Laboratory Evaluation of Hexane:Alcohol Azeotrope-Extracted Soybean Flakes as a Source for Bland Protein Isolates

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ABSTRACT

Most commercial edible soy products in the U.S. are prepared from hexane-extracted soybean flakes. The flavor of these materials prevents their unrestricted use in bland foods and beverages. Studies now indicate that defatted flakes can be improved in flavor by re-extraction with azeotropic mixtures of hexane:methanol, hexane:ethanol, or hexane:2-propanol. The nature and intensities of flavors of defatted flakes, azeotrope-extracted flakes, and protein isolates prepared from both types of flakes were evaluated by a taste panel. Samples were rated for odor and flavor. Flavor responses for laboratory-prepared defatted flakes, azeotrope-extracted flakes, and their isolated proteins were beany, bitter, and astringent; but the flavor intensities of the azeotrope-extracted products were lower than for hexane-defatted products.

Isolated soybean proteins are added to a variety of modern-day foods including ground meats, simulated meats, whipped toppings, infant foods, dried soups, and dietary items (1,2). Usage of isolates is estimated at 22 to 35 million lb. annually (2).

Dairy-type products, such as coffee whiteners, frozen desserts, and synthetic milks, represent a potential area for use of greatly expanded amounts of isolated soy protein (3). A nearly bland soy protein is desirable for this application (2), and this requirement has limited the use of isolated soy protein. Recent organoleptic evaluations of commercial soy flours, concentrates, and isolates confirm that these products are not bland; flavors characteristic of soybeans are still present (4). Use of an azeotropic mixture of hexane-ethanol for removing the residual oil and flavor components from soybean flakes has been reported (5,6). Here we describe results of testing hexane:alcohol (methanol, ethanol, 2-propanol) azeotrope-extracted soybean flakes as sources for bland protein isolates. The character and intensities of flavors of azeotrope-extracted flakes and isolates prepared from these flakes were evaluated organoleptically. Effects of hexane:alcohol extraction on protein solubility in the flakes and on yields of isolates were also studied.

MATERIALS AND METHODS

Preparation of Flakes

Certified, seed-grade soybeans (Amsoy and Hawkeye varieties) were air-dried to 8 to 10% moisture and then cracked between corrugated rolls 0.1 in. apart. The cracked cotyledons (meats) were collected after removal of hulls and fines in a...
Eureka seed cleaner. The meats were tempered 16 hr. at 4°C. with added water to bring the final moisture to 14%, flaked between smooth rolls 0.003 in. apart, and then extracted with pentane:hexane (b.p. 33° to 57°C.), at room temperature (6). After extraction of the oil, the flakes were air-dried to remove the solvent.

Flakes initially defatted with pentane:hexane were further extracted with either hexane:methanol (75:25 v./v., b.p. 51°C.), hexane:ethanol (82:18 v./v., b.p. 59°C.), or hexane:2-propanol (80:20 v./v., b.p. 63°C.) in a Soxhlet extractor. The solvent-to-flake ratio in the extractor was 5:1. The temperature in the Soxhlet thimble (60 X 180 mm.) was the boiling point of the azeotropic mixture. Extractions were conducted for 1, 3, or 6 hr. After air-drying for at least 48 hr., the flakes were ground in a Wiley mill equipped with a 40-mesh screen. The finely powdered flours were than evaluated organoleptically.

Preparation of Isolates

Protein isolates were prepared from the defatted flakes by extracting twice with water, first at a solvent-to-meal ratio of 10:1 and a second time at 5:1. After centrifugation of the two extracts, the supernatant solutions were combined and adjusted to pH 4.5 with hydrochloric acid; the precipitated curd was centrifuged, washed three times with 5 vol. of water, dispersed in water, adjusted to pH 7.0 to 7.2 with NaOH, and freeze-dried.

Analytical Procedures

For ultracentrifuge analysis, pentane:hexane defatted flakes, as well as hexane:methanol-, hexane:ethanol-, and hexane:2-propanol-extracted flakes, were stirred at 25°C. with water at a solvent-to-flake ratio of 10:1. After centrifugation (15 min. at 11,900 X g), the extracts were dialyzed against potassium phosphate-sodium chloride buffer, pH 7.6, 0.5 ionic strength, containing 0.01M 2-mercaptoethanol (7) at 1°C. for 48 hr. The dialyzed extracts were centrifuged (10 min. at 11,900 X g) at 25°C. and then diluted 1:2 with the dialysis buffer.

Ultracentrifuge analyses of the water-extractable proteins were performed at
Fig. 2. Effect of extraction time on flavor scores of soybean flakes extracted with hexane:alcohol azeotropes. Organoleptic evaluations were made on 2% water dispersions.

room temperature in a Spinco Model E ultracentrifuge with a 30-mm. cell and a plastic double-sector center piece at 47,660 r.p.m. Ultracentrifuge pattern areas were corrected for radial dilution, and the compositions expressed as percentages of the total area.

Organoleptic evaluations were conducted on 2% dispersions in charcoal-filtered tap water or fresh whole milk as described by Kalbrener et al. (4). Samples were evaluated at room temperature. Flavors and odors were described and scored for intensity on a 10-point scale where 1 is strong and 10 is bland (4).

Nitrogen solubility index (NSI) of the solvent-extracted flakes was determined according to official procedures (8).

RESULTS AND DISCUSSION

Extraction and Flavor of Soybean Flakes

The results obtained with soy flakes extracted with hexane:methanol, hexane:ethanol, and hexane:2-propanol are plotted in Figs. 1 and 2. The amount of material removed from previously defatted soybean flakes by the azeotropes increases with extraction time. Hexane:methanol removed 7.5% of the starting material after 6 hr., whereas hexane:ethanol or hexane:2-propanol removed only 2.0 to 2.6% in the same time. Variation in the amount of material extracted by the three azeotrope solvents probably reflects solubility differences of the low-molecular-weight materials present in defatted soybean flakes.

Shown in Fig. 2 are the flavor scores of the azeotrope-extracted flakes after grinding and dispersal in charcoal-filtered tap water. Flavor scores of pentane:hexane-defatted flakes were initially 4.2 to 4.3, but increased after a 1-hr. extraction with hexane:alcohol azeotropes, and remained nearly constant during prolonged extraction periods. The hexane:ethanol azeotrope-extracted flakes gave statistically significant higher flavor scores (7.0 to 7.2) than flakes prepared with hexane:methanol (flavor scores 6.1 to 6.2) or hexane:2-propanol (flavor scores 5.0 to 5.4).

Flavor responses noted by the panelists were beany, bitter, astringent, cereal,
and cardboardy. However, the flavor intensities of the azeotrope-extracted products were lower than for pentane:hexane-defatted flakes.

**Flavor of Protein Isolates**

The sodium proteinates of acid-precipitated protein from pentane:hexane defatted flakes and azeotrope-extracted flakes were presented to the taste panel as 2% aqueous dispersions and as 2% milk dispersions. Organoleptic evaluations of the proteinates as water dispersions are shown in Fig. 3.

Proteins isolated from flakes unextracted with the azeotropic solvents scored 5.0 to 5.2, about one unit higher than the corresponding flakes (Fig. 2). Flavor scores of the sodium proteinates from azeotrope-extracted flakes had nearly identical flavor scores. All azeotrope-extracted samples had flavor scores of about 7 after a 3-hr. extraction period. Odor responses given by the panel were beany, while flavor responses were beany, bitter, and astringent.

When the sodium proteinates from the azeotrope-extracted flakes were evaluated as 2% dispersions in milk instead of water, there were differences between the samples (Fig. 4). Extracting for 3 or 6 hr. with hexane:ethanol gave an isolated protein significantly better than the protein from hexane:methanol-treated flakes. Isolated protein from hexane:2-propanol-extracted flakes gave flavor scores intermediate of the values for the other two isolates. Odor responses for all azeotrope-extracted products in milk were milk and slightly beany. Flavor responses by the panelists for the hexane:methanol- and hexane:2-propanol-extracted protein isolates were beany and milk. However, the hexane:ethanol-extracted product was described by the taste panel as having only a milk-like, salty taste; no beany flavor was reported.

**Effect of Extraction on Physical Properties**

Since azeotrope-extracted products were significantly better flavored than materials prepared from flakes defatted with pentane:hexane when tasted in water or milk, more detailed studies were conducted. Effects of azeotropic extraction on NSI and yields of protein isolates were determined (Figs. 5 and 6). Extraction with

![Graph showing effect of extraction time on flavor scores of sodium proteinates from hexane:alcohol-treated soybean flakes. Organoleptic evaluations were made on 2% aqueous dispersions.](image-url)
Fig. 4. Effect of extraction time on flavor scores of sodium proteinates from hexane:alcohol-treated soybean flakes. Organoleptic evaluations were made on 2% dispersions in milk.

Fig. 5. Effect of extraction time on the NSI of soybean flakes extracted with hexane:alcohol azeotropes.

Fig. 6. Effect of extraction time on protein yields from soybean flakes extracted with hexane:alcohol azeotropes.
slightly greater decrease in NSI values occurred with hexane:ethanol and the values decreased greatly when hexane:methanol was used for the extraction. The NSI values for flakes treated with hexane:methanol dropped to 25-30, depending on the time of extraction. These decreases in NSI values as a result of azeotropic extraction were also reflected in lowered yields of protein isolates (Fig. 6). With hexane:2-propanol, yields decreased from 41-43 g. per 100 g. flakes to about 38 g. When hexane:ethanol was used, about 28% of the starting material was obtained as an isolate; but with hexane:methanol-treated flakes, yields of isolate were only about 10% of the starting flakes.

To study further the effects of azeotrope extraction on the flakes, the water-extractable proteins from hexane:alcohol azeotrope- and from pentane:hexane-extracted flakes were analyzed in the ultracentrifuge (Fig. 7 and Table I).

Extraction with hexane:methanol had the most pronounced effect. The quantity of protein extracted from flakes treated with hexane:methanol was much lower than from flakes treated with hexane:ethanol or hexane:2-propanol; a considerable decrease in solubility of 7S and 11S components was noted when hexane:methanol was the extracting solvent. There was little difference between

![Fig. 7. Ultracentrifugal patterns for water-extractable proteins from soybean flakes extracted with pentane:hexane, hexane:methanol, hexane:ethanol, and hexane:2-propanol. Numbers above patterns are approximate sedimentation coefficients in Svedberg units. Differences in areas reflect differences in extractability of the proteins.](image)

<table>
<thead>
<tr>
<th>Extraction Solvent</th>
<th>2S Area cm²</th>
<th>2S %</th>
<th>7S Area cm²</th>
<th>7S %</th>
<th>11S Area cm²</th>
<th>11S %</th>
<th>15S Area cm²</th>
<th>15S %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentane:hexane</td>
<td>3.20</td>
<td>17</td>
<td>7.03</td>
<td>36</td>
<td>7.85</td>
<td>41</td>
<td>1.31</td>
<td>7</td>
</tr>
<tr>
<td>Hexane:methanol</td>
<td>2.72</td>
<td>34</td>
<td>1.53</td>
<td>19</td>
<td>2.90</td>
<td>37</td>
<td>0.74</td>
<td>9</td>
</tr>
<tr>
<td>Hexane:ethanol</td>
<td>2.81</td>
<td>14</td>
<td>7.32</td>
<td>37</td>
<td>8.21</td>
<td>42</td>
<td>1.33</td>
<td>7</td>
</tr>
<tr>
<td>Hexane:2-propanol</td>
<td>3.56</td>
<td>17</td>
<td>6.92</td>
<td>33</td>
<td>8.54</td>
<td>41</td>
<td>2.03</td>
<td>10</td>
</tr>
</tbody>
</table>

*aActual measured area, which includes a tenfold magnification factor.*
the water-extractable proteins from the hexane-defatted flakes and the other two azeotrope-treated flakes. This uniformity indicates that neither hexane:ethanol nor hexane:2-propanol markedly denature the proteins.

**CONCLUSIONS**

This study confirms previous research (5,6) on the quantity of materials removed on re-extraction by hexane:alcohol azeotropes after soybean flakes have been extracted with pentane:hexane. About 3% of material is obtained with hexane:ethanol or hexane:2-propanol and more than twice as much (7%) is soluble in hexane:methanol.

Although hexane:alcohol-azeotrope mixtures remove flavor components from defatted soybean flakes, complete removal of the undesirable flavors is not achieved with these solvents. Extraction with hexane:ethanol or hexane:methanol yields flakes with less flavor intensity than hexane:2-propanol extraction. However, isolates from all three azeotrope-extracted flakes are similar in flavor intensity when tasted in water. The hexane:ethanol-treated product is significantly less flavored than the isolate from hexane:methanol-extracted flakes when both are tasted in milk.

Examination of flavor intensities (Fig. 2) shows that hexane:ethanol azeotrope is probably a better solvent than the hexane:methanol or hexane:2-propanol azeotropes for extracting soybean flakes to produce edible soybean flours. For the production of a protein isolate where NSI, protein yields, and flavor are important, either hexane:ethanol or hexane:2-propanol should give comparable products.

**Literature Cited**


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