ULTRASONIC EXTRACTION OF PROTEINS FROM AUTOCLAVED SOYBEAN FLAKES

INTRODUCTION

WITH MORE and more soybean protein going into human food, efficient extraction from soybean flakes assumes more and more importance. Commercial extraction yields 30% or less of the weight of flakes, and ideal laboratory extraction yields 42% of defatted flakes (Smith, 1958). For maximum yield of proteins, the flakes are unheated; but for maximum nutritive quality, the flakes require a moist heat treatment (Circle and Smith, 1972). Toasting causes protein denaturation which means low yields, diminished functionalities and limited applications.

Factors that affect the dispersion of soybean proteins in water include temperature and time of extraction, meal age, particle size, solvent-to-meal ratio, pH, salt type and concentration and rate of stirring (Circle, 1950). High-speed stirring has been reported to grind the sample during extraction (Paulsen et al., 1960). Other factors affecting protein solubility have been reviewed (Circle, 1950; Wolf, 1972).

Although sonication has been applied in the past to disintegrate and solubilize animal and plant tissue components (El’Piner, 1964; Ensminger, 1973), a search of literature indicates that it has not been applied to improve the extraction of proteins in soybean flakes. This paper reports the comparison between ultrasonic extraction on a laboratory scale with conventional-stir extraction upon heated and unheated soybean flakes.

MATERIALS & METHODS

Preparation of flakes

Whole soybeans from a 1971 crop of Kanrich variety were cracked, dehulled and flaked. The flakes were defatted by extraction with a hexane-pentane mixture on a laboratory scale. The defatted flakes, which contained 46.4% protein based on 7.41% total nitrogen (air-dry basis, moisture content 10.7%) were stored in a refrigerator at 4°C before use. A portion of flakes was autoclaved at 120°C for 20 min and stored at room temperature. The respective nitrogen solubility indexes (NSI) were 25 and 91 for autoclaved and unautoclaved flakes (AOCS, 1970).

Extraction of proteins

Sonication. To 5g of flakes in a 2-oz glass jar were added 50 ml distilled water. The jar was chilled in an ice slurry, and the mixture was sonicated usually for 8 min (except in one experiment for a varied length of time) with a Sonifer Model S125 manufactured by Heat Systems-Ultrasonics, Inc., Plainview, N.Y. The sonifer contains a standard mechanical transformer with a step horn and a power unit with a meter measuring input power. The step horn was dipped about 1 in. deep into solution, and the sonication was tuned to a maximum input power at which point the instrument operates at a frequency of 20 kHz and delivers an output power of 125 watts. After sonication, the mixture was centrifuged at 10,000 x G for 15 min, and the supernatant was poured through a thin layer of glass wool into a cylinder. The volume of the supernatant was recorded, and a portion was taken to measure the amount of proteins by the biuret method (Layne, 1957). The amount of solubled protein was calculated on the basis of 50 ml solvent used. Averages of duplicated protein determinations are reported.

Conventional stir. In 50 ml of distilled water, 5g of sample were stirred mechanically at 1200 rpm (three 1/2-in. bladed type) at room temperature for 1 hr. The sample was treated the same way as in the sonication-extraction regarding centrifugation, filtration and measurement of protein. This treatment was used because of its convenience. In one experiment the amount of protein extracted in three consecutive stirrings was compared with sonication-extractions.

Protein fractions

Cold-insoluble fraction (CIF). A portion of water extract from 5g of flakes was chilled overnight in a refrigerator (4°C) and then centrifuged at more than 10,000 × G at 4°C to collect the proteins (Wolf and Sly, 1967). The proteins were dissolved in 0.03M phosphate buffer -0.04M NaCl (pH 7.6, µ = 0.5) for further analyses.

Acid-precipitated fraction (APF). A portion of water extract was dialyzed with 1N HCl to pH 4.2 and then centrifuged at 34,000 × G for 10 min to collect the proteins. The proteins were treated the same way as described for CIF.

Centrifugation

Proteins in CIF, APF and water extracts were dialyzed with two successive 3-liter batches of distilled water at 4°C for 48 hr. After dialysis the proteins were equilibrated with the same phosphate buffer as described above for 4 hr before adjusting the protein concentration to 7 mg per ml. The protein samples were centrifuged at room temperature with a Spinco Model E centrifuge at 47,600 rpm and their schlieren patterns were examined qualitatively for their protein components.

RESULTS & DISCUSSION

Extraction with sonication and conventional-stir

Experiments to compare sonication and the conventional-stir...
stir method included those with (a) three consecutive extractions of proteins with water and (b) single extraction with water or 1N NaOH as solvents.

(a) In three consecutive extractions 70% total protein in unautoclaved flakes was solubilized in water by the conventional-stir method, and 90% by sonication-extraction (Fig. 1). When autoclaved flakes were used, on the one hand, conventional-stir extracted only 32% protein. Sonication, on the other hand, solubilized 69% of the total protein. Proteins in the autoclaved flakes were denatured, and obviously conventional-stir extraction was ineffective in dispersing the proteins in water. By comparison, sonication effectively dispersed more of the denatured proteins in autoclaved flakes in water.

(b) In a single extraction, results with water were compared to that with 1N NaOH (Table 1). When water was used in a single extraction, sonication dispersed 58% of the proteins in autoclaved flakes, whereas conventional stirring extracted only 16% of the proteins.

The NaOH solvent extracted 73% and 91% proteins, respectively, from autoclaved and unautoclaved flakes with conventional-stir, whereas sonication with NaOH solvent recovered 99% and 95%, respectively. Although water was less efficient in protein extraction than the NaOH solvent, sonication extracted more proteins in either solvent whether a single extraction or three consecutive extractions.

Dispersion of denatured proteins

The amounts of protein in autoclaved flakes dispersed in water by sonication are affected by extraction time and meal-to-solvent ratios (Fig. 2). With a meal-to-solvent ratio of 1:10 the amount of protein extracted with sonication reached a maximum of 48% at 6 min and then leveled off. When the ratio was varied to 1:40, a new maximum of 78% was reached at 10 min. Further increase of the solvent or length of time did not improve the percentage significantly. Other factors, such as pH of solvent, salt type and concentration and extraction temperature, were not tested extensively; nevertheless, data indicate that an irreversible loss of protein in autoclaved flakes is about 20% of the total and that sonication extracted nearly 80% of the total proteins in autoclaved flakes.

Yield of protein fractions

To compare the qualitative differences in proteins dispersed by sonication and conventional-stir, the yields of protein fractions in CIF, APF and water extract were measured (Table 2). Sonication improved the yields of protein in CIF, APF and water extract from autoclaved or unautoclaved flakes, compared to the respective categories by conventional-stir. With sonication the yield (179 mg) of CIF from autoclaved flakes was significantly lower than that from unautoclaved (259 mg). The differences in APF and water extract were not so great as in CIF. By conventional-stir extraction, all three categories were lower from autoclaved flakes. Regardless of which method of extraction was used, the loss of CIF in autoclaved flakes was more than 60% (61% by conventional-stir, 69% by sonication).

The similarities of components in these three categories of proteins (CIF, APF and water extract) are seen in Figure 3. There are no qualitative differences in their components between the methods used. The quantitative yields of individual components in these patterns were not determined.

Sonication represents a new way to isolate soybean proteins. It not only extracts more proteins from flakes, but more significantly, it does so from autoclaved flakes analogous to ones produced commercially (Fig. 1). Up to now, extraction of soybean proteins has been mostly done by conventional-stir (Circle and Smith, 1972). Results from that method are good on unautoclaved flakes, but poor on autoclaved flakes. If sonication is applied on autoclaved flakes, the efficiency of protein extraction can be improved.

Soybeans used in this study are of Kanrich "vegetable" variety which are larger in size than field varieties. Soybeans for industrial use are of field varieties. Although the varietal difference has not been studied, the efficiency of protein extraction by sonication as affected by other factors—variety of beans, size of flakes, extraction time, temperature, salt, pH, etc.—will be investigated later.

Sonication may also provide a partial solution to the flavor.

Table 1—Comparison of soy protein extracted by sonication and conventional-stir with water and with 1N NaOH as solvents

<table>
<thead>
<tr>
<th>Defatted flakes (5g)</th>
<th>Sonication</th>
<th>Conventional-stir</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sonication</td>
<td>Water</td>
</tr>
<tr>
<td>Autoclaved</td>
<td>1350 (58%)</td>
<td>2290 (99%)</td>
</tr>
<tr>
<td>Unautoclaved</td>
<td>2050 (88%)</td>
<td>2200 (95%)</td>
</tr>
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</table>

Table 2—Yield of soybean protein in cold-insoluble fractions, acid-precipitated fractions and water extracts

<table>
<thead>
<tr>
<th>Defatted flakes (5g)</th>
<th>Yield of protein (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cold insoluble</td>
</tr>
<tr>
<td>Unautoclaved, conventional-stir</td>
<td>155</td>
</tr>
<tr>
<td>Autoclaved, conventional-stir</td>
<td>92</td>
</tr>
<tr>
<td>Unautoclaved, sonication</td>
<td>250</td>
</tr>
<tr>
<td>Autoclaved, sonication</td>
<td>179</td>
</tr>
</tbody>
</table>

Fig. 2—Percentages of total soybean proteins from 5g autoclaved Kanrich defatted flakes in a single extraction with water at varied time and meal-to-solvent ratios.
Ultrasound Extraction of Soy Proteins

Schlieren Patterns of Soybean Protein Fractions

<table>
<thead>
<tr>
<th>Defatted Flakes</th>
<th>Cold Insoluble</th>
<th>Acid Precipitated</th>
<th>Water Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unautoclaved, conventional stir</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
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<tr>
<td>Autoclaved, sonicated</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>Unautoclaved, sonicated</td>
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<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Fig. 3—Ultracentrifuge patterns (48 min after starting centrifugation) of soybean protein in cold-insoluble fractions, acid-precipitated fractions and water extracts from autoclaved and unautoclaved Kanrich defatted flakes extracted by sonication and conventional-stir.

problem of soybean proteins. Since autoclaving increases the nutritional value of proteins and also removes undesirable flavors in soybeans, proteins solubilized by sonication should have improved flavor and good functionality. These proteins are similar to those extracted by conventional-stir in some of their chemical and physical properties.

REFERENCES


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The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Dept. of Agriculture over other firms or similar products not mentioned. Presented at the 34th Annual Meeting of the Institute of Food Technologists, New Orleans, La., May 12—15, 1974.