 μ m o.d., 12-cm length), was collected in a vial, containing 10 mL dichloromethane, at 15-min intervals.

The amount of oil extracted during a 15-min supercritical-fluid extraction was determined gravimetrically by evaporating the dichloromethane in the collection vial and weighing the remaining oil. The oil was then dissolved in 30 mL of ethanol/diethyl ether (1:1, vol/vol) and titrated with a 0.01 M NaOH solution. The amount of extracted FFA was calculated as oleic acid for each fraction. By assuming that the extracted oil is mainly composed of triglycerides, partial glycerides, unsaponifiable matter, and FFA, the difference between the amount of extracted oil and extracted FFA is the amount of extracted neutral oil (NO).

RESULTS AND DISCUSSION

Enzymic hydrolysis of NO to FFA. The enzymic hydrolysis of the valuable triglycerides to the undesirable FFA during vegetable oil production is of significant economic importance because the cost of removing FFA increases with increasing FFA concentration. This increase in FFA content when the seeds are ground is clearly shown in Figure 1. Enzymatic hydrolysis of triglycerides proceeds rapidly after grinding, though the rate varies with the grinding procedure and storage conditions. Most of the triglyceride degradation occurs

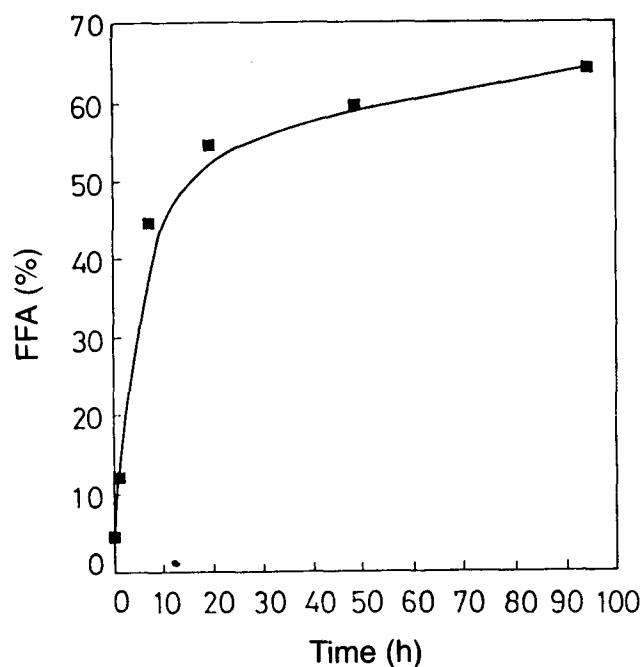


FIG. 1. Change of free fatty acid (FFA) wt% of Black cumin oil in ground seeds stored at 33 \pm 1°C.

within the first 10–15 h, with over 50% of the triglyceride having been hydrolyzed to the FFA. Seeds collected at various storage times were used to investigate the effect of FFA content on the selectivity and extraction efficiency of the SFE process. The ability of the SFE technique to deacidify seeds with a wide range of FFA compositions is discussed below.

Effect of the FFA content of the oil on the SFE deacidification process. Black cumin seeds with relatively high (e.g., 38 wt%) and low (e.g., 13 wt%) FFA contents were extracted with SC-CO₂ at 60°C and 20 MPa. The results are given in

TABLE 1
Supercritical CO₂ Extraction of Black Cumin Seeds at 60°C and 20 MPa

FFA content of seed oil (wt%)	Volume of CO ₂ used in SFE (mL) ^a	Amount extracted		
		Oil (g)	FFA (g)	NO (g)
13.0	28.5	0.100	0.040	0.060
	52.5	0.156	0.049	0.107
	67.8	0.185	0.052	0.133
24.9	23.7	0.112	0.069	0.043
	45.5	0.187	0.095	0.092
	66.1	0.236	0.099	0.137
33.1	27.7	0.156	0.105	0.051
	54.8	0.235	0.129	0.106
	73.6	0.272	0.132	0.140
37.7	31.6	0.188	0.116	0.072
	61.5	0.273	0.144	0.129
	88.9	0.313	0.148	0.165

^aVolume of CO₂ measured as a liquid from the pump. FFA, free fatty acid; SFE, supercritical fluid extraction; NO, neutral oil.

Table 1 and show that the total amount of oil extracted increased with increasing FFA content of the seed, even though all seeds were extracted at the same temperature and pressure. This increase in total oil recovery is due to the relatively high solubility (e.g., $ca. 5 \times 10^{-3}$ mole fraction) of the FFA, which are quickly solvated in the fluid and so can be rapidly extracted to exhaustion (20). Conversely, the amount of neutral oil recovered from the seed remained reasonably constant, and the recoveries were independent of the seed's FFA content. This consistent NO recovery is related to the relatively low solubility of the triglyceride (e.g., $ca. 1 \times 10^{-5}$ mole fraction), which can be two orders of magnitude less than for FFA (20). Hence, only a fraction of the NO was extracted compared to the quantitative FFA recoveries.

This significant difference in solubility of FFA and NO components in the supercritical fluid is clearly demonstrated with the extraction profile of the analytes in Figure 2. The FFA compounds have a typical extraction curve associated with an analyte that has sufficient solubility in the supercritical fluid. Thus, the FFA are initially rapidly extracted from the sample matrix, but as the extraction continues and the analytes at the surface of the sample matrix are depleted, this results in a decreasing extraction rate as diffusion becomes the rate-limiting factor. This ability of CO_2 to solvate the FFA in the seeds is confirmed by the solubility of FFA. For example, oleic acid is soluble in CO_2 at 0.026 g/g under the extraction conditions employed (e.g., 20 MPa, 60°C, CO_2) (19), and this is more than sufficient to recover the 0.005 g/g of FFA extracted from the seeds (Table 1). Conversely, NO compounds have virtually a linear extraction rate with time, which is indicative of a solubility-limited extraction process, where the amount of analyte removed is directly related to the amount of supercritical fluid passing through the sample matrix. This trend is also confirmed with the literature solubility values for NO, such as triolein, which is 0.004 g/g soluble in CO_2 at the 20 MPa and 60°C extraction conditions (19), and is comparable to the 0.003 g/g of NO that was extracted from

the seeds (Table 1). This solubility/diffusion phenomenon has been observed and discussed in detail in the literature (24).

The importance of these oil recoveries becomes even more apparent when the data are expressed as the percentage of FFA and NO extracted from the Black cumin seeds, as shown in Figure 2. It is clear that FFA are quantitatively (e.g., >90%) recovered from the seeds with $ca. 50$ mL supercritical CO_2 in $ca. 30$ min, whereas only $ca. 30$ – 40% of the corresponding NO was extracted. A similar FFA extraction rate was obtained for both the low- and high-acidity seeds, and this demonstrates that the supercritical fluid has sufficient solvating capability to extract FFA over a wide range of FFA concentrations. To exhaustively recover the FFA, so that less than 1% remained in the seeds, requires a large (70–80 mL) volume of SC- CO_2 , but this also dramatically increases the amount of NO, which is co-extracted with the FFA, with the result that nearly half of the seeds' NO is removed. This demonstrates that a partially selective supercritical fluid extraction of FFA from the seeds can be obtained if the appropriate extraction time is used. However, a significant percentage of commercially valuable NO is simultaneously extracted. Thus, a more selective extraction process was required and this was attempted by using both temperature, pressure, and organic modifier parameters.

Effect of pressure on deacidification. The potential of using pressure to obtain selective FFA extraction was investigated by analyzing highly acidic (e.g., 38% FFA) Black cumin seeds with 60°C carbon dioxide at low (15 MPa) and medium (20 MPa) pressures. The results are shown in Figure 3 and Table 2. By decreasing the extraction pressure from 20 to 15 MPa, the extraction rate of FFA decreased so that the amount of FFA recovered per mL of CO_2 was reduced. However, if a sufficiently large enough volume of CO_2 is used (e.g., 100 mL) quantitative FFA recoveries (e.g., >90%) can still be achieved at both pressures. The advantage of using a low extraction pressure is that a more selective FFA extraction can be obtained because the amount of NO co-ex-

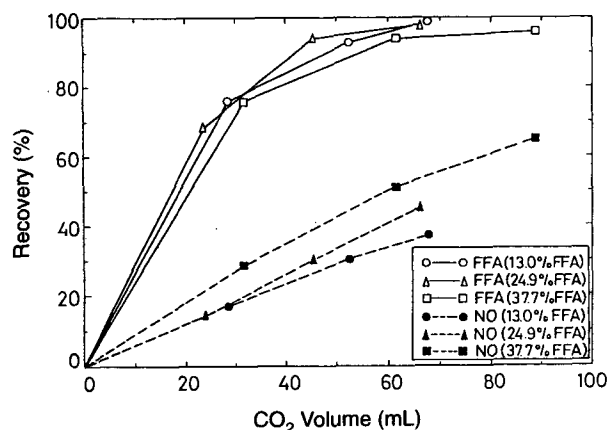


FIG. 2. Dependence of extracted neutral oil (NO) and FFA on the volume of CO_2 , at 60°C and 20 MPa for seeds that contain oils of initial acidity 13.0, 24.9, and 37.7 wt%. See Figure 1 for other abbreviation.

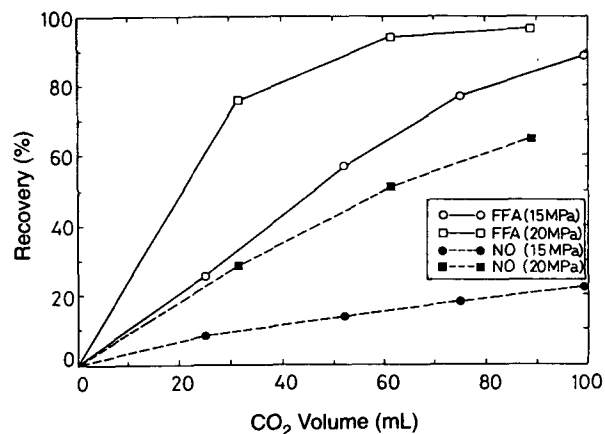


FIG. 3. Dependence of extracted NO and FFA on the volume of CO_2 at 60°C and 20 and 15 MPa with seeds of 37.7 wt% FFA. See Figures 1 and 2 for abbreviations.

TABLE 2

Selective Extraction of Free Fatty Acids from Black Cumin Seeds with Supercritical CO₂ at Various Conditions

SFE conditions	Volume of CO ₂ used in SFE (mL) ^a	Seed oil composition before SFE (wt%) ^b		Seed oil composition after SFE (wt%) ^c		Oil remaining in seeds after SFE ^d (wt%)	Percentage of non-extracted ^e	
		FFA	NO	FFA	NO		FFA (wt%)	NO (wt%)
15 MPa, 60°C, CO ₂	99	37.7	62.3	7.8	92.2	52.7	10.8	77.5
20 MPa, 60°C, CO ₂	89	37.7	62.3	4.6	95.4	22.5	2.8	34.5
60°C, 20 MPa, CO ₂	74	33.1	66.9	2.5	97.5	33.3	2.5	48.5
40°C, 20 MPa, CO ₂	103	28.2	71.8	21.5	78.5	5.8	4.4	6.2
CO ₂ , 60°C, 15 MPa	99	37.7	62.3	7.8	92.2	52.7	10.8	77.5
CO ₂ /MeOH, 60°C, 15 MPa	43	33.1	66.9	11.6	88.4	42.3	14.8	55.9

^aVolume of CO₂ measured as a liquid from the pump. See Table 1 for abbreviations.^bComposition of the seed oil before SFE as determined by Soxhlet extraction and titration of the organic solvent extracted with NaOH.^cComposition of the seed oil after SFE as calculated based on the material balance.^dPercentage of seed oil (e.g., FFA + NO) that was not extracted during SFE.^ePercentage of FFA and NO present in the SFE residue relative to the original FFA and NO content.

tracted with the FFA is greatly reduced (Fig. 3). By decreasing the extraction pressure by 5 MPa, the amount of neutral oil co-extracted during the SFE procedure can be decreased from 64 to 23 wt%. This significant decrease in the NO extraction rate is related to the oil's relatively low solubility at the 15 MPa extraction pressure (20) and was confirmed by the linear extraction profile obtained with the extraction volume (Fig. 3). At the lower extraction pressure, and hence density, the extraction profile of the FFA compounds is more reminiscent of the linear extraction profile of NO, suggesting that extraction of FFA is also becoming a solubility-limited extraction process.

Both 15 and 20 MPa extraction pressures were capable of decreasing the FFA content of the remaining oil in the seed from a highly acidic (e.g., 38 wt% FFA) oil to a relatively low acidity (e.g., <10 wt% FFA) oil, but at the higher pressure, over 75% of the total oil had been extracted from the seeds (Table 2). Therefore, to obtain a selective extraction, where most (e.g., >75%) of the valuable NO remains in the seed and most (e.g., >90%) of the FFA is removed, a low (15 MPa) extraction pressure is required to produce a selective 2.4:1 FFA/NO extract. If the higher extraction pressure of 20 MPa is used, a poorly selective 0.9:1 FFA/NO extract is obtained, which is more indicative of total extraction, rather than selective extraction.

Effect of temperature on deacidification. The effect of temperature on the extraction of highly acidic (ca. 30% FFA) oil from Black cumin was investigated with SC-CO₂ at temperatures of 40 and 60°C, and the results are presented in Figure 4 and Table 2. By decreasing the extraction temperature to 40°C, quantitative recoveries of both FFA and NO from the seed were achieved with ca. 100 mL of CO₂ in 60 min (e.g., flow rate ca. 1.6 mL/min). This increase in the extraction rate of NO is associated with the increase in density of the supercritical fluid at the lower temperature and corresponds to an increase in the solubility of triglycerides with increasing supercritical fluid density (20). This increase in NO solubility can be observed by examining the extraction rate profile of NO, which has a less linear dependence on the extraction vol-

ume of CO₂ and is similar to the extraction profile of FFA, which is not solubility-limited. The relationship between solubility of an analyte with density and temperature of the supercritical fluid has been previously reported and is referred to as the cross-over point (19,24). In contrast, the extraction rate of FFA appears to be independent of the extraction temperature, which is to be expected because the FFA have sufficient solubility in the CO₂, at both 40 and 60°C. Thus, decreasing the extraction temperature results in the loss of over 94% of the total oil from the seeds, and the remaining unextracted oil is still highly acidic (e.g., 21.5 wt% FFA). (Table 2.) This suggests that a more selective extraction may be obtained by employing slightly higher extraction temperatures, such as 80°C.

Effect of a modifier with SF-CO₂ on deacidification. In an attempt to increase the selectivity of the SFE procedure for FFA, 0.5 mL of methanol was added to 1 g of ground seeds that contained highly acid oil (33.1 wt% FFA), and the mixture was extracted with SC-CO₂ at 60°C and 15 MPa. Methanol was chosen as the modifier because it was envis-

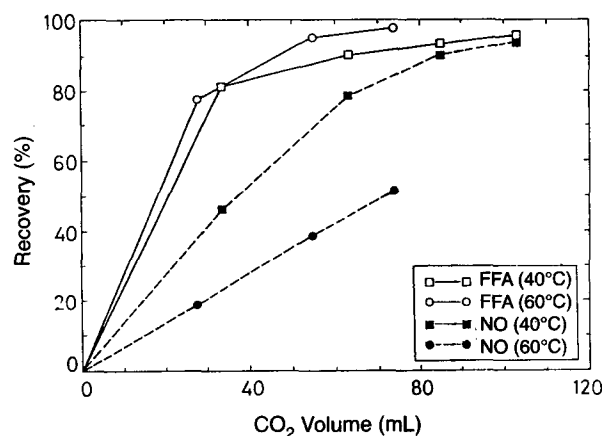


FIG. 4. Dependence of extracted NO and FFA on the volume of CO₂ at 40°C and 20 MPa for seeds of 28.2 wt% FFA, and at 20 MPa and 60°C for seeds of 33.1 wt% FFA. See Figures 1 and 2 for abbreviations.

aged that the polar FFA would be more soluble in methanol than the triglycerides. Modifier addition significantly enhances the extraction rate of both FFA and NO from the seeds, with the greatest increase occurring in the first 15 min of extraction when the methanol was present in the extraction cell (Fig. 5). Once the methanol had been removed from the cell, the extraction rate of FFA and NO became comparable to the extraction rates obtained with pure CO₂, as shown in Figure 5. Ideally, methanol should be dynamically added to the CO₂ throughout the entire extraction process, so that continuous CO₂/MeOH extraction could be undertaken. However, sufficient results were obtained to demonstrate that the presence of a polar organic modifier decreased the selectivity of the extraction because the remaining oil in the seed still contained a significant amount (e.g., 11.6 wt% FFA) of FFA and nearly 50% of the NO had been co-extracted (Table 2.). Organic modifiers also may be undesirable due to the potential of organic solvent residue in the sample matrix after SFE.

With SC-CO₂ at relatively low (15 MPa) pressure and relatively high (60°C) temperature, a selective and quantitative (e.g., 90%) FFA extraction was obtained from Black cumin seeds. It is envisaged that the resulting unextracted low-acidity oil which is rich in neutral oils (e.g., 92 wt%) will be quantitatively recovered by reextracting the SFE residue with high-pressure (e.g., 40 MPa) SC-CO₂. Enhancing the solvation properties of the supercritical fluid by varying temperature, pressure, or modifier reduced the selectivity of the extraction because a greater percentage of NO was co-extracted with the FFA. Selectivity of the extraction appears to depend on solubility differences between the two components, and therefore, by employing mild extraction conditions, solubility of the NO was minimized while maintaining quantitative recovery of FFA.

ACKNOWLEDGMENT

The financial support of British Council is acknowledged.

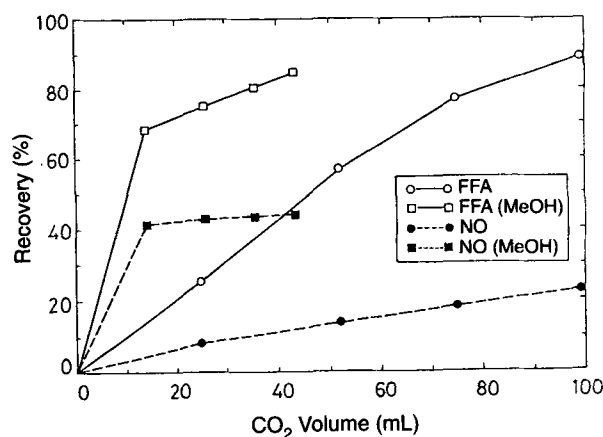


FIG. 5. Dependence of extracted NO and FFA on the volume of CO₂ at 60°C, and 15 MPa for the seeds of 33.1 wt% FFA containing MeOH and for the seeds of 37.7 wt% FFA without MeOH. See Figures 1 and 2 for abbreviations.

REFERENCES

1. Üstün, G., L. Kent, N.Çekin, and H. Civelekoglu, Investigation of the Technological Properties of *Nigella sativa* (Black cumin) Seed Oil, *J. Am. Oil Chem. Soc.* 67:958–960 (1990).
2. Dandik, L., and H.A. Aksoy, The Kinetics of Hydrolysis of *Nigella sativa* (Black cumin) Seed Oil Catalyzed by Native Lipase in Ground Seed, *Ibid.* 69:1239–1241 (1992).
3. Patterson, H.B.W., *Handling and Storage of Oil Seeds, Oils, Fats and Meal*, Elsevier Applied Science, London, 1989, pp. 83–85.
4. Anderson, A.J.C., *Refining of Oils and Fats for Edible Purposes*, 2nd revised edn., Pergamon Press Ltd., 1962, p. 92.
5. Türkay, S., and H. Civelekoglu, Deacidification of Sulfur Olive Oil. I. Single-Stage Liquid-Liquid Extraction of Miscella with Ethyl Alcohol, *J. Am. Oil Chem. Soc.* 68:83–86 (1991).
6. Türkay, S., and H. Civelekoglu, Deacidification of Sulfur Olive Oil. II. Multi-Stage Liquid-Liquid Extraction of Miscella with Ethyl Alcohol, *Ibid.* 68:818–821 (1991).
7. Stahl, E., E. Schütz, and H.K. Mangold, Extraction of Seed Oils with Liquid and Supercritical Carbon Dioxide, *J. Agric. Food Chem.* 28:1153–1157 (1980).
8. Friedrich, J.P., and G.R. List, Characterization of Soybean Oil Extracted by Supercritical Carbon Dioxide and Hexane, *Ibid.* 30:192–193 (1982).
9. List, G.R., J.P. Friedrich, and J. Pominski, Characterization and Processing of Cottonseed Oil Obtained by Extraction with Supercritical Carbon Dioxide, *J. Am. Oil Chem. Soc.* 61:1847–1849 (1984).
10. List, G.R., J.P. Friedrich, and D.D. Christianson, Properties and Processing of Corn Oils Obtained by Extraction with Supercritical Carbon Dioxide, *Ibid.* 61:1849–1851 (1984).
11. Favati, F., J.W. King, and M. Mazzanti, Supercritical Carbon Dioxide Extraction of Evening Primrose Oil, *Ibid.* 68:422–427 (1991).
12. Muuse, B.G., F.P. Cuperus, and J.T.P. Derksen, Extraction and Characterization of *Dimorphotheca pluvialis* Seed Oil, *Ibid.* 71:313–317 (1994).
13. Kuk, M.S., and R.J. Hron, Sr., Supercritical Carbon Dioxide Extraction of Cottonseed with Co-Solvents, *Ibid.* 71:1353–1356 (1994).
14. Taylor, S.L., J.W. King, and G.R. List, Determination of Oil Content in Oilseeds by Analytical Supercritical Fluid Extraction, *Ibid.* 70:437–439 (1993).
15. Walker, D.F.G., K.D. Bartle, and A.A. Clifford, Determination of the Oil Content of Rapeseed by Supercritical Fluid Extraction, *Analyst* 119:1471–1474 (1994).
16. Maness, N.O., D. Chrz, T. Pierce, and G.H. Brusewitz, Quantitative Extraction of Pecan Oil from Small Samples with Supercritical Carbon Dioxide, *J. Am. Oil Chem. Soc.* 72:665–669 (1995).
17. Bartle, K.D., and A.A. Clifford, Supercritical Fluid Extraction and Chromatography of Lipids and Related Compounds, in *Advances in Applied Lipid Research*, edited by F.B. Padley, JAI Press, London, Vol. 1, 1992, pp. 217–264.
18. Clifford A.A., and K.D. Bartle, Chemistry Goes Supercritical, *Chemistry in Britain* 6:499–502 (1993).
19. Brunetti, L., A. Daghetta, E. Fedeli, I. Kikic, and L. Zanderighi, Deacidification of Olive Oils by Supercritical Carbon Dioxide, *J. Am. Oil Chem. Soc.* 66:209–217 (1989).
20. Bartle, K.D., A.A. Clifford, S.A. Jafar, and G.F. Shilstone, Solubilities of Solids and Liquids of Low Volatility in Supercritical Carbon Dioxide, *J. Phys. Chem. Ref. Data* 20:713–756 (1991).
21. Bondioli, P., C. Mariani, A. Lanzani, E. Fedeli, A. Mossa, and A. Muller, Lampante Olive Oil Refining with Supercritical Carbon Dioxide, *J. Am. Oil Chem. Soc.* 69:477–480 (1992).
22. Zhao, W., A. Shishikura, K. Fujimoto, K. Arai, and S. Saito,

- Fractional Extraction of Rice Bran Oil with Supercritical Carbon Dioxide, *Biol. Chem.* 51:1773–1777 (1987).
23. Rossell, J.B., Classical Analysis of Oils and Fats, in *Analysis of Oils and Fats*, edited by R.J. Hamilton and J.B. Rossell, Elsevier Applied Science, London, 1987, pp. 12–15.
24. Bartle, K.D., A.A. Clifford, S.B. Hawthorne, J.J. Langenfeld, D.J. Miller, and R. Robinson, A Model for Dynamic Extraction Using a Supercritical Fluid, *J. Supercritical Fluids* 3:143–149 (1990).

[Received December 13, 1995; accepted May 6, 1996]