Off-Flavor Removal from Soy-Protein Isolate by Using Liquid and Supercritical Carbon Dioxide

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ABSTRACT: The efficacy of liquid carbon dioxide (L-CO_2), supercritical carbon dioxide (SC-CO_2), and SC-CO_2 containing 5% ethanol in the removal of off-flavors from soybean protein isolate was studied. Medium-chain aldehydes: *n*-butanal, *n*-pentanal, and *n*-hexanal; ketones: 2-butanone, 2-pentanone, and 2-hexanone; and alcohols: 1-butanol and 2-butanol; were the major compounds extracted. The extractions were performed at a constant fluid density of 901 kg/m^3 with 100, 500, and 1000 standard liter of carbon dioxide. None of the treatments had a detrimental effect on soy-protein functionality. Headspace gas chromatography (GC) and sensory analysis of the treated samples were compared with the untreated soy isolate (control). In general, L-CO_2 was the least effective, and SC-CO_2 was the most effective in removing the off-flavor volatiles. Addition of ethanol as an entrainer did not improve the efficiency of off-flavor removal by SC-CO_2. The results of sensory analysis correlated well with the GC analysis. Sensory analysis of a 33% (wt/vol) slurry of treated soy-protein isolate had more off-flavor notes than the dry soy isolate. Dry and slurried treated soy-protein isolates had significantly less off-flavors and significantly more acceptability than the untreated control. *JAOCS 72*, 1107–1115 (1995).

KEY WORDS: Gas chromatography, liquid and supercritical carbon dioxide, off-flavor, removal, sensory analysis, soy-protein.

A recent trend in the consumer preference for "cholesterol-free" food could help increase the usage of plant proteins, such as soy proteins, over the traditional animal proteins. However, the off-flavor associated with soy proteins makes them undesirable for use in human foods. The off-flavor in soy proteins is caused by aldehydes, ketones, furans, and alcohols. Medium-chain aldehydes (pentanal, hexanal, and heptanal) are the major class of compounds contributing to beany and grassy flavors of soy proteins. The low cost and high nutritional value of soy proteins have been motivating researchers to resolve the flavor problem.

These off-flavor compounds generated by peroxidation of linoleic and linolenic acid by lipoxygenase were reported by Axerod *et al.* (1). Rapid inactivation of lipoxygenase was attempted by using wet or dry heat treatment (2). However, the heat treatment resulted in protein denaturation, i.e., low nitrogen solubility index (NSI) value, and in cooked and toasted flavors that were not desirable. Protein denaturation is usually measured by NSI or protein dispersibility index (PDI) values (3). Man *et al.* (4,5) used acid treatment with HCl, H_3PO_4, and tartaric acid to inactivate lipoxygenase in soy flour. These investigators obtained a bland soy flour after acid treatment, but the PDI decreased to 49% compared with the control. Furthermore, the neutralization of soy flour after the acid treatment resulted in the accumulation of salt in the flour, which was unacceptable to sensory panelists. Srinivas *et al.* (6) used hexane containing 3 and 5% (vol/vol) acetic acid and achieved a 55–63% reduction of the beany flavor and complete inactivation of lipoxygenase. However, NSI was reduced by 39% compared with the hexane-extracted meal (control). Eldridge *et al.* (7) used hexane/methanol (75:25, vol/vol), hexane/ethanol (82:18, vol/vol), and hexane/isopropanol (80:20, vol/vol) solvent mixtures effectively in removing the off-flavors, but they reduced the NSI values significantly and, hence, denatured the soy-protein. Enzyme treatment with aldehyde dehydrogenase (8) was successful in removing the green and beany flavor from an aqueous solution of soy-protein isolate. However, it would be impractical and uneconomical to use this method because NAD+ is required as a cofactor for the enzyme reaction. A method involving genetic modification of soybean seed to remove the genes encoding for lipoxygenases (L_1, L_2, and L_3) (9) has been used to prevent the formation of off-flavors. Removal of L_2 isozyme from cultivars resulted in significantly less beany and rancid flavors. However, the L_2-null soybean preparation had more dairy and cereal flavors; the genetic modification method was not very efficient in producing a bland product. In addition, autoxidation of soybean oil still remained a problem in the lipoxygenase-null variety of soybean seed.

Recently, supercritical carbon dioxide (SC-CO_2) has gained popularity as a fluid for extraction and fractionation in the food and pharmaceutical industries (10,11). The SC-CO_2 technology was investigated in the removal of off-flavors from soybean and corn proteins (10,12). SC-CO_2 was used instead of hexane to extract the oil and to produce a food-

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grade germ flour from corn (12). Although there was a significant improvement of the flour flavor, the authors reported a 52% reduction of NSI, and an even greater decrease (58%) was observed in the corn germ flour containing 8% moisture. Eldridge *et al.* (13) extracted full-fat soybean flakes with SC-CO_2 to produce defatted protein flakes with improved flavor characteristics and high protein functionality. Good flavor scores at pressures greater than 83 MPa and temperatures above 80°C were obtained, but these conditions also had a detrimental effect on the protein quality. Wu *et al.* (14) improved the flavor profile of corn distillers' grain by treating it with SC-CO_2 at rather high pressures (64–83 MPa) and temperatures (82–102°C).

This study is a continuation of our previous work (10,15), which showed a significant reduction of *n*-butanal, *n*-pentanal, and *n*-hexanal in soy flour after SC-CO_2 extraction at 27.6 MPa and 40°C. The goal of this work was to compare the efficiencies of SC-CO_2, SC-CO_2/ethanol mixture, and liquid CO_2 in desorbing the off-flavor from a soy-protein isolate. We also attempted to identify and quantify the off-flavor compounds removed by the CO_2 extraction.

EXPERIMENTAL PROCEDURES

Materials. The soy-protein isolate (Supro 710) selected for this study was obtained from Protein Technologies International (St. Louis, MO). Tenax-GC and the external standards (greater than 99.9% purity) used in gas chromatography (GC) were purchased from AllTech Associates Inc. (Deerfield, IL).

Extraction equipment. A custom-assembled supercritical-fluid extraction system was used in the extraction of soy-protein isolate with liquid carbon dioxide (L-CO_2) and SC-CO_2. The schematic diagram of the flow-through apparatus is shown in Figure 1. Carbon dioxide gas (Matheson Gas Products, Chicago, IL) of 99.9% purity was delivered at a pressure of 5.5 MPa to the air-driven gas booster compressor (Haskel Inc., Burbank, CA). The CO_2 was compressed beyond the operating pressure in a stainless-steel surge tank maintained at a pressure around 41.4 MPa to obtain a pulse-free flow of SC-CO_2 to the extractor. The operating pressure of the system was controlled by a Alphagaz Model 2612 regulator (Cooks Inc., Algona, IA). For the extraction of soy-protein isolate with SC-CO_2 containing 5 wt% ethanol (SC-CO_2/EtOH mixture), the extraction system was used along with a Milton Roy Simplex miniPump Model 2396-31 (LDC Analytical, Riviera Beach, FL) to deliver the cosolvent (ethanol). The SC-CO_2 and ethanol were passed through a static mixer. The CO_2 or CO_2/EtOH mixture was heated to the process temperature by flowing through a stainless-steel coil immersed in a constant-temperature water bath before entering the extraction vessel (16).

Extraction procedure. About 30 g of soy-protein isolate was packed in three beds separated by silane-treated glass wool (AllTech Associates Inc.) in a 20-cm long and 2.2-cm i.d. mirror-finished 316 stainless-steel column (AllTech Associates Inc.). The packing of soy-protein isolate in three beds was used to prevent channeling CO_2 (10,11). The operating conditions used in the removal of the off-flavor compound from the soy-protein isolate were: L-CO_2—17.2 MPa, 25°C, 901 kg/m^3; SC-CO_2—27.6 MPa, 40°C, 901 kg/m^3; SC-CO_2/EtOH (5%)—27.6 MPa, 40°C, 915 kg/m^3; treatment, pressure, temperature, and density, respectively.

The extraction vessel was immersed in a constant-temperature water bath, and the system was allowed to reach equilibrium temperature and pressure for at least two hours before the extraction process started. Two-hour equilibrium was optimum for our system (10). The extraction was started by opening the micrometering valve allowing the CO_2 to flow. The extraction was performed by using 100 (18.1 g), 500 (90.5 g), and 1000 (181 g) standard liters (SL) of CO_2 for each condition. An SL is defined as the volume of CO_2 at 25°C and 1.01 kPa. The flow rate of CO_2 during the extraction was maintained between 0.25–0.35 SL/min (0.4–0.6 g/min).

The SC-CO_2 was passed through a glass U-tube containing five grams of Tenax-GC to entrap solutes from the gas stream. Upon completion of the experiment, the lines were flushed with high-performance liquid chromatography-grade diethyl ether (AllTech Associates Inc.) to remove any residual volatiles in the lines. The extracted soy-protein isolate and glass U-tube were stored in the refrigerator at −18°C until further analysis.

pH Determination. About one gram of the sample was transferred into a scintillation vial along with 10 mL of nano pure water. The soy isolate slurry of 10 wt% was mixed vig-

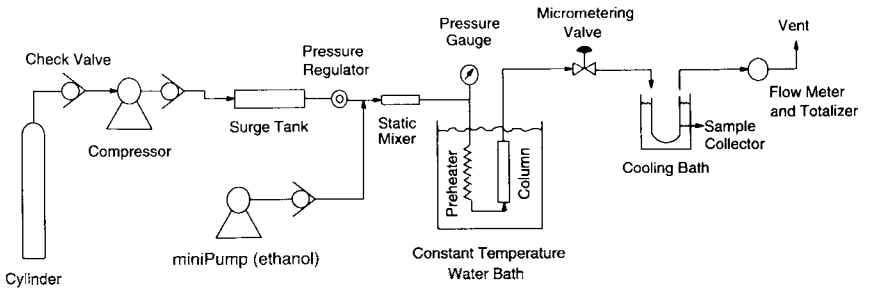

FIG. 1. Schematic diagram of the supercritical-fluid extraction system.

orously, and the pH was measured by a Corning pH meter Model 340 (Fisher Scientific, Pittsburgh, PA). The pH of untreated and treated soy-protein isolate was determined.

Total protein and NSI determination. The total protein and NSI of the soy-protein isolate were expressed as per dry weight basis of protein. The moisture content was determined by drying under vacuum at 80°C for 5 h according to AOAC Method 925.09 (17). Both treated and untreated soy-protein isolate were analyzed in triplicate for total protein content by using Kjeldahl AOCS Method Ac 4-41 (18), using a N-conversion factor of 5.71. For the determination of the water-soluble nitrogen, the official AACC Method 46-23 (19) was modified to measure the NSI from a 2.5-g sample. The analysis was performed in triplicate for both the control and treated Supro 710 soy-protein isolate.

GC headspace analysis. About 2 g of soy-protein isolate was weighed into a 50-mL GC bottle. The three beds of treated Supro 710 soy-protein isolate were mixed to homogeneity for analysis. The GC bottle was sealed with a Teflon septum and aluminum seal (Supelco, Bellefonte, PA) and stored in the dark at room temperature for 24 h and then equilibrated for at least 2 h at 35°C in a Precision Model 183 water bath (Fisher Scientific) before headspace analysis. Equilibrium headspace analysis of the control and treated Supro 710 soy-protein isolate was performed by using a Varian 3400 (Sunnyvale, CA) gas chromatograph equipped with a flame-ionization detector (FID). The column was a 30-m long DB-WAX fused-silica capillary column with 0.25-mm i.d. and 0.25-μ film (J&W Scientific Inc., Rancho Cordova, CA). The column injector temperature and FID temperature were set at 210°C. The column temperature was maintained at 40°C for 10 min, then the temperature was raised to 200°C at 10°C/min and held at 200°C for 10 min. The flow rate of the carrier gas (H_2) was 2 mL/min. Nitrogen, used as a make-up gas, was delivered at a rate of 30 mL/min. The air and hydrogen flow rates in the FID were set at 300 and 30 mL/min, respectively. The attenuation was set between 4–8, and the detector sensitivity was 10^{-12} amps/s. The peak rejection-value was set at 2000 integration units (IU).

A 5.0-mL gas-tight Hamilton syringe (AllTech Associates Inc.) was used to inject a 1-mL aliquot at a rate of 2 mL per minute (20). After 2 min of cryofocusing, the temperature program was initiated, and the oven was turned on. The cryofocusing was carried out by placing a loop of capillary column from the injector side in a styrofoam cup containing liquid nitrogen inside the GC oven, and it was removed at the end of a two-minute period. For each treatment, three 50-mL GC-bottles containing the mixtures of Supro 710 were analyzed, and three injections were made from each GC bottle. The extracted volatiles were eluted from the Tenax-GC using double-distilled ether followed by distillation of ether. The volatiles were analyzed by injecting 500 μL of the headspace sample. The volatile compounds were identified by using *n*-butanal, *n*-pentanal, *n*-hexanal, 2-butanone, 2-pentanone, 2-hexanone, 1-butanol, and 1-pentanol as external standards.

Aromagram. A 1-mL headspace sample was withdrawn and injected with cryofocusing into the gas chromatograph. After sampling, the flame was turned off, and a panelist participating in the analysis sniffed the aroma released from the detector. The panelist described the aroma perceived from the detector and the intensity of the aroma detected.

Sensory evaluation. The sensory evaluation of the dry and the water-slurry of soy-protein isolate was performed by utilizing a 15-cm unstructured line-scale test (21). The sensory panel consisted of seven panelists selected and screened for their ability to detect the beany and off-flavors from the soy-protein isolate. We chose panelists of different ethnic origins to establish whether any differences in soy-flavor perception exist. Three panelists were of Oriental origin, two of Asian–Indian origin, and two of North American origin. The sensory analysis was conducted by giving the panelists two grams of soy-protein sample in 20-mL screw-capped scintillation vials labeled with random 3-digit codes. The samples were kept at room temperature for 24 h and then at 35°C for two hours prior to the testing. The samples were evaluated by the panelists under a white fluorescent light in isolated and well-ventilated booths. Five samples were given to the panelists every session: one untreated sample as a control, three treated samples, and one sample of odorless water. For the sensory evaluation of the wet soy-protein isolate, 4 mL of distilled water was added to the sample to make a 33% (wt/vol) slurry. The following scale was used: for beany and off-flavors, 0 cm on the line scale indicated no flavor, and 15 cm indicated strong flavor. For overall acceptability, 0 cm indicated the least desirable, and 15 cm indicated the most desirable sample.

Statistical analyses. The General Linear Model Procedure, Least Significant Differences, and Pearson's Correlation were used to analyze the data by using Statistical Analysis System Package (version 6.03).

RESULTS AND DISCUSSION

Protein analysis. The effect of CO_2 treatments on the moisture content, pH, total protein, and NSI are given in Table 1. Except for the 1000 SL of L-CO_2 treatment, L-CO_2-treated samples were not significantly ($P > 0.05$) different in moisture content from the control. All SC-CO_2-treated samples had significantly less ($P < 0.05$) moisture content except the treatment performed with 100 SL SC-CO_2. The greater moisture content of the L-CO_2-treated samples compared with the SC-CO_2 ones parallels the lower solubility of water in L-CO_2 (0.1 wt%) (22) than in SC-CO_2 (0.2 wt%) (23). SC-CO_2/EtOH, being more polar, removed a greater amount of moisture than the other two treatments.

These results parallel the observations of Christianson *et al.* (12) that corn germ extracted with SC-CO_2 had less moisture than the control. All treated samples had a reduced moisture content proportional to the volume of CO_2 used in the extraction. The change in moisture content after CO_2 processing is important from a commercial point of view. A 1–2% drop in moisture can result in lower profits to an industry because finished product is sold close to maximum allow-

TABLE 1
Effect of Subcritical and Supercritical CO_2 (SC-CO_2) Extraction on the Moisture Content, pH, Total Protein, and NSI of Soy-Protein Isolate

Treatment[a]	Moisture (wt%)	pH	Total protein (wt%)	NSI (w/w %)
Control	5.02 ± 0.04[b]c,d	7.03c	82.1 ± 0.6c	61.7 ± 0.5c,d,e
L-CO_2				
100 SL	5.32 ± 0.01c	6.82e	80.1 ± 0.3d	63.4 ± 0.2c
500 SL	4.64 ± 0.03d	6.77e	80.4 ± 0.3d	62.8 ± 0.3c
1000 SL	3.38 ± 0.01f,g	6.78e	80.1 ± 0.4d	62.5 ± 0.8c,d
SC-CO_2				
100 SL	5.04 ± 0.08c,d	6.70e	80.8 ± 0.4c,d	61.4 ± 1.3c,d
500 SL	3.64 ± 0.07f	6.75e	80.0 ± 0.3d	61.9 ± 0.4c,d,e
1000 SL	2.70 ± 0.45h,i	6.77e	80.3 ± 0.4d	59.5 ± 1.4f
SC-CO_2/EtOH				
100 SL	4.16 ± 0.02e	6.86e	80.9 ± 1.0c,d	62.0 ± 0.6c,d,e
500 SL	3.06 ± 0.04g,h	6.76e	79.1 ± 0.6d	60.4 ± 0.3e,f
1000 SL	2.44 ± 0.13i	6.76e	80.5 ± 0.2c,d	60.7 ± 1.0d,e,f

[a]$n \geq 2$.
[b]Standard error. Means with letters c, d, e, f, g, h, and i for % moisture, pH, total protein, and nitrogen solubility index (NSI) within a column with the same letter are not significantly different ($P > 0.05$) from each other, either among different treatments, or within the treatment; L-CO_2, liquid CO_2; SL, standard liters.

able moisture level. Therefore, conditioning of soy-protein isolate will be required to raise the moisture level to industry standards for a profitable product.

Table 1 also shows the effect of the different treatments on the final pH of soy-protein isolate. The CO_2-treated samples had a significantly lower ($P < 0.05$) pH compared with the control. Carbon dioxide reacted with water (moisture) present in soy-protein isolate producing carbonic acid (H_2CO_3). Although the decrease of pH in the treated samples was statistically significant, it did not affect the NSI. The pH decrease of the treated samples was not large enough to shift the flavor-binding equilibrium and to enhance the desorption of off-flavors from soy proteins (24).

Similar to our previous finding (10), none of the treatments substantially affected the NSI. The NSI values for most of the treatments were not significantly different from the control. The samples extracted with 1000 SL of SC-CO_2, and 500 and 1000 SL SC-CO_2/EtOH resulted in significantly lower NSI, but we believe that a 4% reduction in NSI would not be a major obstacle in terms of reduced protein solubility. Our results demonstrated that extraction of soy-protein with L-CO_2, SC-CO_2, and SC-CO_2/EtOH did not markedly affect protein solubility under processing conditions which were milder (lower temperature and pressure) than those used by Eldridge *et al.* (13). They observed a drastic reduction in the NSI values of soybean flakes extracted at 97.3 MPa and 90°C.

Flavor study. We performed the flavor study by using GC and sensory analysis. In the GC analysis, we were mainly interested in the peak area of total volatiles (TV), total identified volatiles (TIV), and off-flavor compounds, such as 2-butanone, *n*-butanal, 1-butanol, 2-pentanone, *n*-pentanal, 2-hexanone, *n*-hexanal, and 1-pentanol. The presence of these off-flavor-causing volatiles was determined by using an aromagram because GC has a detection limit an order of magnitude lower than that of the human nose (25–27). To determine which peaks of the chromatogram should be included in the TV, a panelist was asked to sniff the odor compounds released from the GC detector after sample injection. The panelist described the released odor in the first 12 min as rancid-oil odor. The intensity of the odor compounds decreased in min 13 of analysis. The panelist was not able to detect any odor coming from the GC column after min 15 of chromatography, even though additional peaks were recorded on the chromatogram. This test was used as a basis for determining the retention time cut-off value for TV present in the soy-protein isolate. Because we were only interested in the compounds that have an undesirable flavor, the volatiles retained more than 13 min on the GC column were not included in the calculation of TV.

The peak area of TV and TIV (external standards) in the soy-protein isolate before and after the CO_2 treatments are shown in Figure 2. We used a peak rejection value of 2,000 in order to eliminate the noise and the minor peaks detected by the FID. L-CO_2 was the least effective, and SC-CO_2 was the most effective in reducing TV from the soy isolate (Fig. 2A). All treated samples had significantly ($P < 0.05$) lower TV compared with the control. L-CO_2-100 SL treatment removed 32% of TV, whereas 1000 SL of SC-CO_2 reduced TV to 60%. SC-CO_2/EtOH treatment removed more volatiles than L-CO_2; however, the latter was not as effective as SC-CO_2. In all the treated samples, TV were reduced with an increase of measured volume of CO_2 used. The TV for the SC-CO_2/EtOH treatment were greater than for the control because of the alcohol adsorbed to the soy-protein (28) during the extraction of off-flavors. Therefore, in calculating TV, we eliminated the ethanol peak area. In general, the SC-CO_2-treatment was the most effective in the removal of volatiles. The addition of a cosolvent (ethanol) to SC-CO_2 had no effect on the flavor desorption. Figure 2B describes the peak area of TIV. We calculated TIV as the sum of peak areas of *n*-butanal, *n*-pentanal, *n*-hexanal, 2-butanone, 2-pentanone, 2-

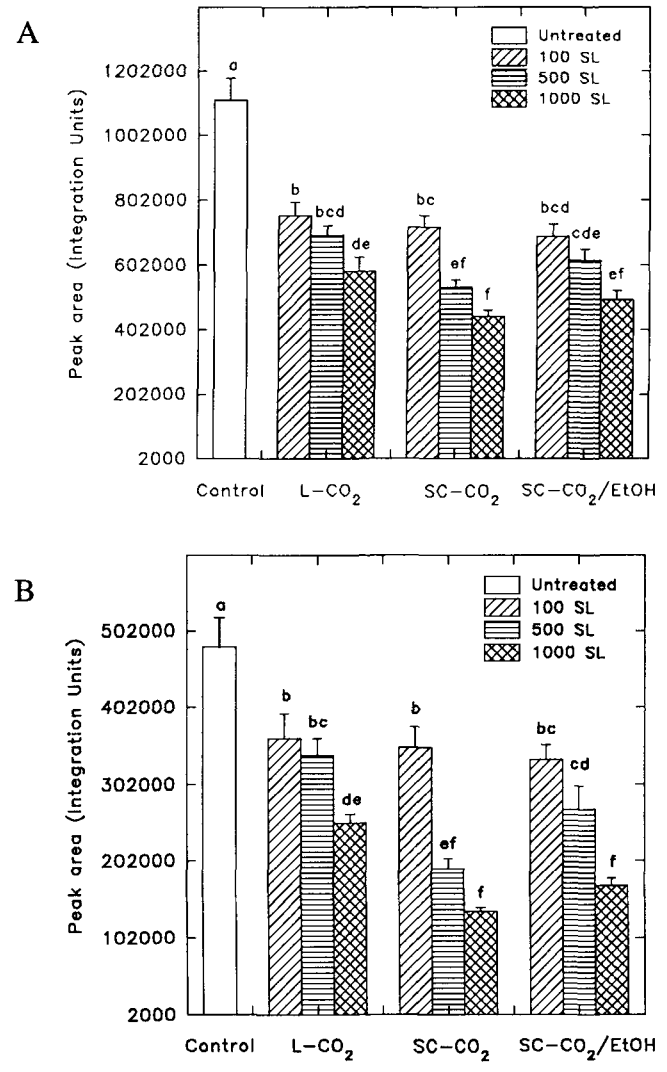

FIG. 2. Total peak area of total and total identified volatiles in the treated and untreated soy-protein isolate: (A) total volatiles and (B) total identified volatiles. Different letters represent treatments which are significantly different at $P < 0.05$. For the treatments, $n \geq 3$; SL, standard liter; SC-CO_2, supercritical CO_2; L-CO_2, liquid CO_2.

hexanone, 1-butanol, and 1-pentanol. The GC results showed that TIV in the control soy-protein isolate (Supro 710) were 43% of TV. The trend in reduction of TIV by the different treatments was similar to that of TV. The extraction with 1000 SL SC-CO_2 reduced TIV as much as 72%. Therefore, all L-CO_2, SC-CO_2, and SC-CO_2/EtOH treatments removed TV and TIV from the soy-protein isolate in a similar manner.

The effects of various treatments on the removal of medium-chain aldehydes (major off-flavor contributor) are shown in Figure 3. We detected butanal (Fig. 3A) as the major aldehyde in the control followed by hexanal (Fig. 3C) and pentanal (Fig. 3B). Butanal is the most polar, and hexanal is the least polar aldehyde among the three aldehydes studied.

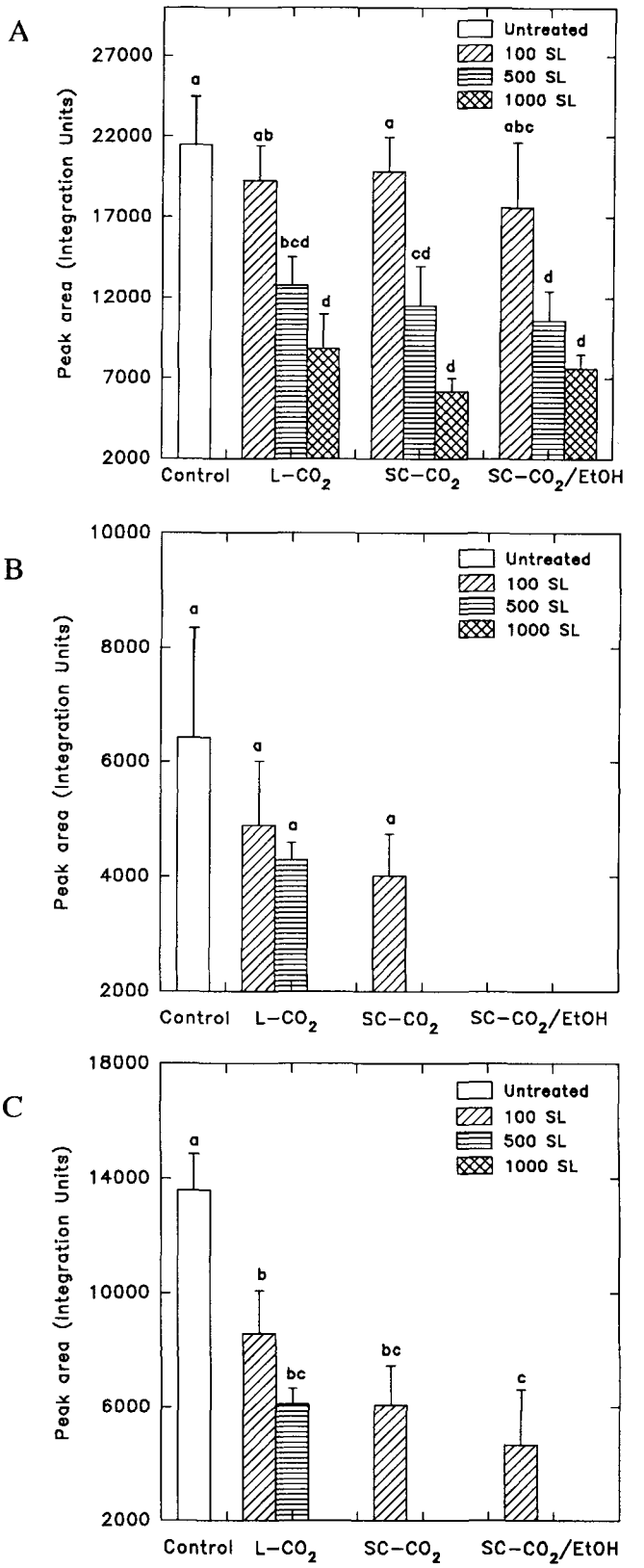

FIG. 3. Total peak area of identified aldehydes in the treated and untreated soy-protein isolate: (A) *n*-butanal, (B) *n*-pentanal, and (C) *n*-hexanal. Different letters represent the treatments significantly different at $P < 0.05$. Missing bars indicated that aldehydes were not detected by gas chromatography at the peak-area cutoff of 2000. For the treatments, $n \geq 3$. See Figure 2 for abbreviations.

L-CO_2 was not effective in solubilizing polar compounds, which is reflected by the data in Figure 3 (A and B). L-CO_2 at 100 SL did not significantly reduce the amount of butanal and pentanal in the soy isolate compared with the control. However, Figure 3C indicates that the peak area of the more nonpolar hexanal was significantly lower compared with the control. The SC-CO_2-1000 SL-treated sample had a reduction of 72% in butanal. Moderately polar pentanal was not detected in the treatments with SC-CO_2/EtOH, 500 SL and 1000 SL of SC-CO_2, and 1000 SL of L-CO_2. Ethanol was useful in removing pentanal from the soy-protein isolate; however, it did not have a significant effect on the removal of hexanal from the soy-protein isolate when compared with the SC-CO_2. In summary, SC-CO_2/EtOH treatments were slightly better than the SC-CO_2 in removing aldehydes. Our recommendation would be to use SC-CO_2 because the process is simpler and there will be no residual alcohol left in the protein isolate to impart its own flavor.

The total areas for 2-butanone, 2-pentanone, and 2-hexanone are shown in Figure 4. The control contains medium-chain ketones in substantially larger amounts than corresponding aldehydes. Again, in most cases, SC-CO_2 was a better treatment to remove ketones. Up to 94% of 2-butanone, 86% of 2-pentanone, and 71% of 2-hexanone were removed by 1000 SL (181 g) of SC-CO_2. Previous binding studies with aldehydes and ketones (29,30) showed that flavor adsorption to soy-protein increased with increasing hydrophobicity (chainlength). In a recent study, Cooray (24) suggested that the binding of flavor components to soy-protein was hydrophobic in nature. This would explain why 2-butanone was the easiest ketone to remove and 2-hexanone the most difficult to remove with CO_2 extraction.

The amount of desorbed medium-chain alcohols (1-butanol and 1-pentanol) was the greatest among the off-flavor compounds of the corresponding chainlength (Fig. 5). Aspelund and Wilson (28) and Crowther *et al.* (31) studied the gas–solid interaction of flavor compounds with dry soy-protein isolate. Their work showed that alcohols adsorbed more strongly to the protein isolate than carbonyls because of the significantly greater heat of adsorption of alcohols compared with carbonyls with a similar carbon number. We did not study 1-hexanol because the odor threshold for 1-hexanol in water is 500 ppb (32), more than 100 times greater than the odor threshold of 1-hexanal, 4.5 ppb (33). Therefore, 1-hexanol is not a major contributor to the off-flavor in soy-proteins. For alcohol removal, L-CO_2 treatment was the least efficient. Butanol was most effectively removed by SC-CO_2/EtOH, probably because ethanol displaced the weakly bound butanol and aided the SC-CO_2 extraction. We were able to remove as much as 72% of 1-butanol, but only 56% of 1-pentanol by using 1000 SL of SC-CO_2. The addition of alcohol to SC-CO_2 apparently did not aid 1-pentanol removal. This is probably due to the difference between the dielectric constants (polarity) of ethanol and 1-pentanol. 1-Pentanol binds more tightly to the soy-protein isolate than butanol and is more difficult to displace by ethanol. The reported binding constants of stud-

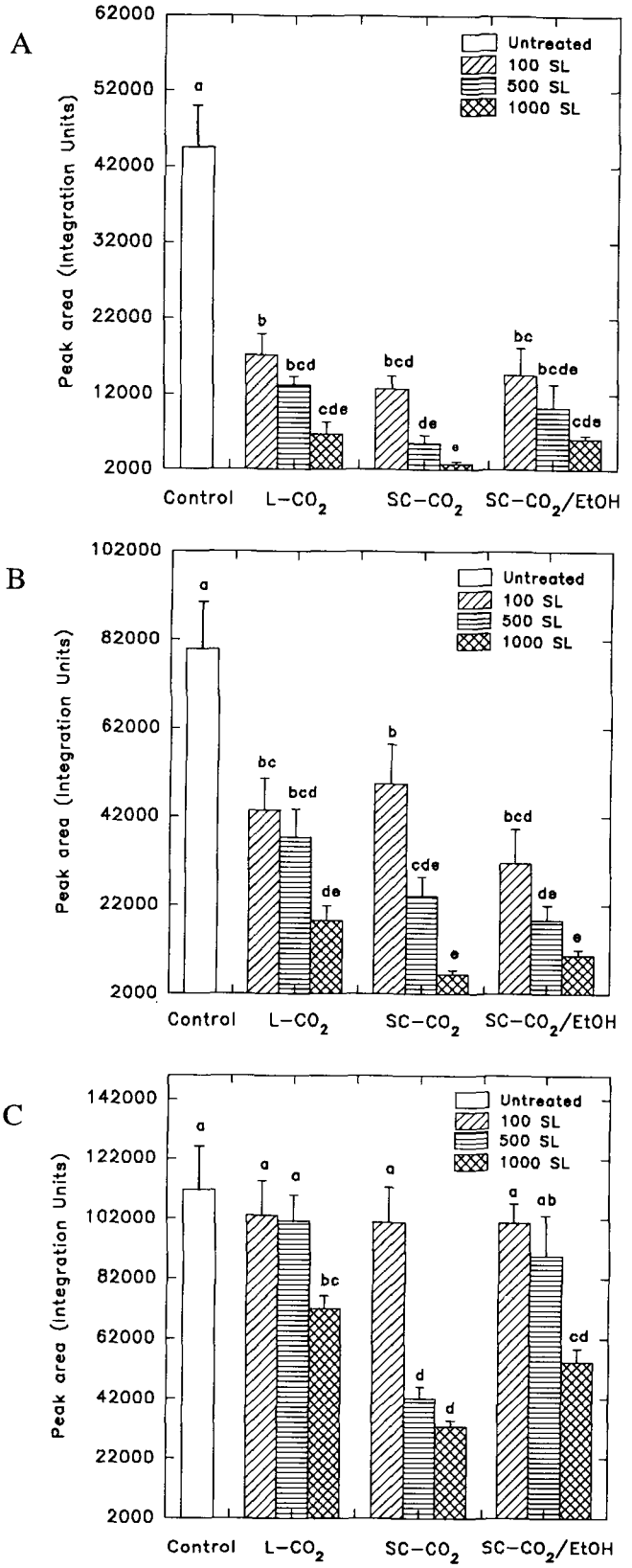

FIG. 4. Total peak area of identified ketones in the treated and untreated soy-protein isolate: (A) 2-butanone, (B) 2-pentanone, and (C) 2-hexanone. Different letters represent the treatments significantly different at $P < 0.05$. For the treatments, $n \geq 3$. See Figure 2 for abbreviations.

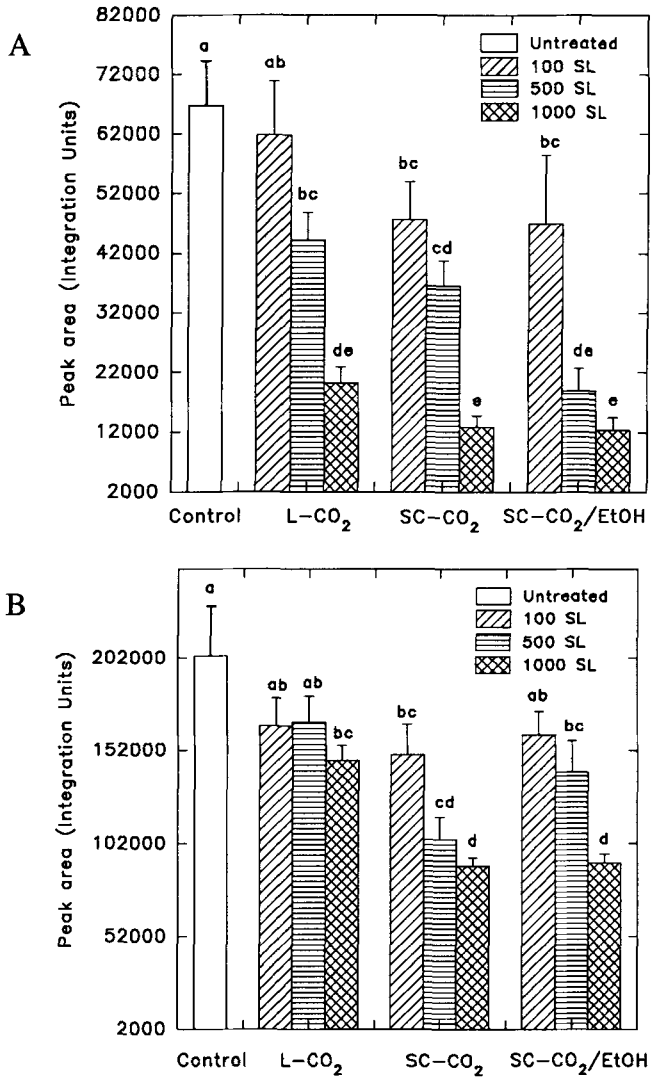

FIG. 5. Total peak area of identified alcohols in the treated and untreated soy-protein isolate: (A) 1-butanol and (B) 1-pentanol. Different letters represent the treatments significantly different at $P < 0.05$. For the treatments, n ≥ 3. See Figure 2 for abbreviations.

TABLE 2
Sensory Analysis of Dry Soy-Protein Isolate (Supro 710)[a]

Treatments significance (LSD)	Beany odor LSD = 2.28	Off-flavor LSD = 2.40	Overall acceptability LSD = 2.24
Control	12.16a	11.71a	1.72e
L-CO_2			
100 SL	8.88b	7.48b,c	5.81c,d
500 SL	6.60b,c,d	5.94b,c,d,e	7.21b,c
1000 SL	4.66d,e	3.55e,f	9.50a
SC-CO_2			
100 SL	8.29b	8.10b	4.85d
500 SL	4.99d,e	5.10c,d,e	8.70a,b
1000 SL	4.22e	3.86e,f	9.46a
SC-CO_2/EtOH			
100 SL	8.08b,c	6.39b,c,d	5.16c,d
500 SL	5.84c,d,e	4.23d,e,f	7.16b,c
1000 SL	3.75e	2.30f	10.50a

[a]Protein Technologies International (St. Louis, MO). Different letters (a–f) within the same column are significantly different at $P > 0.05$. For beany odor and off-flavor: 0 = none and 15 = strong. For overall acceptability: 0 = least desirable and 15 = most desirable. LSD, least significant difference. See Table 1 for other abbreviations.

ied off-flavor ligands decrease in the following order: aldehydes, ketones, and alcohols (34). Our results are in good agreement with this order because aldehydes were the easiest to remove from soy-protein isolate followed by the respective ketones and alcohols.

The U-tube extract containing trapped off-flavors was analyzed by using GC. We detected the presence of all the identified volatiles removed from the soy-protein isolate. This confirmed that various CO_2-treatments were able to desorb and solubilize the off-flavor compounds.

Sensory evaluation. The results of the sensory evaluation of the dry soy-protein isolate are shown in Table 2. The beany odor and off-flavors for all the treated samples were significantly ($P < 0.05$) lower than for the control. The off-flavors perception decreased significantly when the volume of CO_2 in each of the treatments was increased. This correlates well with the GC analysis. The extractions with 100 SL of CO_2 had a significantly lower TIV (Fig. 2B) and significantly higher overall score (Table 2) compared with the control. However, the treated samples were not preferred by the panelists because beany odor and off-flavors were detected. The beany odor and off-flavors were minimal in the treatments with 500 SL of SC-CO_2 and SC-CO_2/EtOH, and 1000 SL of L-CO_2, SC-CO_2, and SC-CO_2/EtOH. The results were in agreement with the GC, which indicated that a significant reduction of off-flavor compounds was achieved with the five treatments. As expected, the beany odor and off-flavors were highly correlated (Pearson correlation) with the content of butanal (r = 0.95 and 0.92, respectively), pentanal (r = 0.83 and 0.85, respectively), and hexanal (r = 0.95 and 0.94, respectively). As the peak areas for butanal, pentanal, and hexanal decreased (Fig. 3), the beany odor and off-flavor scores also decreased. The results for overall flavor acceptability were highly correlated with TV (r = 0.93). The best treatments with the most desirable flavor were associated with the greatest reduction in TV. The panelists were able to detect the presence of ethanol in the SC-CO_2/EtOH-treated samples; however, they found that 1000 SL-treated samples were still desirable and relatively bland in flavor.

There was no significant difference in perception of the beany odor and off-flavors of the soy-protein isolate between the panelists of different ethnic origins. The panelists from the Orient preferred the beany flavor, whereas the Asian–Indian and North American panelists liked the bland soy-protein isolate.

Because the soy-protein isolate is exposed to a significant amount of water in food preparations, we studied the flavor profile difference between dry and water-mixed samples. Soy-proteins have been shown to generate more off-flavors in the presence of moisture (35). Therefore, a 33% slurry of soy-pro-

tein isolate was prepared, and sensory analysis was carried out. The results of the sensory evaluation of the slurry are shown in Table 3. The beany odor and overall acceptability for L-CO_2- and SC-CO_2-treated samples were very similar for both the dry and slurried form, as shown in Tables 2 and 3. The off-flavor score was slightly higher for all slurried samples because the panelists could detect the "mealy" and "cerealy" odor released from the slurry. O'Keefe *et al.* (35) reported that the presence of water may shift the predicted Schiff's base equilibrium of aldehyde-protein binding, allowing more free aldehydes to be released in the soy-protein suspension. We believe that this was the reason why the panelists detected more off-flavor in the soy slurry than in the dry samples.

The slurry of SC-CO_2/EtOH-treated samples had even more off-odor than the other treatments because ethanol was released from the SC-CO_2/EtOH-treated samples. The binding of alcohols is weaker in aqueous systems than in the dry state (28); therefore, the SC-CO_2/EtOH-treated samples (with the exception of 1000 SL of SC-CO_2) were not desirable in the presence of water.

Even though the addition of moisture (Table 3) increased the odors, the overall acceptability of the soy-protein slurries was still high for 500 SL of SC-CO_2 and L-CO_2, and 1000 SL of L-CO_2, SC-CO_2, and SC-CO_2/EtOH treatments which were most efficient as determined by the sensory evaluation of soy-protein isolate in the dry form.

In conclusion, SC-CO_2 desorption offers a possible solution to solve the off-flavor problem of soy-protein for human consumption. It is evident from our study that a significant improvement in the flavor profile of soy-proteins can be achieved by using the SC-CO_2 extraction technology without impairing protein functionality. L-CO_2 was the least effective and SC-CO_2 the most effective fluid in desorbing off-flavor compounds from soy isolate. The addition of ethanol as a polarity modifier (entrainer) to SC-CO_2 did not increase the off-flavor removal. The ethanol entrainer partially adsorbed to the soy-protein, and it was detected in the samples by the sensory panelists.

The sensory panelists found no difference in acceptability between the CO_2-treatments of both dry and water-slurried soy-protein isolate when the extraction was performed with 1000 SL of CO_2. Therefore, either L-CO_2 or SC-CO_2 could be used for off-flavor removal. L-CO_2 would probably be more economical to use on a large scale because lower pressure and temperature are required to obtain the same solvation power as that of SC-CO_2. Further optimization of the process is needed to fine-tune the process temperature and initial protein moisture. In our opinion, the off-flavor process could only be economically viable if combined with L-CO_2 or SC-CO_2 soy-oil extraction.

TABLE 3
Sensory Analysis of Soy-Protein Isolate (Supro 710)[a] in Slurry Form

Treatments significance (LSD)	Beany odor LSD = 4.63	Off-flavor LSD = 4.15	Overall acceptability LSD = 4.34
Control	8.18a	9.57a	4.38c,d,e
L-CO_2			
100 SL	7.28a,b	9.26a	4.54c,d,e
500 SL	5.48a,b	6.50a,b,c,d	8.12a,b,c,d
1000 SL	4.84a,b	5.02b,c,d	9.74a
SC-CO_2			
100 SL	7.52a,b	9.48a	4.04d,e
500 SL	4.72a,b	6.28a,b,c,d	9.00a,b
1000 SL	2.98a,b	3.88d	11.08a
SC-CO_2/EtOH			
100 SL	8.76a	9.02a,b	3.52e
500 SL	5.60a,b	8.08a,b,c	5.12b,c,d,e
1000 SL	4.46b	4.70c,d	8.40a,b,c

[a]See Table 2 for company source and Tables 1 and 2 for abbreviations. Different letters (a–e) within the same column are significantly different at $P > 0.05$. For beany odor and off-flavor: 0 = none and 15 = strong. For overall acceptability: 0 = least desirable and 15 = most desirable.

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