Preparation and Evaluation of Supercritical Carbon Dioxide Defatted Soybean flakes

A. C. ELDREDGE, J. P. FRIEDRICH, K. WARNER, and W. F. KWOLEK

ABSTRACT

Full-fat soybean flakes were extracted with supercritical carbon dioxide (SC-CO₂) at pressures of 10,600–12,400 psi, temperatures from 80–100°C, and moisture levels of 5–13.5%. Conditions could be selected to produce defatted soybean meals with nitrogen solubility indices greater than 70% and flavor scores greater than 6.5 on a scale of 1 to 10 (1 = strong and 10 = bland). The usual grassy-beany and bitter flavors of hexane-defatted soybean flours were only minimally detectable in the optimally SC-CO₂-extracted materials. Bland, defatted soybean meal prepared by SC-CO₂ extraction was further processed into high-quality protein concentrates and isolates that were stable when stored under adverse conditions.

INTRODUCTION

THIRTY MILLION TONS of soybeans are extracted each year in the United States for domestic use. Virtually all these soybeans are extracted with hexane or, perhaps, hexane-alcohol mixtures. Hexane extraction of soybeans produces defatted protein products that generally have grassy-beany and bitter tastes associated with them (Kalbrenner et al., 1971), which renders them undesirable for food use. Investigators have suggested that these flavors are not only breakdown products of lipid oxidation (Sessa et al., 1969), but may also be caused by traces of hexane residues in the meal products (Warner et al., 1983). Liquid CO₂ has been used to remove volatile substances such as aroma constituents from fruits and other food products (Schultz et al., 1974).

Supercritical carbon dioxide (SC-CO₂) has many advantages over hexane extraction because it is nontoxic, nonexplosive, cheap, readily available, and easily removed from extracted products. SC-CO₂ is also a more versatile solvent than liquid CO₂ because extraction conditions can be varied over a wider range.

The SC-CO₂ extraction of oilseeds, including soybeans, has been reported (Friedrich et al., 1982), but analytical and flavor studies of SC-CO₂-extracted soybean meals are needed to evaluate the potential of these defatted products.

The purpose of this study is to determine the optimum extraction parameters for SC-CO₂ extraction of full-fat soybean flakes to produce defatted protein products with improved flavor characteristics and high protein solubility.

MATERIALS & METHODS

Extraction equipment and conditions

The general apparatus for SC-CO₂ extractions in a laboratory has been discussed by Friedrich et al. (1982), Friedrich and List (1982), and Friedrich and Pryde (1984) and is shown in Fig. 1. In this study, we required accurate temperature control. To accomplish this, a small 316 S3 extractor (22” × 0.56” i.d.) with a pressure rating of 20,000 psig was placed in a gas chromatography oven and controlled within ±0°C of desired extraction temperature.

Experimental design

To optimize the extraction parameters, a central composite rotatable design was used to produce response surface contours (Cochran and Cox, 1957). Three conditions (pressure, temperature and moisture) were varied while CO₂ flow and extraction time were constant. To establish the time parameter, an extraction was run at the lowest combination of pressure and temperature (84°C and 11,000 psi) and a flow rate of 15 standard L of CO₂ per min. The time required to reach a residual oil content of <1% in the meal was determined to be 20 min. At combinations of higher pressure and temperature the time required for extraction was less, but the extraction was continued for the full 20 min to eliminate the time variable. The pressure was varied from 10,600 to 12,400 psi, temperature from 80 to 100°C, and moisture from 5 to 12.4%. In addition to the moisture in the flakes, 1 mL of water was added to a glass wool plug at the inlet of the extractor for each of the extractions. This was necessary because in the absence of additional water the dry CO₂ entering the column carries the moisture away from the initially contacted flakes before the flavor-improving effects of moisture, pressure and temperature are realized. After 20 extractions conditions were defined, their order was randomized, and the extractions were conducted.

Full-fat flakes (40g) with known moisture levels were removed from cold storage and placed in the tube extractor. The extractor was sealed and brought to desired pressure while being heated to a controlled temperature. With a CO₂ supply pressure of 1,100–1,200 psi, a flow of 15–18 standard L/min was maintained at extraction conditions. After 20 min the extractor was depressurized, and the defatted flakes were removed.

To verify the time, flow, moisture, temperature, and pressure relationships, several larger confirmatory extractions (i.e., 1 kg) at selected conditions were conducted. Parameters for the extractions were selected based on the data in Fig. 2. CO₂ flow was adjusted (85–95 Std L/min) to allow complete extraction in 20 min.

Sensory evaluation

A 15-member trained panel, experienced in tasting soybean protein products, evaluated the samples for flavor quality. The samples were prepared as 2% dispersions in 25°C carbon-filtered tap water. Panelists were served 10 mL aliquots of each dispersion in 50 mL glass beakers covered with watch glasses. All sensory testing was conducted under red fluorescent light to mask color differences between samples. Further details on testing procedures were described previously by Warner et al. (1983). Overall scores were based on a 10-point scale, with 10 as bland (excellent quality) and 1 as strong (poor quality). Individual flavor descriptions (cereal, cooked bean, grassy, bitter, astringent and toasted) of the samples were rated on a scale of 0 = none, 1 = weak intensity, 2 = moderate intensity, and 3 = strong intensity. Flavor intensity values (FIV's) were calculated by a procedure described by Warner et al. (1983). A wheat flour control (2% dispersion) was included in all tests.

A balanced incomplete block design (Cochran and Cox, 1957) was used as a testing pattern for the 20 samples. The samples were randomly divided into two groups of 10 samples each. Each tester evaluated three samples at a panel session. A total of nine scores for each sample was used in calculating overall means. An analysis of variance...
determined statistical significance among means at the 95% confidence level (P>0.05). Concentrates and isolates were evaluated in groups of two or three and data was analyzed statistically by a two-way analysis of variance (P>0.05). Least significant difference (LSD) among samples was also calculated (P>0.05).

Analytical methods

The following American Oil Chemists’ Society Methods (1975) were used in this study: oil, Ba 3-38; moisture, Bc 2-49; protein, Ba 4-38; residual oil, Ac 3-44; nitrogen solubility index, Ba 11-65; and urease, Ba 9-58. Lipoxygenase (EC 1.13.1.13) was determined by the method described by Christopher et al. (1972). Soybean trypsin-inhibitor (TI) activity of the defatted meals was determined by the method of Hamerstrand et al. (1981).

RESULTS & DISCUSSION

Extraction of flakes

The extraction conditions, nitrogen solubility index (NSI), flavor scores (FS’s), and lipoxygenase activity of 20 samples are shown in Table 1. Where a high NSI and FS are desired, the best extraction conditions were at least 12,000 psi, about 85°C, and moisture levels of 10.5 to 11.5%. Such conditions should give defatted soybean flakes with NSI’s approaching 70 and FS’s near 7.0.

Samples with high NSI’s but very low FS’s had lipoxygenase activities very similar to undenatured hexane-defatted soybean flakes. Apparently the presence of moisture in the flakes caused denaturation of lipoxygenase, with a concurrent increase in flavor score but a decrease in NSI (see samples 11 and 12, Table 1, for example). However, one sample (sample 17) had a high NSI and a low flavor score but had no lipoxygenase activity. This result is unexplainable at this time.

Full urease activity was indicated in all 20 samples by a consistent pH change of 2.0–2.1. The reasons why lipoxygenase was denatured and urease was unaffected are of interest.
The second defatted soy moisture. SC-CO$_2$-extracted = sed pre­pre­pre­
pres at 80°C. The process should have potential in the
flow rate (Table 2). These
Significant at Probability >0.01 level. 37°C. The process should have potential in the
id;IDd FS contours when tem­
however, the
multiple correlation. Correlation of observed and predicted NSI or FS values,
(repeating)

**Table 2—Equations relating NSI and FS to process condition*\(^{a}\)**

<table>
<thead>
<tr>
<th>Term</th>
<th>Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSI</td>
<td>$-863.31 + 6.62058P + 10.79633T$</td>
</tr>
<tr>
<td></td>
<td>+ 33.28007M - 0.03715S²</td>
</tr>
<tr>
<td></td>
<td>- 0.07172T - 0.17133M²</td>
</tr>
<tr>
<td></td>
<td>+ 0.02875P² - 0.06407PM</td>
</tr>
<tr>
<td></td>
<td>- 0.31688TM</td>
</tr>
<tr>
<td>R²</td>
<td>0.962**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Term</th>
<th>Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS</td>
<td>$-132.13 + 0.84087P + 1.71314T$</td>
</tr>
<tr>
<td></td>
<td>+ 1.55462M + 0.00012SM²</td>
</tr>
<tr>
<td></td>
<td>- 0.00005T - 0.00444M²</td>
</tr>
<tr>
<td></td>
<td>+ 0.01256P + 0.00753PM</td>
</tr>
<tr>
<td></td>
<td>- 0.01549TM</td>
</tr>
<tr>
<td>R</td>
<td>0.934**</td>
</tr>
</tbody>
</table>

*\(^{a}\) P = pressure/100, T = temperature, M = % moisture.  
*\(^{b}\) R = multiple correlation. Correlation of observed and predicted NSI or FS values.  
** Significant at Probability >0.01 level.  

**Fig. 3—Contour diagram showing effect of moisture and temperature on nitrogen solubility index (---) and flavor score (---) when full-fat soybean flakes are extracted at 11,000 psi for 20 min with constant carbon dioxide flow.**

and currently under investigation. Weder (1980, 1984) reported that ribonuclease and lysozyme were changed slightly when treated with wet SC-CO$_2$ at ~5000 psi at 80°C. The changes were mainly in the disulfide bonds of the proteins, and this may be the phenomenon we have observed.

The NSI and FS data in Table 1 were analyzed statistically, and equations were derived that predicted either NSI or FS when pressure (P), temperature (T), and moisture (M) were controlled for a given time and CO$_2$ flow rate (Table 2). These equations were used to create NSI and FS contours when temperature and moisture were varied. Figure 2 is the plot for 12,000 psi and Fig. 3 is for 11,000 psi. Products with NSI’s greater than 70 and FS’s greater than 6.5 can be obtained by extracting under conditions designated by crosshatching in Fig. 2. Products with these qualities were unattainable when the pressure of the extraction was decreased, the NSI’s generally remained the same, but there was a significant decrease (probability >0.05) in FS’s. Table 3 shows the NSI and flavor scores of two 1 kg batches of defatted soybean flour. The NSI of sample 1 was lower than anticipated (~68); however, the FS were as predicted (~7) by Fig. 2. The second defatted soy sample had the expected NSI (~47) and flavor score (~7). The FS of soy flour #1 (7.3) was rated as not significantly different than a wheat flour control (FS = 7.9) whereas the FS of sample #2 (6.6) was significantly lower than the control.

There are three procedures for preparing soybean protein concentrates (Kalbrenner et al., 1971) which contain 70% protein. The above two defatted soybean flours were used to prepare concentrates by (a) water leaching of toasted defatted soybean flakes, (b) alcohol washing of underdenatured defatted soybean flakes, or (c) acid leaching of defatted soybean flakes. Flavor scores ranged from 6.1 for the acid-leach concentrate to 7.2 for the alcohol wash concentrate (Table 3). The scores for concentrates prepared by acid or water leaching were rated significantly lower than the wheat flour control (FS = 7.9). There were no significant differences between the score of the control and those of the two alcohol-washed concentrates. The acid-leached concentrate had a predominant grassy flavor and the water-leached sample was described as having cereal and toasted flavors, whereas both the wheat flour control and the alcohol-washed concentrate were only described as cereal-like. All samples had low FIV’s for bitter and astringent characteristics.

Soybean protein isolates, i.e., 90% protein, were also prepared from the same two flours by procedures described by Kalbrenner et al. (1971). The flavor evaluation of these two isolates is also shown in Table 3. Flavor scores were 7.0 and 6.1, respectively, for the samples extracted at 12,000 psi. Both isolates were rated as significantly lower in score than the wheat flour control (FS = 7.9) possibly because of the bitter taste in both isolates and the astringency in isolate #2. Neither of the isolates had any undesirable grassy flavors.

The flours, concentrates, and isolate (Table 3) were also evaluated for flavor after storage at 37°C for 2 months. No significant differences were observed between the flavor scores of the aged flours, concentrates or isolates and their corresponding control samples held at 2°C. The intensities of predominant flavors in the samples also did not change significantly after sample aging for 2 mo.

Flavor scores of the flours, concentrates and isolates listed in Table 3 compared favorably with scores of commercial soy products published by Warner et al. (1983). The authors reported flavor scores of commercial soy flours ranging from 5.5 to 6.3 whereas scores for optimally processed SC-CO$_2$ samples in this study were 7.3 and 6.6. The concentrates and isolates (Table 3) also received higher scores than the top-rated corresponding commercial products. Flavor intensity of descriptions such as cereal and toasted were low in SC-CO$_2$ processed samples because the heat treatment steps typically required for commercially prepared soy products were not necessary.

To examine the storage stability further, we prepared a partially defatted soybean flour (2.8% residual oil) by SC-CO$_2$ and compared the flavor of a portion of the sample stored at 2°C for 2 months against a portion stored at 37°C for the same time. No significant difference was noted in the flavor scores (6.9 and 6.7, respectively). The FIV’s on the sample were also nearly identical. This observation, and the fact that flours prepared by SC-CO$_2$ extraction had less lipoygenase activity than hexane-extracted flakes would suggest that the enzyme inactivation may protect the defatted soybean meal from developing undesirable flavors during storage.

In conclusion, it has been observed that full-fat soybean flakes can be extracted with supercritical carbon dioxide at pressures of 10,600-12,500 psi at various temperatures and moisture. By carefully controlling parameters, products with high protein solubility and excellent flavor scores and profiles can be produced. Flours, concentrates and isolates prepared from SC-CO$_2$-extracted flakes were also stable after 2 months storage tests at 37°C. The process should have potential in the food industry for the extraction of vegetable oils to yield proteins of superior flavor and physical qualities. The high NSI opens opportunities for high protein beverages and the bland

586—JOURNAL OF FOOD SCIENCE—Volume 51, No. 3, 1986
Table 3—Flavor scores and Flavor Intensity Values (FIV) of fresh and stored soybean products prepared by supercritical carbon dioxide extraction

<table>
<thead>
<tr>
<th>Samples</th>
<th>Flavor scoresa</th>
<th>Flavor intensity values¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cereal</td>
<td>Cooked bean</td>
</tr>
<tr>
<td>1 Soy flour*</td>
<td>7.3 (7.1)d</td>
<td>0.6 (0.6)</td>
</tr>
<tr>
<td>2 Soy flour*</td>
<td>6.6 (6.7)</td>
<td>0.9 (0.5)</td>
</tr>
<tr>
<td>3 Heat concentratef</td>
<td>6.8 (6.7)</td>
<td>0.6 (1.0)</td>
</tr>
<tr>
<td>4 Heat concentrateg</td>
<td>6.8</td>
<td>1.3</td>
</tr>
<tr>
<td>5 Alcohol concentratef</td>
<td>7.2 (7.8)</td>
<td>0.4 (0.7)</td>
</tr>
<tr>
<td>6 Alcohol concentranb</td>
<td>7.1</td>
<td>0.9</td>
</tr>
<tr>
<td>7 Acid concentratenf</td>
<td>6.1 (6.5)</td>
<td>0.3 (0.7)</td>
</tr>
<tr>
<td>8 Acid concentranb</td>
<td>6.2</td>
<td>0.3</td>
</tr>
<tr>
<td>9 Isolatef</td>
<td>7.0 (6.1)</td>
<td>0.4 (0.8)</td>
</tr>
<tr>
<td>10 Isolatef</td>
<td>6.1</td>
<td>0.7</td>
</tr>
</tbody>
</table>

¹ Flavor Intensity Value (FIV) 1 = weak; 2 = moderate; 3 = strong.

b Flavor Scores (FS) 1 = strong; 10 = bland; Least significant difference (LSD) = 0.7 (P>0.05).

c 12,000 psi; 82°C; 11.3% moisture, NSI = 58.

d Values in ( ) are for samples stored at 37°C for 2 months.

f 12,000 psi; 90°C; 13.5% moisture, NSI = 53.

The authors are indebted to J. Johnson, Lynn Black, and M. E. Hockridge for their assistance.

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

---

flavor should encourage the use of these storage stable products in enriched flours for soups, baked goods, etc.

REFERENCES


Ms received 8/24/84; revised 12/6/85; accepted 12/8/85.