Extraction of Cholesterol and Other Lipids from Dehydrated Beef Using Supercritical Carbon Dioxide†

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Supercritical carbon dioxide (SC-CO_2) was used to extract cholesterol and other lipids from dehydrated beef powders and chunks. When powders were extracted at 55 °C and a fluid density of 0.90 g/cm^3, the cholesterol and total fat contents of the product could both be reduced by as much as 87%. Lipids were more easily extracted from chunks than from powders, allowing for successful removal at lower temperatures and pressures. Extracted samples were lighter in color than the control. The residual lipids in the extracted samples also contained higher relative percentages of linoleic and linolenic acids than did the lipids in the control samples. Taste panel evaluation of control and extracted powders found no significant differences in beef flavor, the presence of off-flavors, or overall acceptability.

INTRODUCTION

The reduction of total fat and cholesterol levels in meat and meat products continues to be of interest to the food industry and health professionals. A processing technology that has potential for removing cholesterol and other lipids from animal-derived foods is extraction with supercritical fluids. Carbon dioxide (CO_2) is inert, nontoxic, and nonflammable and has a low critical temperature, making it the fluid of choice for food applications. Several researchers have used supercritical carbon dioxide (SC-CO_2) to alter the contents of cholesterol and other lipids of foods of animal origin.

Wong and Johnston (1986) investigated the solubility of various sterols, including cholesterol, in SC-CO_2. SC-CO_2 has been used to separate butteroil into fractions with different fatty acid compositions, with evidence that cholesterol can be concentrated into selected fractions (Kaufmann et al., 1982; Shishikura et al., 1986; Arul et al., 1987; Bradley, 1989). The technology has also been used to extract cholesterol and other lipids from spray-dried egg yolk powders (Leiner, 1986; Froning et al., 1990; Rossi et al., 1990). Cholesterol and triacylglycerols were preferentially removed from egg powders, while retaining the phospholipids that contribute to the functionality of the product (Froning et al., 1990). Interest has also been shown in applying supercritical fluid extraction to meats and meat byproducts. Zosel (1979) has described a process for extracting fats from animal sources such as bone meal and a process for deodorizing animal fats (Zosel, 1982).

Several reports of extraction of lipid components from animal and fish muscle appear in the scientific literature. Yamaguchi et al. (1986) have extracted lipids from Antarctic krill with SC-CO_2. The recovered oils were composed largely of nonpolar triacylglycerols and contained very few phospholipids. Hardardottir and Kinsella (1988) were able to extract 97% of cholesterol and 78% of other lipids from trout muscle with SC-CO_2. King et al. (1989) investigated the technique for performing analytical extraction of lipids from meat products. Efficient extraction of lipids from intact muscle presents serious technical difficulties due to the fibrous nature of the muscle structure and the high moisture content, both of which serve as barriers to penetration by the carbon dioxide. King et al. (1989) found that comminuting and/or dehydrating the muscle prior to extraction improved the efficiency of the extraction process. Chao et al. (1991) have investigated the ability of SC-CO_2 extraction to reduce the cholesterol and total lipid contents of ground beef. They found that up to 36.9% of the cholesterol and 71.2% of the total lipid present could be removed during extraction. Extraction with CO_2 may also tend to dehydrate the muscle tissue, resulting in decreased solubility of some muscle proteins and other changes in functional properties (Hardardottir and Kinsella, 1988).

The preceding factors appear to limit the applicability of supercritical fluid extraction for intact muscle foods. However, the process is far more successful with dehydrated meat products, due to the physical nature and low moisture contents of these products. Also, dehydrated meats are usually cooked products, so protein denaturation during extraction is not a concern. Initial work in our laboratory has shown that the fat and cholesterol levels in dehydrated beef powders can be significantly reduced as a result of extraction with SC-CO_2 (Wehling, 1991). Dehydrated beef powders and chunks are widely used as ingredients in many prepared foods, such as soups and convenience foods. Current annual production of dehydrated beef products in the United States is approximately 15 million kilograms. Development of a product with lowered cholesterol and fat contents would likely expand this market and could improve demand for lower grade meat and meat trimmings.

The objective of this work was to investigate various factors that affect the extraction of lipid components from dehydrated beef into SC-CO_2, so that the extraction process could be optimized. Also, the effects of various extraction conditions on the composition and sensory properties of the products were evaluated.

MATERIALS AND METHODS

Samples. Products used for extraction were cooked beef type I (Henningsen Foods, Omaha, NE). Both a spray-dried powder and air-dried chunks of intact meat were extracted. The chunks were irregularly shaped, with dimensions ranging from 0.5 to 1.0 cm for length, width, and thickness. The dehydrated beef products were stored at −18 °C, and subsamples were removed from frozen storage as needed for extraction.

Extraction Conditions. The supercritical fluid extraction apparatus used in this study has been described in detail in a

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Table I. Effect of SC-CO_2 Extraction, at Various Combinations of Temperature, Fluid Density, and Extractant Quantity, on the Composition of Dehydrated Beef Powders[a]

treatment	moisture, %	total lipid, %	protein, %	cholesterol, mg/g
control	3.19[c]	34.47[c]	56.96[a]	1.56[c]
55 °C; 0.85 g/cm³; 45 g/g[b]	1.91[c]	24.17[d]	70.97[d]	0.69[d]
55 °C; 0.90 g/cm³, 30 g/g	2.10[c]	10.22[e]	86.91[c]	0.49[de]
55 °C; 0.90 g/cm³; 45 g/g	1.41[c]	4.23[e]	93.82[c]	0.19[e]

[a] Means within a column that have different superscripts are significantly different at the 5% level. [b] Extraction temperature, density of CO_2, and number of grams of CO_2 used per gram of sample, respectively.

Table II. Effect of SC-CO_2 Extraction, at Various Combinations of Temperature, Fluid Density, and Extractant Quantity, on the Hunter Color Values of Dehydrated Beef Powders[a]

treatment	L	a_L	b_L
control	56.07[a]	2.38[c]	15.58[c]
55 °C; 0.85 g/cm³; 45 g/g[b]	59.44[d]	2.16[cd]	15.63[c]
55 °C; 0.90 g/cm³; 30 g/g	64.44[c]	1.62[e]	14.56[cd]
55 °C; 0.90 g/cm³; 45 g/g	64.02[c]	1.87[de]	13.93[d]

[a] Means within a column that have different superscripts are significantly different at the 5% level. [b] Extraction temperature, density of CO_2, and number of grams of CO_2 used per gram of sample, respectively.

Table III. Effect of SC-CO_2 Extraction, at Various Combinations of Temperature, Fluid Density, and Extractant Quantity, on the Composition of Dehydrated Beef Chunks[a]

treatment	moisture, %	total lipid, %	protein, %	cholesterol, mg/g
control	0.97[d]	29.03[c]	67.03[a]	1.57[c]
45 °C; 0.85 g/cm³; 45 g/g[b]	0.66[d]	11.61[d]	85.72[d]	0.37[e]
45 °C; 0.90 g/cm³; 30 g/g	0.88[d]	10.85[d]	86.43[d]	0.59[d]
45 °C; 0.90 g/cm³; 45 g/g	1.67[c]	0.61[e]	97.38[c]	0.24[e]
55 °C; 0.90 g/cm³; 45 g/g	1.65[c]	0.69[e]	98.74[c]	0.23[e]

[a] Means within a column that have different superscripts are significantly different at the 5% level. [b] Extraction temperature, density of CO_2, and number of grams of CO_2 used per gram of sample, respectively.

previous paper (Froning et al., 1990). Samples of dehydrated beef powder or chunks (ca. 150 g) were placed in the extraction vessel and extracted with commercial grade CO_2. Samples were extracted at temperatures of 45 or 55 °C, with backpressures selected to provide a fluid density of either 0.85 or 0.90 g/cm³. Pressure/temperature combinations used to obtain a density of 0.85 g/cm³ were 231 atm/45 °C and 286 atm/55 °C, while conditions used to obtain the 0.90 g/cm³ density were 299 atm/45 °C and 381 atm/55 °C. Pressures in the extraction vessel were regulated to ±3.5 atm, and temperatures were regulated to ±1 °C. Pressure in the separation vessel was maintained at 34 ± 3.5 atm for all extractions.

The flow rate of carbon dioxide through the system was maintained between 5 and 10 std L/min, as measured at ambient conditions. For each density/temperature combination, samples were extracted with 30 ± 1 or 45 ± 1 g of CO_2/g of sample. Sample weights were recorded before and after extraction. Each treatment was replicated at least twice. Analytical tests were performed in duplicate on each extracted or control sample, unless otherwise noted.

Compositional Analysis. The crude fat contents of all samples were determined by extracting the fat with petroleum ether in a Soxtec extractor (Tecator Co., Herndon, VA). Prior to extraction, samples were dried for 5 h at 95 °C in a vacuum oven. Moisture contents were determined by a vacuum oven drying method (AOAC, 1991, No. 950.46), while protein was measured according to the Kjeldahl method (AOAC, 1991, No. 981.10) using a conversion factor of 6.25.

Measurement and Characterization of Lipid Components. Cholesterol was determined in control and extracted samples by high-performance liquid chromatography (HPLC), following cold saponification, as previously described in detail by Froning et al. (1990). Modifications to the procedure included the use of an HPLC mobile phase with a composition of 1% 2-propanol in hexane and dissolution of the extracted cholesterol in 2–5 mL of mobile phase (depending on the expected concentration) prior to injection into the chromatograph.

Fatty acid profiles were determined by gas chromatography, following methylation, as described by Froning et al. (1990).

Lipid oxidation was monitored by measurement of thiobarbituric acid (TBA) reactive substances, according to the method of Pikul et al. (1983). The method was modified to use a 5% butylated hydroxytoluene (BHT) stock solution rather than a 1% BHT solution. Increasing the concentration of BHT in the reaction mixture did not increase the background absorbance at the wavelength used. The sample size was 0.1000 g in all cases.

Color Measurement. Color values (L, a_L, b_L) of control and extracted beef powder samples were measured using a Hunterlab Tristimulus colorimeter Model D25 M-9 (Hunter Associates, Reston, VA). Samples were packed into 50 mm (diameter) × 9 mm glass Petri dishes for color measurement. Each sample was repacked and measured in triplicate.

Sensory Evaluation. For sensory evaluation, samples of dehydrated beef powder were extracted with 30 or 45 g of CO_2/g of sample at a fluid density of 0.90 g/cm³ and a temperature of 55 °C. Samples were extracted on two different days, creating duplicate sets of samples. All extracted samples, along with a control, were stored in closed polyethylene bags. Samples were subjected to sensory analysis after 0, 14, and 28 days of storage at 21 °C. Measurement of TBA reactive substances was made at these same times.

The extracted products, along with an unextracted control, were evaluated using sensory analysis for their level of beef flavor, the development of off-flavors, and overall desirability. Sample preparation for taste panels was as follows:

A 20-g sample of beef powder was blended, using a hand blender, into 200 mL of boiling water. The solubilized sample was boiled for an additional 1 min and then removed from the heat and allowed to partially cool. When the sample was sufficiently cool to handle, it was transferred to 50-mL centrifuge tubes and centrifuged at 2000g for 10 min at 4 °C. After centrifugation, the liquid fraction was transferred to a 250-mL beaker, and any fat residue in the tubes was also transferred. The sample was heated in a 650-W microwave oven for 30 s, after which time the sample was mixed to reincorporate the fat. Aliquots of 10 mL were then transferred to plastic cups (30 mL) and allowed to cool to room temperature. These portions were reheated in a microwave oven (650 W) for 7 s immediately prior to serving to taste panelists. Panelists were served three samples (control plus two levels of extraction), one sample at a time in a random order. A nine-member panel participated, and evaluations were performed using a standard 15-cm scale. Panelists were asked to evaluate the intensity (0 = lacking; 15 = strong) of beef flavor and off-flavor. In addition, overall acceptability was evaluated (0 = very undesirable; 15 = very desirable). A complete block design, using nine panelists per session and three treatments with two replications, was employed.

Statistical Analysis. An analysis of variance (ANOVA) was performed on all data with the Statistical Analysis System (SAS Institute, 1985). Duncan's multiple range test was used to measure differences between means. A 5% level of significance was used throughout the study.

RESULTS AND DISCUSSION

When an extraction temperature of 45 °C was used, there was a high degree of variability in the amount of cholesterol and total lipid removed from beef powder during replicate extractions (data not shown). At 45 °C, the beef lipids are partially, but not completely, melted. Therefore, small variations in temperature can cause substantial changes in the proportion of lipids in the liquid state, which in turn likely affects their degree of solubility in the extracting fluid. Increasing the temperature of the

Table IV. Effect of SC-CO_2 Extraction, at Various Combinations of Temperature, Fluid Density, and Extractant Quantity, on the Fatty Acid Profiles of the Lipids in Dehydrated Beef Chunks[a,b]

treatment	C14:0, %	C16:0, %	C16:1, %	C18:0, %	C18:1, %	C18:2, %	C18:3, %
control	3.46[d]	29.88[d]	5.45[d]	17.26[e]	41.19[e]	2.21[e]	0.56[e]
45 °C; 0.85 g/cm³; 45 g/g[c]	2.39[de]	26.61[de]	4.42[d]	19.31[d]	43.42[d]	3.27[e]	0.65[e]
45 °C; 0.90 g/cm³; 30 g/g	2.42[de]	26.33[e]	4.36[d]	18.95[de]	43.29[d]	3.88[e]	0.71[e]
45 °C; 0.90 g/cm³; 45 g/g	1.50[ef]	22.42[f]	4.29[d]	18.95[de]	37.30[f]	13.80[d]	1.74[d]
55 °C; 0.90 g/cm³; 45 g/g	1.00[f]	21.99[f]	4.17[d]	17.74[de]	35.54[f]	17.70[d]	1.85[d]

[a] Values for each fatty acid are expressed as a percentage of the total fatty acids present. [b] Means within a column that have different superscripts are significantly different at the 5% level. [c] Extraction temperature, density of CO_2, and number of grams of CO_2 used per gram of sample, respectively.

Table V. Effect of SC-CO_2 Extraction on the Sensory Evaluation of Dehydrated Beef Powders[a,b]

treatment[c]	beef flavor[d]	off-flavor[d]	overall acceptability[e]
day 0			
control	7.26[f]	4.86[f]	9.24[f]
30 g/g	7.87[f]	3.93[f]	9.40[f]
45 g/g	7.93[f]	3.45[f]	10.62[f]
day 14			
control	6.71[f]	5.24[f]	7.78[f]
30 g/g	8.53[f]	4.89[f]	9.73[f]
45 g/g	8.36[f]	4.54[f]	9.83[f]
day 28			
control	7.33[f]	4.43[f]	9.77[f]
30 g/g	7.98[f]	4.88[f]	10.05[f]
45 g/g	8.57[f]	4.42[f]	11.98[f]

[a] Rehydrated as a 10% broth before serving. [b] For a given day, means within a column that have different superscripts are significantly different at the 5% level. [c] Samples were extracted at a temperature of 55 °C and a fluid density of 0.90 g/cm³ with the quantity of CO_2 indicated and stored at a temperature of 21 °C. Days listed are the number of days after extraction. [d] 0 = lacking and 15 = strong. [e] 0 = very undesirable and 15 = very desirable.

Table VI. Effect of SC-CO_2 Extraction on the Levels of TBA Reactive Substances in Dehydrated Beef Powders Stored at 21 °C[a]

	mg of malonaldehyde/g of sample		
extraction conditions[b]	0 days	14 days	28 days
control	1.42[c]	1.53[c]	1.79[c]
30 g/g	1.22[c]	1.73[c]	1.78[c]
45 g/g	1.27[c]	1.75[c]	1.72[c]

[a] Means within a column that have different superscripts are significantly different at the 5% level. [b] Samples were extracted at a temperature of 55 °C and a density of 0.90 g/cm³ with the quantity of CO_2 indicated.

extraction vessel to 55 °C improved both the efficiency and the reproducibility of the extraction process.

The compositions of powder samples extracted at 55 °C, but with different densities and different quantities of CO_2, are shown in Table I. It can be observed that increasing the fluid density significantly increased the amount of total lipid and cholesterol removed from the beef powder. With a density of 0.90 g/cm³ and 45 g of extractant used/g of sample, the levels of total lipid and cholesterol in the product were both reduced by more than 87%.

Extracting the dehydrated beef powders with CO_2 also altered their color properties. The L value was significantly increased for the extracted samples, while a_L and b_L decreased significantly at the higher fluid densities and volumes (Table II). This change may be attributable to removal of pigments. A product with lighter color could be desirable as a protein source in various prepared foods.

The results of extracting dehydrated beef chunks with SC-CO_2 are shown in Table III. The lipid components were extracted more easily from the chunks than from the powder, as demonstrated by the fact that improved reproducibility and extraction levels were obtained when chunks were extracted at 45 °C, as compared to powders extracted with the same conditions. The irregularly shaped beef chunks do not pack as tightly in the extraction vessel as the powder particles, thereby providing less resistance to flow. When beef chunks were extracted with 45 g of CO_2/g of sample and a fluid density of 0.90 g/cm³, only small amounts of residual lipid material remained after extraction at either 45 or 55 °C. There were no statistically significant differences in the amounts of either cholesterol or total lipid remaining in the products, indicating that the higher temperature is not necessary to achieve effective removal of lipids from beef chunks. Another interesting observation is that when chunks were extracted at a lower fluid density (0.85 g/cm³), more total lipid remained in the product than when a higher density was used, but there was no statistically significant difference in the amount of cholesterol remaining. For the chunk product, the solubility of cholesterol in CO_2 seems to be less sensitive to density differences than is the solubility of the triacylglycerol fraction that constitutes the major portion of the lipids. Equivalent results were not obtained with the powder (Table I), as lowering the density resulted in both more cholesterol and more total lipid being retained in the product. Decreasing the pressure and density may make penetration of the fluid into the bed of tightly packed powder particles more difficult. This factor may affect the extraction of cholesterol from powders to a greater degree than do the solubility differences that occur as the density is changed.

Extraction with CO_2 altered the fatty acid composition of the residual lipids remaining in the dehydrated beef. Fatty acid composition data for the beef chunks are presented in Table IV. When the chunks were extracted at the lower fluid density, or at the higher density but with a lower total fluid volume such that approximately one-third of the total lipid remained in the product, small but statistically significant changes in the fatty acid ratios can be observed. The most notable change is a small increase in the percentage of oleic acid (C18:1) present, relative to the control. However, when the beef chunks were extracted with higher volumes and densities, such that only a small amount of residual lipid remained, the fatty acid composition of that residual lipid differed substantially from the initial fatty acid profile. The percentages of myristic (C14:0), palmitic (C16:0), and oleic acids were all significantly decreased compared to the control, while the relative percents of linoleic (C18:2) and linolenic (C18:3) acids present increased significantly. These higher percentages of polyunsaturated fatty acids are consistent with the fatty acid composition of muscle phospholipids (Hultin, 1985), indicating that the polar phospholipids are not extracted by the nonpolar CO_2 and are thus concentrated in the residual lipid material. Extraction of beef powders at high fluid densities and total volumes also resulted in a decrease in the relative amounts of the saturated fatty acids and oleic acid and an

increase in the ratios of linoleic and linolenic acid present (data not shown).

Dehydrated beef powders, extracted at 55 °C and a density of 0.90 g/cm^3 with either 30 or 45 g of CO_2/g of sample, were used for taste panel evaluation. The samples were evaluated for level of beef flavor, presence of off-flavors, and overall acceptability of the product. Samples were evaluated immediately after extraction and after 14 and 28 days of storage at 21 °C. The storage study was performed to determine if off-flavors developed to a different degree in extracted samples as compared to a control stored for the same length of time. The taste panel data are shown in Table V. Numerically, the extracted samples had slightly better scores for beef flavor and overall acceptability and slightly less off-flavor than the control. However, the differences were too small to be statistically significant at the 5% level. The results of the sensory evaluation indicate that compounds responsible for the "beefy" flavor are apparently not removed to a significant degree during extraction with CO_2 and that the extracted samples store at least as well as the control material.

Thiobarbituric acid reactive substances were measured in the extracted and control samples simultaneously with the taste panels. The data are given in Table VI. When TBA values were compared for a given storage time, no significant differences were noted as a result of extraction with different quantities of CO_2. This lack of differences is consistent with the inability of the taste panelists to detect any significant differences in off-flavors. It should be noted, however, that while values for TBA reactive substances in both control and extracted samples were very similar, the extracted samples contained much less total lipid. For TBA values to be similar, it is likely that the residual lipid in the extracted samples had undergone extensive oxidation. The relatively high levels of linoleic and linolenic acids in the residual lipid may increase its susceptibility to development of oxidative rancidity. Additional research is needed in this area to more completely characterize this phenomenon.

In summary, extraction with SC-CO_2 can be used to remove most of the cholesterol and fat from dehydrated beef products without significantly changing the flavor. A high-protein low-fat ingredient that retains a desirable beef flavor should find many applications in prepared and convenience foods and may provide an expanded market for lower grade beef and beef trimmings.

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