Soybean protein flavor components: a review

J.C. COWAN, J.J. RACKIS and W.J. WOLF, Northern Regional Research Laboratory,2 Peoria, Illinois 61604

This review on the flavor components of soybean protein products examines primarily our studies in sensory evaluation of commercial flours, concentrates and isolates; on extraction of flavor components from soybean flakes with hexane-alcohol azeotropic mixtures; on the application of proteolytic enzymes to improve flavor; and on the effect of inactivating lipoxygenase on soy beverage flavor. Evidence indicates that enzymatic reactions affect the flavor of final products. Presumably lipoxygenase is a primary culprit and linolenic acid a primary precursor when soybeans are partially processed before destroying enzymatic activity.

The use of soybean protein food products has increased considerably during the last 10 years. Eley (1) estimated that 244-275 million pounds of soybean protein products were utilized in foods in 1967; Wilding (2), Wolf and Cowan (3) and Burket (4) reported extensions of these estimates (Table I). Although actual data are hard to obtain, new plants for protein products to food manufacturers. The four major types, other than forms of the isolate, vary in fat, carbohydrate and protein content, as well as in macrostructure of the soybean is completely destroyed in all these protein procedures used did raise the thresholds for sample detection and for sample contained something besides soy from our taste panel to record values generally in the range of 6 to 7. Data on flavor thresholds of a raw flour, concentrate and two isolates are given in Table IV.

Data in Tables III and IV might lead some to believe that processing besides steaming did not improve flavor. This assumption is not true. Note...
from Table IV that raw defatted flour at 0.005% or 50 ppm can be detected by the taste panel, and the concentrates and isolates at 400 to 600 ppm. At 300-400 ppm as a slurry, raw flour tastes beany and bitter; the concentrate tastes beany and bitter at 2000 ppm. One isolate is beany at 12,500 ppm but bitter at 30,000 ppm. These data emphasize that some of the flavor factors can either be removed or reduced in intensity, or both. Although the flour used in those comparisons was a raw flour, toasting usually improved its flavor to a higher score. The flavor scores of toasted flour are generally, but not always, the same as the scores for concentrates and isolates. These data also serve to emphasize a point that we frequently make to manufacturers of foods and soy food products: There is flavor left that is difficult to remove. But with the many flavor additives available, the food manufacturer can often mask or retard any recognition of soy flavor. Thus it is possible in a number of products now to use the available soy flours, soy concentrates and isolates in varying amounts.

Even though toasting or cooking of soybeans is known to improve their flavor substantially, industry is seeking means of achieving better flavor. Research at NRRL seeks the way and means without any substantial loss in functionality.

Before we review some of the practical approaches to improving the flavor, identifying flavor components and their potential precursors appears desirable. Identification of Flavor Components

Demonstration that measurable amounts of lipid material remain in protein isolated from hexane-extracted soybean flakes suggests that oxidation of lipid components may be a factor in the flavor of soybean protein products (7). Removal of much of the flavor and lipid material by extraction of dehulled, defatted soybean flakes with alcohols supports this suggestion (8).

Early studies on removal of lipid materials and flavors from defatted flakes with ethanol gave us the opportunity to investigate the materials extracted by this solvent. Teeter and coworkers (9) fractionated the “alcoholic extract” in an attempt to isolate beany and bitter fractions. When the concentrated extract was repeatedly fractionated by dissolving in acetone and precipitating, a bitter concentrate was obtained. It appeared, however, to result from the interaction of carbohydrates present. At least part of the bitter material isolated resulted from a browning reaction that occurred after extraction with alcohol. The alcoholic extract was steam-distilled and beany volatiles were collected. The beany distillate contained ketones, carboxylic acids, esters, phenols and some other neutral compounds not further identified. Acetone, methyl n-propyl ketone and an 11 carbon ketone were found along with free acids from 3 to 9 carbon atoms with caproic present in the largest amounts but no 4 carbon acid. Also esters of acids from 6 to 10 carbon atoms were found with the acids containing 8 and 9 carbon atoms predominant. Steam distillation appeared to hydrolyze esters of acids containing 6 to 8 carbon atoms and the esters may exist as the components in the original flakes with little free acid present. Also they could have been formed from alcohols and acid present in the extract since it did stand for a long time prior to fractionation.

### TABLE I

<table>
<thead>
<tr>
<th>Product</th>
<th>Millions of lb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour and grits (total)</td>
<td>232-237 a</td>
</tr>
<tr>
<td>Bakery</td>
<td>60</td>
</tr>
<tr>
<td>Meat products</td>
<td>38</td>
</tr>
<tr>
<td>Beverages</td>
<td>12</td>
</tr>
<tr>
<td>Dey cereals and baby foods</td>
<td>8</td>
</tr>
<tr>
<td>Brewers' flakes</td>
<td>4</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>8-13</td>
</tr>
<tr>
<td>Exports</td>
<td>12</td>
</tr>
<tr>
<td>U.S. government (CSM and other foods)</td>
<td>81 b</td>
</tr>
<tr>
<td>Concentrates</td>
<td>20-35</td>
</tr>
<tr>
<td>Isolates</td>
<td>25-40</td>
</tr>
</tbody>
</table>

aProduction in 1970 reported as 325-500 but includes dog food, a relatively large outlet (4).

bCorn-soy-milk (CSM) and other blends projected at 97 and 187 million lb for 1971 and 1972 (4).

### TABLE II

Effect of Steam on Flavor Score Given by Taste Panel for Different Soy Flours

<table>
<thead>
<tr>
<th>Flour sample</th>
<th>Treatment</th>
<th>Flavor score</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full fat</td>
<td>No steaming</td>
<td>1.5</td>
<td>Raw flour, beany, bitter, toasted</td>
</tr>
<tr>
<td>Full fat</td>
<td>3 min steaming b</td>
<td>4.5</td>
<td>Beany, bitter, nutty, sweet, toasted</td>
</tr>
<tr>
<td>Full fat</td>
<td>10 min steaming</td>
<td>6.0</td>
<td>Beany, nutty, bitter</td>
</tr>
<tr>
<td>Full fat</td>
<td>20 min steaming</td>
<td>6.3</td>
<td>Beany, nutty, bitter</td>
</tr>
<tr>
<td>Full fat</td>
<td>40 min steaming</td>
<td>6.1</td>
<td>Beany, nutty, bitter</td>
</tr>
<tr>
<td>Hexane defatted</td>
<td>Washed with dilute acid at pH 4.6</td>
<td>3.6</td>
<td>—</td>
</tr>
<tr>
<td>Hexane defatted</td>
<td>80% methanol extraction</td>
<td>6.3</td>
<td>—</td>
</tr>
<tr>
<td>Hexane defatted</td>
<td>80% ethanol extraction</td>
<td>7.3</td>
<td>—</td>
</tr>
<tr>
<td>Hexane defatted</td>
<td>80% ethanol extraction plus 20 min steaming</td>
<td>8.0</td>
<td>Highest score</td>
</tr>
</tbody>
</table>

aScoring 1-10 where 10 is bland and 1 is a strong flavor or odor.
bLive steam treatment in preheated autoclave at atmospheric pressure.

### TABLE III

Odor and Flavor of Some Commercial Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Odor</th>
<th>Flavor</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6.8 b</td>
<td>NP c</td>
</tr>
<tr>
<td>C</td>
<td>7.0 b</td>
<td>Beany</td>
</tr>
<tr>
<td>F</td>
<td>7.4 b</td>
<td>Toasted</td>
</tr>
<tr>
<td>Concentrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>6.4 a</td>
<td>CW c</td>
</tr>
<tr>
<td>C</td>
<td>6.9 ab</td>
<td>NP</td>
</tr>
<tr>
<td>E</td>
<td>7.4 b</td>
<td>NF</td>
</tr>
<tr>
<td>Isolates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>7.7 a</td>
<td>Musty, corn meal</td>
</tr>
<tr>
<td>F</td>
<td>7.4 a</td>
<td>Flour</td>
</tr>
</tbody>
</table>

aSee Table II for scoring
bWithin each group, i.e., flours, concentrates or isolates, scores with letters in common are not significantly different at the 0.05% level.
cNP, none predominant; CW, odor similar to oat cereal-singed wool in water.
Later Rackis and coworkers (10,11) investigated lipid oxidation during preparation of raw and toasted full-fat and defatted flakes. They identified four products and developed strong evidence for a fifth in raw full-fat products. Arai et al. (12) at our laboratory, and was established work by C.D. Evans shows that these were not the products responsible for the bitter and beany responses of soy flour. Unpublished work by C.D. Evans shows that gas chromatograms of volatiles from defatted soy flour. Among this series we find papers on: aliphatic carbonyl compounds (13), phenolic acids (14), volatile fatty acids and volatile amines (15), and volatile neutral compounds (16) and alcohols (17).

The volatile carbonyl compounds (13) were isolated by passing carbon dioxide through an aqueous suspension of 2 kg defatted soybean meal in 10 liters water. Volatile carbonyl compounds separated by this procedure are listed in Table V.

Soybeans contain a number of phenolic acids that could be directly related to flavor, odor and other effects—such as throat-catching—known to be associated with soybean protein products. Arai et al. (14) carried out an extensive fractionation of defatted soybean flour. Their work indicates that there are at least seven phenolic acids in soybeans that can be extracted with ethanol and separated from the concentrated extract into ether- and water-soluble fractions. In the ether-soluble fraction, they found ferulic and coumaric acids, derivatives of cinnamic acid: coumaric acid is p-hydroxycinnamic acid and ferulic acid is m-methoxy-p-hydroxycinnamic acid. The other five in the ether-soluble fraction were all hydroxybenzoic acids: syringic, vanillic, gentisic, salicylic and p-hydroxybenzoic. The water-soluble fraction contained chlorogenic, isochlorogenic, neochlorogenic and probably cryptochlorogenic acids. They are caffeic acid derivatives of quinic acid. A report by Corse and associates (18) indicates that some chlorogenic acids do survive the roasting of coffee, but some are destroyed. Although a much higher temperature is involved in roasting (ca. 200-250 C) than in toasting (100-122 C), decomposition does occur. Van der Meer and Spaans (19) indicate that hydroxycinnamic acid derivatives in soybean flour may be important factors in its odor when this product is cooked. They autoclaved a dough of soy flour. The dough was then steamed and the volatiles were collected. Among the compounds isolated, 4-vinyl phenol and 4-vinyl guaicol were identified. Their source was found to be p-coumaric and ferulic acids, respectively. Attempts to reproduce the cooked soy odor were made by mixing these two vinyl phenols with diacetyl, maltol, hexanal and other compounds. The Dutchmen were unable to reproduce fully the cooked soybean odor. They did conclude that 4-vinyl phenol and 4-vinyl guaicol were integral parts, but that other compounds must be involved.

The Japanese workers (15) extracted raw meal with methylene dichloride and prepared the odor components by the following steps: extraction of solvent layer with dilute alkali, concentration of ether-soluble fraction into ether- and water-soluble fractions. In the ether-soluble fraction, they found ferulic and coumaric acids, derivatives of cinnamic acid: coumaric acid is p-hydroxycinnamic acid and ferulic acid is m-methoxy-p-hydroxycinnamic acid. The other five in the ether-soluble fraction were all hydroxybenzoic acids: syringic, vanillic, gentisic, salicylic and p-hydroxybenzoic. The water-soluble fraction contained chlorogenic, isochlorogenic, neochlorogenic and probably cryptochlorogenic acids. They are caffeic acid derivatives of quinic acid.
neutralization with phosphoric acid to pH 8, evaporation of aqueous solution to dryness and conversion of sodium salts to free fatty acids. They were dissolved in ether and GLC was used to identify the acids present. Acids from acetic through n-caproic were identified. The workers characterized isocaproic, n-caproic and n-caprylic acids as green bean-like. Like Teeter and coworkers (9), the Japanese found that the largest amount was n-caproic. The Japanese did not assign any practical effect on soybean flavor to the quantities of isocaproic, caproic and caprylic acids present.

The volatile amines were separated from raw meals by the following steps: Raw ground soybeans were extracted in 10% trichloroacetic acid, and the resulting solution was adjusted to pH 5 by sodium hydroxide (15). After filtration, concentration and adjustment of pH to 8, the extract was steam distilled. Volatile amines were trapped in hydrochloric acid and analyzed by paper and gas chromatography. Evidence was found for ammonia, methylvamine, dimethylamine, piperidine and cadaverine. Dimethylamine was believed to contribute to a fishy flavor associated with soy flour products. The amount of volatile amine was ca. 1.5 ppm, with dimethylamine being about one-third of the total.

In another approach, ground raw soybeans were packed in a glass cylinder, warmed to 50°C, swept with nitrogen gas, and the volatiles trapped. A 5 hr UV irradiation did lead to an intense bitter-biting-choking effect, and the thiobarbituric acid number was substantially increased from an original value of 5.5 to 75.9. The oxidized products of the lipid fractions of defatted or full-fat flour appear to be associated with at least a portion of the flavor of some of these flours (16).

Honig and coworkers (21) isolated ethyl-β-D-galactopyranoside as a bitter component of hexane-ethanol-extracted soy flake solutions. Evidence shows that this compound is probably formed during extraction. Although bitter, this compound is present at levels below its threshold value for bitterness. While present at 0.2% in full-fat flakes and 0.03% in defatted flakes when both were treated with hexane-ethanol, in an aqueous disp. it requires ca. 5000 ppm or 0.5% to give a bitter response. It is not likely to be much of a factor for bitterness, even in alcohol-extracted flour. Pinotol, also found in this fraction, was sweet.

Another addition to the compounds known to be present in soy protein products is ethyl vinyl ketone, reported by Mustakas and coworkers (31). They isolated this ketone from Clark soybeans and identified it by GC and mass spectrometry. The beans were ground and treated in ways similar to the preparation of soy milk. Indeed, the authors believe this compound is an important factor in the green bean-like flavor of both raw soybeans and soy milk from raw uncooked soybeans. At the same research laboratory, Badenhop and Wilkens (23) showed that 1-octen-3-ol formed during soaking of the beans. It causes a mushroom flavor in dairy products (24). In soy milk it was detected when added at levels 0.5 to 1.0 ppm, and was formed in greatest amounts in milks made at pH 6-7.

Workers (25) at Peoria have shown that linoleic and linolenic acids may be implicated as the source of flavors. Hydroperoxides of linoleic and linolenic acid were prepared with the use of soybean lipoxygenase. After purification by silicic acid chromatography, these hydroperoxides or their decomposition products were characterized by a taste panel as grassy-beany, musty-stale and bitter for linoleic hydroperoxide at 50 ppm, and grassy-beany, bitter and astringent for linolenic hydroperoxide at 10 ppm. Although these flavors are similar to what the taste panel reports for flour, concentrate and isolate, other materials may be involved. The work does show that these acids must be considered as a primary source of off-flavors. Any procedure that deactivates lipoxygenase readily without denaturing the protein should be given careful commercial consideration.

Preventing Formation of Flavor

The simplest procedure inactivates enzymes before the hull of the soybean is cracked. Historically, both the Chinese and Japanese have soaked beans, cooked them and used them in a variety of ways (26,27). This procedure denatures the protein so that more of its functionality is lost, and if the oil is extracted the fine flavor remaining is difficult to handle. The desirability of steaming (28), cooking (29) or treating whole beans with dilute acid (30) was early recognized in patents issued during the 1920's and 1930's. Since then, several research groups have suggested modification of these procedures that should be considered in making soybean protein products.

Mustakas and coworkers (31) of our Northern Regional Research Laboratory adopted the procedure of extrusion cooking immediately after cracking and dehulling beans. Since the process is continuous, the enzymes have only a short period to act. Improvement in flavor is reported over standard procedures for making full-fat flour when the beans are steamed. After tempering, cracking and flaking. In other work at Peoria, a simple method for making full-fat soy flour (32) was devised, which included water soaking and immersion cooking. Washed beans are placed in a cloth sack, soaked overnight, cooked in boiling water for 10 min, air-dried, cracked, dehulled and ground to a flour. In related work (33) it was shown that ca. 30 min of steaming was required to inactivate urease in whole beans containing 20-68% moisture, whereas 60 min steaming was required for beans with 8% moisture. Only 5 min of immersion in boiling water was required to destroy urease in beans with 62-65% moisture, whereas ca. 30 min was required if they had 8% moisture. Nitrogen solubility indexes (NSI) and urease activities are contained in Table VII. After 5 and 10 min immersion in
boiling water the NSI was reduced to 35 and 13, respectively. Similarly, Mustakas et al. (34) showed that a number of different heat treatments of dehulled beans gave rapid deactivation of lipoxygenase and relatively higher flavor scores for the flours, but reduced protein solubility.

Cornell researchers (35) applied the technique of early denaturation of enzymes to the preparation of soy milk. By grinding soybeans in hot water, they were able to improve flavor over soy milk made by cooking after extraction of the beans. Superior flavor in the milk was achieved when beans were hydrated at 121°C for 15 min.

Kon and associates (36) at the Western Regional Research Laboratory extended the work of Cohn (30) to show that soybeans could be ground in acidic solutions of pH 1.0-3.85 and that the resulting slurry cooked on a steam bath gave a better flavored product than one made at a more neutral pH of 6.7. A research group at the University of Illinois suggested some modification of these procedures that involves blanching (37). Two approaches were recommended: (a) soaking beans for 4 hr and either cooking or blanching at 210°F for 10 min; and (b) blanching for 20 min before any further treatment.

Commercially, deactivating enzymes in soybeans before dehulling may cause some problems in handling. Also, as seen from the NSI data in Table VII, some of the most desired functional properties of soybean protein may be lost. Also, cracked beans might have to be separated before soaking, and any hard beans that do not absorb water fast might resist the heat treatment and contaminate some of the soybean protein product with lipoxygenase.

Procedures for Removing Flavor

Steaming (toasting) of defatted soybean flakes is the generally accepted commercial method for removing some of the flavor from soybean protein products. As shown in studies previously mentioned (6), although this treatment helps, more improvement is needed for use in many applications.

Earlier we mentioned some studies involving extraction with alcoholic solvents. Alcoholic treatment does partly inactivate enzymes in soybeans and does remove substantial amounts of lipids and flavor (5, 7, 8, 20). Alcohol suffers from some of the same disadvantages as cooking and steaming. Denaturation of at least some of the major protein occurs and functionality is partly lost. It reminds one of a once popular song that still is heard on occasion—"Love and marriage, love and marriage...You can't have one without the other"—and have a completely satisfactory product. In soybean processing it appears most difficult to achieve the marriage of excellent flavor and functionality. This difficulty might be resolved if a method of inactivating lipoxygenase and other enzymes could be found that did not insolubilize the major storage proteins; or conversely, if a practical method of solubilizing these proteins were found that was applicable to steamed or alcohol-treated soybean protein products.

Alcoholic extraction of soybeans was practiced in Manchuria in the 1930's (38) and studied extensively in the U.S. after World War II. As reported in Table II, the combination of ethanolic extraction and steaming gave as high flavor score as the NRRL taste panel has been able to report with a soy protein product. The hexane-alcohol azeotropes (hexane-methanol, ethanol, 2-propanol) have now been studied, and hexane-ethanol azeotrope gave the best flavored soybean flakes and derived sodium proteinates of the three azeotropes. Aqueous dispersions (2%) of the flakes and sodium proteinates both received

FIG. 1. Effect of extraction time on amount of material removed with hexane-alcohol azeotropes from defatted soybean flakes.

FIG. 2. Effect of extraction time on flavor scores of soybean flakes extracted with hexane-alcohol azeotropes. Organoleptic evaluations made on 2% water dispersions.
scores of ca. 7 after 1 hr extraction with hexane-ethanol (39). Figures 1 and 2 show the effect of extraction time on the amount of material removed with hexane-alcohol azeotropes and on the mean flavor score of the resulting flakes in 2% water dispersions, respectively. Use of alcohol for washing soy flakes to make a concentrate is practiced by one company (40).

In another series of papers, Fujimaki and coworkers found a ninhydrin-negative bitter peptide in the peptic hydrolyzate of soybean protein (41), evidence for the occurrence of protein-flavor binding (42) and enzymes capable of removing the bitter flavor from soybean protein products (43). Hydrolysis with Molsin, a fungal enzyme preparation, decreased the bitter flavor (44,45). The acid carboxypeptidase from \textit{Aspergillus saitoi} gave the lowest bitterness of several tested, apparently because its hydrolyzate had less bitter peptides (46); these workers believe the more bitter hydrolyzates were rich in peptides ending in leucine, i.e., a hydrophobic amino acid.

REFERENCES

35. Wilkens, W.F., L.R. Mattick and D.B.