Functional Properties of Edible Protein Concentrates from Alfalfa

Benny E. Knuckles* and George O. Kohler

Functional properties of an edible spray-dried alfalfa protein concentrate were evaluated. The nitrogen solubility of this product was greater than that of the soy protein isolate. The nitrogen solubility and gelation were adversly affected by a high spray dryer outlet temperature (140 °C). The alfalfa protein concentrate absorbed at least 20% more oil than soy protein. Emulsions formed with 2% alfalfa protein solutions and oil were stable and had the consistency of mayonaise. Heating solutions of 3% alfalfa protein to 72 °C and cooling resulted in the formation of firm gels. Whipping alfalfa protein solutions (2.5%) formed large volumes of foam (about 10 times the solution volume). When stabilized with sucrose and baked, these foams resembled egg meringue.

Potential shortages of food protein and the desire to more fully utilize the nutrients of forages have prompted considerable research on the preparation of protein from leafy crops, particulary alfalfa (Pirie, 1971; Kohler et al., 1968; Edwards et al., 1978; Stahmann, 1975). A whole green leaf protein concentrate (LPC) from alfalfa has been shown to cure symptoms of Kwashiorkor, the protein deficiency disease (Olatunbosun et al., 1972; Kamalanathan and Devadas, 1976; Devadas et al., 1978), but it has not been generally accepted as a food because of its texture, color, and grassy flavor. So that these objectionable characteristics of LPC could be overcome, processes for separation of alfalfa LPC into green and edible "white" fractions were developed. The white fraction LPC produced by one process was insoluble (Edwards et al., 1975) and that produced by another process was soluble (Knuckles and Kohler, 1981). White fraction LPCs from alfalfa have been shown to have a good balance of amino acvids and PERs which were not significantly different from those of casein (Bickoff et al. 1975; Knuckles et al., 1979).

In addition to having good nutritional quality, protein concentrates must have certain critical characteristics which enable them to be used and accepted in food systems (Kinsella, 1976). These characteristics, called functional properties, include solubility, emulsifying and foaming capacity, and fat- and water-binding capacity. Evaluation of various preparations of alfalfa LPC has shown that the heat-coagulated LPC had very low solubility over a wide pH range and was limited in other functional properties (Betschart, 1974, 1975). Laboratory preparations of acid-precipitated alfalfa LPC had increased solubility and improved functionality (Betschart, 1974; Wang and Kinsella, 1976). This paper reports the evaluation of this soluble LPC for functional properties and, in appropriate cases, compares it with soy protein isolate and egg white.

EXPERIMENTAL SECTION

Protein Concentrates or Isolates. The alfalfa protein concentrate was prepared as described by Knuckles and Kohler (1981). Briefly, protein-rich juice was extracted from alfalfa by grinding and pressing. The juice was heated (62 °C), centrifuged, and filtered to produce a clear yellow-brown solution. This clear solution was concentrated by ultrafiltration, and the concentrate was passed through a gel filtration column to remove impurities. The bulk of the protein solution (pH 6.0) from the column was spray-dried to a light tan bland-tasting powder. A small freeze-dried sample was prepared for comparison.

The soy protein isolate (Promine D, Central Soya Co.) and frozen egg white were used for comparisons. The soy protein isolate was a commericial preparation. The egg white was hand separated from cracked eggs, frozen, and thawed as needed. Typical compositions of the protein sources are given in Table I.

Functional Properties. Unless otherwise noted, all analyses were done in duplicate. The nitrogen solubility profiles (pH 2–9) were determined by a modification of Betschart's (1974) method. The sample (400 mg) and distilled water (30 mL) were added to a 50-mL centrifuge tube, and the sample was dispersed with a spatula. The mixture was stirred by a magnetic stirrer for 0.5 h while adjusting the pH with 0.1 N NaOH or HCl as needed to maintain the selected pH. The volume was then adjusted to 40 mL with distilled water. After centrifugation (48000*g*; 20 min; 4 °C), the clear supernatant (10 mL) was analyzed for Kjeldahl nitrogen.

$$\%\ \text{nitrogen solubilized} = \frac{N\ \text{in aliquot} \times 4}{N\ \text{in 400 mg}} \times 100$$

The water-binding capacity was determined by the method of Betschart et al. (1979) except a 1-g sample and 20 mL of distilled water were used.

$$\%\ \text{water-binding capacity (\% by vol)} = \frac{\text{20-mL decanted vol (mL)}}{\text{sample wt (g)}} \times 100$$

Fat-binding (absorption) was measured as described by Lin et al. (1974). The emulsification activity (EA) was determined by a modification of the method reported by Wang and Kinsella (1976). It is as follows: a 3.5-g sample was dissolved or suspended in 50 mL of distilled water and blended (Waring blender; 150 mL bowl) for 1 min. Then two 10-mL protions were centrifuged in 12-mL tubes at 1300*g* for 5 min. EA was expressed as (height of emulsion layer/height of total contents of tube) × 100. Emulsion stability was determined as described by Wang and Kinsella (1976).

Emulsion capacity (EC) was determined by a modification of the method developed by Crenwelge et al. (1974). Two-gram samples were added to 100 mL of distilled water and mixed. This solution was blended (Waring blender; 1-qt bowl) for 1 min with 100 mL of vegetable oil. As blending continued, oil was added at 1 mL/s until inversion occurred. The inversion point was indicated by a sudden decrease in current drawn by the blender. The current, measured by a clip-on meter (Amprobe Instru-

Western Regional Research Center, Science and Education Administration, U.S. Department of Agriculture, Berkeley, California 94710.

Table I. Composition of Protein Concentrate or Isolate[a]

material	moisture, %	nitrogen, %	crude protein,[b] %	fat, %	fiber, %	ash, %
alfalfa leaf protein concentrate	7.4	14.2	88.6	1.1	<0.5	1.9
soy protein isolate	4.7	14.6	91.3		0.2	4.2
egg white	88.5	14.6	91.3			4.3

[a] Except for moisture, data are reported on a dry basis. Data on alfalfa leaf protein concentrate are averages from four different preparations; data on soy protein isolate (Promine D) were supplied by Central Soya Co.; data on egg white are calculated from data from Gorman (1978). [b] $N \times 6.25$.

Table II. Some Functional Properties of Alfalfa Leaf Protein Concentrate and Soy Protein Isolate[a]

material	bulk density, g/mL	fat-binding capacity, mL/100 g	water-binding capacity, % by vol
alfalfa leaf protein concentrate			
freeze-dried	0.01	516	0
spray-dried (outlet, 140 °C)	0.27	208	428.1
spray-dried (outlet, 85 °C)	0.285	239	0
soy protein isolate	0.47	159	612.9

[a] Average of duplicate analysis; reported on a dry basis.

ments, Model KS-3), attached to the blender cord, was about 8 A for the premixture. As additional oil was added, the current increased to about 15 A and then suddenly dropped to 8 A at the inversion point. The EC is reported as milliliters of oil emulsified per gram of protein in the sample.

The gelling ability of proteins is generally measured with a Brookefield viscometer (Kinsella, 1976). In these studies it was more convenient to measure the gel strength by using a penetrometer because the gels of alfalfa protein concentrate were very firm and resilient at low concentrations. The gels were formed by making water solutions of the samples at concentration of 1, 3, 5, and 7% (w/v). Soy protein isolate was also tested at 8.5, 11, and 15% concentration. Before final adjustment to the concentration by dilution with water, 0.1 M HCl or NaOH was used for adjustment to the desired pH. Aliquots (40 mL) of the solutions in 50-mL beakers were heated in a water bath at 72 °C for 30 min and then cooled to ambient temperature in a cold water bath. Other conditions, heating and ionic, are discussed in the next section. The gel strength, measured by a Bloom gelometer (Precision Scientific Co.), was expressed as the grams required to cause 4-mm penetration by a 2.5-cm disk.

Whippability and foam stability were determined by using modifications of the methods of Lawhon et al. (1972) and Lin et al. (1974). The conditions used were as follows: 60 mL of solution (at desired pH) was whipped for 6 min in a Hamilton Beach mixer at 90 rpm (setting no. 8). The foam was immediately transferred to a 1000-mL graduated cylinder and the total volume recorded. The volume of foam was measured after standing 0.5, 1, and 2 h. Foaming capacity was calculated as

$$\% \text{ volume increase} = \frac{\text{volume after whipping} - 60 \text{ mL}}{60 \text{ mL}} \times 100$$

Foam stability is reported as foam volume remaining after a holding time or as percent of initial volume remaining.

Meringues were made by using 120 mL of alfalfa protein solution (2% w/v) or thawed-frozen egg white. The solutions were whipped until foamy, and then sugar (80 g) was added slowly as whipping continued until the foam was stiff. Cream of tartar (0.4 g) was mixed in. The meringue was put on cooled lemon pie fillings and heated in an oven for 5 min at 425 °F.

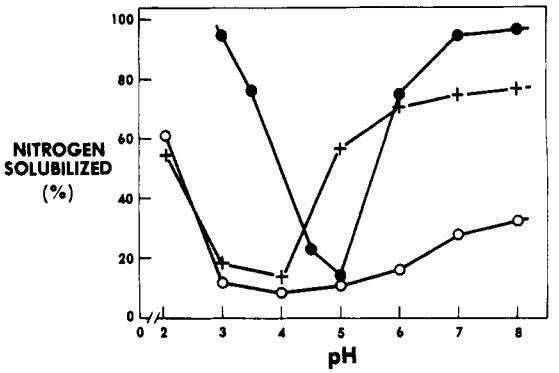

Figure 1. Nitrogen solubility profiles of spray-dried alfalfa leaf protein concentrates. Spray dryer outlet temperatures were 85 (●), 95 (+), and 140 °C (○).

General Methods. Nitrogen, fat, fiber, and ash were determined by standard methods (Association of Official Analytical Chemists, 1975). Moisture was determined by drying at 110 °C for 2 h in a forced draft oven.

RESULTS AND DISCUSSION

Nitrogen Solubility. Moist heat adversely affects the solubility of soy protein (Smith and Circle, 1978). This was also demonstrated for alfalfa LPC. Nitrogen solubility profiles in Figure 1 show alfalfa LPC to be more soluble when spray-dried at lower temperatures. The alfalfa LPC spray-dried with an outlet temperature of 85 °C had a nitrogen solubility profile similar to that of freeze-dried alfalfa LPC purified by diafiltration (Knuckles et al., 1975). Its solubility was also greater than the solubility of some freeze-dried alfalfa LPCs prepared in the laboratory (Betschart, 1974; Wang and Kinsella, 1976).

The nitrogen solubility profiles of the alfalfa LPCs which were spray-dried at outlet temperatures of 95 and 140 °C are essentially the same as those obtained in a preliminary study. Spray drying at 140 °C caused the nitrogen solubility to be greatly reduced. At pH levels between 5 and 8, the nitrogen solubility of the less-soluble LPC (dried at 140 °C) was similar to that of the soy protein isolate. Although the nitrogen solubility of the LPC dried at 140 °C and the soy protein isolate were low, they were still more soluble than het-coagulated alfalfa LPC (Betschart, 1974).

Fat- and Water-Binding Capacity. Fat-binding capacity of the alfalfa LPC was greater than that of soy

Table III. Emulsifying Properties of Alfalfa Leaf Protein Concentrate and Soy Protein Isolate

	emulsifying[a] act., %	emulsion[b] stability, %	emulsifying[c] capacity, mL/g of crude protein
alfalfa leaf protein concentrate			
freeze-dried	64.3	95.5	311.8
spray-dried (outlet, 85 °C)	61.5	94.4	289.1
soy protein isolate	64.3	94.1	263.9

[a] Determined by the method of Wang and Kinsella (1976). [b] Similar to emulsion activity except the emulsion was heated to 80 °C for 30 min before centrifugation. [c] Oil was added until the inversion point was reached.

protein isolate (Table II). The values for the spray-dried alfalfa LPC and the soy protein isolate are within the range reported by others (Lin et al., 1974; Wang and Kinsella, 1976). The very high fat-binding capacity of the freeze-dried alfalfa LPC and the difference in the fat-binding capacity among the LPCs and soy protein isolate are probably caused by differences in bulk density. The method used measures mostly physically entrapped oil (Kinsella, 1976), and there is an 0.95 correlation coefficient between bulk density and fat absorption (Wang and Kinsella, 1976).

Alfalfa LPCs which had been freeze-dried or spray-dried with an outlet temperature of 85 °C had zero water-binding capacities (Table II). These zero values are due to the high solubility which reduces structural entrapment of the water. The alfalfa LPC, spray-dried at a 140 °C outlet temperature, had a water-binding capacity about 30% less than that of soy protein isolate. The values obtained for the latter LPC and soy protein isolate are similar to those reported earlier (Wang and Kinsella, 1976).

Emulsification Properties. The emulsion activity and emulsion stability of the alfalfa LPC and soy protein isolate were similar (Table III). The EA of the alfalfa LPC was higher and the EA of the soy protein isolate were lower than EAs reported for similar preparations (Wang and Kinsella, 1976). The high EA of the LPC is attributed to processing in a manner to reduce denaturation. The lower EA for soy protein is attributed to a difference in the blending action of the blendors used in the two studies. The emulsion capacity of the alfalfa LPC decreased with an increase in concentration. The ECs of alfalfa LPC at 0.5, 1.0, and 2.0% concentration were >700, 521, and 246 mL of oil/g alfalfa LPC. (At 0.5% concentration, the volume of oil to cause inversion exceeded the capacity of the blender bowl.) The decrease in EC with an increase in concentration was observed earlier with acid-precipitated alfalfa LPC, meat proteins, and soy protein isolates (Wang and Kinsella, 1976; Acton and Saffle, 1972; Pearson et al., 1965).

Emulsions formed from alfalfa LPC solutions (2% w/v) and oil had high viscosity (1260 poise) and were very stable. When vinegar and spices were added to the emulsion, the mixture was similar to mayonaise in taste, appearance, and consistency. This synthetic mayonaise, stored in a refrigerator, was stable for more than 3 months.

Gelation. An earlier evaluation showed acid-precipitated alfalfa LPC to have poor gelling properties (Lu and Kinsella, 1972). The the gelling properties of the alfalfa LPC used in this study were better than those of the soy protein isolate (Figure 2). The alfalfa LPC spray-dried at 85 °C outlet temperature formed a gel at a concentration of 1%, but the gel was too weak for penetrometer measurements. As the concentration of LPC was increased above 2%, the gel strength rapidly increased so that at 5% the gel strength was more than twice that of the soy protein isolate at 15% concentration. The results obtained on the soy protein isolate are similar to those reported by Circle and Smith (1972). They reported that the soy protein

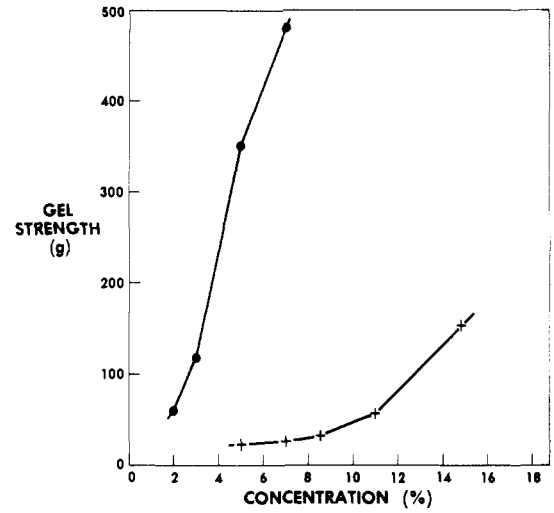

Figure 2. Gel strength of alfalfa leaf protein concentrate and soy protein isolate. Gel strength is expressed as the grams required to cause 4-mm penetration of a 2.5-cm disk. Alfalfa leaf protein concentrate (●) was spray-dried at an outlet temperature of 85 °C. The soy protein isolate (+) was Promine D (Central Soya Co.).

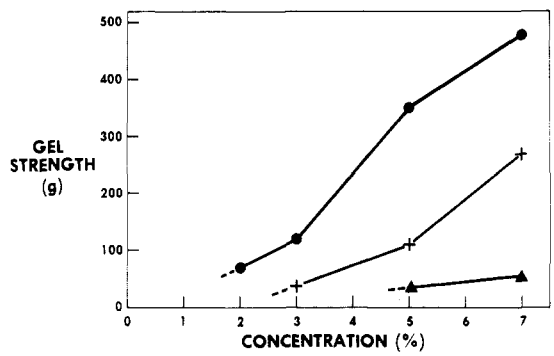

Figure 3. Gel strength of alfalfa leaf protein concentrates spray-dried at different outlet temperatures. Gel strength is expressed as the grams required to cause 4-mm penetration of a 2.5-cm disk. Spray dryer outlet temperatures were 85 (●), 95 (+), and 140 °C (▲).

isolate would form gels at concentrations above 7% and that the gels at 16–17% concentration were firm and resilient.

The gelation of alfalfa LPC was greatly affected by the temperature used in drying the concentrate (Figure 3). As the outlet temperature of the spray dryer was increased from 85 to 140 °C, the protein concentration to form gels of measurable strength increased from 2 to 5%. At 5% concentration, the gel strength of alfalfa LPC spray-dried at 85 °C was about 3.5 and 9.6 times the strength of the gels of LPC dried at 95 and 140 °C. The conditions under which the gels were formed also affected the gel strength. The alfalfa LPC solutions formed gels upon heating to 60 °C for 15–30 min, with the firmest gels being produced by the longest heating time. The gels, except that from LPC

Table IV. Foam Stability of Alfalfa Leaf Protein Concentrate and Egg White[a]

time, h	foam volume, mL: alfalfa protein concentrate	foam volume, mL: egg white
0	600	600
0.5	345	324
1	195	175
2	115	60

[a] 60 mL of frozen-thawed egg white or a 2.5% solution of spray-dried alfalfa protein concentrate was whipped for 5 min in a Hamilton Beach mixer.

Table V. Effect of pH on Volume and Stability of Foams Produced from Alfalfa Leaf Protein Concentrate[a]

pH	foam volume, mL	foam volume retained after 0.5 h, %
3.0	600	78.1
4.5	600	89.0
6.0	600	32.2
7.0	500	21.8
8.0	400	16.5

[a] 60 mL of a 2% solution of alfalfa LPC was whipped for 5 min in a Hamilton Beach mixer.

spray-dried at 140 °C, became stronger as the temperature of gelation was increased from 60 to 95 °C. Salt caused a decrease in gel strength. At 1.0 M NaCl, the gel strength was reduced by 15–20%. The strength of gels were also lower when the pH was lower than 7 and above 8. The effects of conditions on gels are similar to those reported for soy protein (Catsimpoolas and Meyer, 1970; Kinsella, 1979).

Foaming Capacity and Stability. Betschart (1975) suggested that alfalfa LPC could have good foaming properties which could be useful for aeration in food systems to give texture and leavening. Whipping of alfalfa LPC (spray-dried; outlet temperature 85 °C) solutions of 2, 4, and 10% concentrations produced stiff foams which were 10 times the solution volumes. This volume increase was the same as that obtained with frozen–thawed egg white.

Measurement of foam stability over a 2-h period showed alfalfa LPC foams to be more stable than egg white foams (Table IV). The addition of sucrose at 40 g/60 mL of solution stabilized the foams of both LPC and egg white so that only a 10% decrease in foam volume was observed at the end of 2 h. The volume and stability of the alfalfa LPC foams were affected by pH (Table V). Foam volume was reduced at pH levels above 6 and was most stable at a pH near 4.5. The greater stability of foams at pH levels near the protein's isoelectric point was observed with foams of soy protein isolates (Kinsella, 1979).

The studies reported above showed alfalfa LPC had promise as a substitute for egg white in making meringue. Figure 4 shows the meringues made from egg white, alfalfa LPC, and a mixture of the two.

The foam from the alfalfa LPC was stable to the heat of baking but differed somewhat in appearance from the egg white foam. This difference would not be so obvious if the egg white sample were not in close proximity. The foam of the LPC and egg white mixture (120 mL), containing 1.2 g of alfalfa LPC solids and 7.4 g of egg white solids, closely resembled that of the egg white. The texture and taste of the pies with alfalfa protein meringue were quite acceptable to the 12–15 peaple who consumed the pies. These data suggest that the alfalfa LPC could substitute for a portion of the egg white while yielding an acceptable meringue.

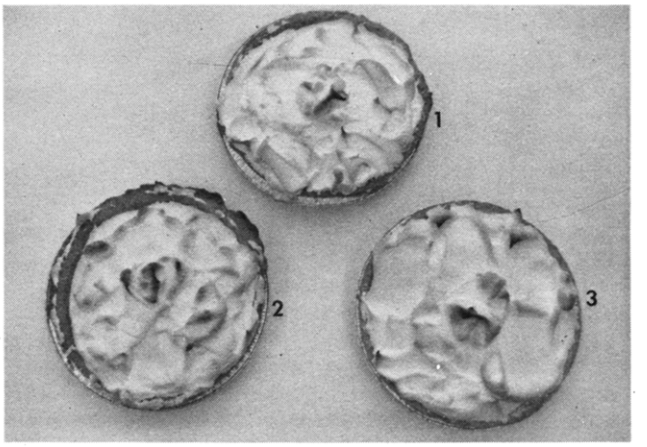

Figure 4. Pies covered with meringue made from alfalfa leaf protein concentrate and egg white. Pie no. 1 was made from egg white only, no. 2 was made from alfalfa LPC only, and no. 3 was made from a mixture of alfalfa LPC and egg white.

Conclusions. The evaluations reported here show that properly prepared alfalfa LPC can have excellent functional properties. These functional properties and good nutritional quality should make the alfalfa LPC a desirable component in various food systems.

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Fermentability of High-Moisture Corn Treated with Chemical Preservatives

James E. VanCauwenberge,* Rodney J. Bothast, and Lynn T. Black

Chemical preservation of high-moisture corn is one alternative to the conventional method of high-temperature drying and has contributed to increased use of high-moisture corn. The present study investigated the use of chemically preserved corn as feedstock for the production of alcohol by fermentation. Preservatives tested were formaldehyde, ammonia, sulfur dioxide, methylene dipropionate (MBP), acetic acid, and propionic acid. Acetic and propionic acids and ammonia-treated corn samples were converted at all concentrations tested, with alcohol production at 80–90% of maximum theoretical alcohol possible. Sulfur dioxide treated corn yielded more alcohol than the other preservatives tested when SO_2 treatments were kept at low concentrations (0.1–0.5%). MBP- and formaldehyde-treated corn yielded low amounts of alcohol and should be avoided as feedstocks for alcohol production.

Increased fossil-fuel prices have stimulated investigations into more economic alternative procedures for conventional high-temperature drying of freshly harvested, high-moisture corn (24–28% moisture content). Chemical preservation of high-moisture corn is one alternative to the conventional method and has contributed to increased use of high-moisture corn. Volatile fatty acids and their salts have received the most attention as preservatives. Propionic acid and mixtures of propionic and acetic acids are presently marketed and prevent mold growth and spoilage in corn containing up to 30% moisture (Hall et al., 1974). Other preservatives which have been investigated include formaldehyde (Muir and Wallace, 1972), ammonia (Bothast et al., 1973; Nofsinger et al., 1977, 1979), sulfur dioxide (Eckhoff et al., 1980), and methylene dipropionate (MBP) (Bothast et al., 1978; Montgomery et al., 1980).

One potential use for preserved corn is as a feedstock for the production of alcohol by fermentation. This study was undertaken to determine the fermentability of high-moisture corn treated with each of six preservatives (ammonia, sulfur dioxide, MBP, propionic acid, acetic acid, and formaldehyde) at four concentrations (0.1, 0.2, 0.5, or 1.0% w/w) with untreated corn as a control. The four concentrations used are those that might actually be employed to preserve corn "in the field". It should be noted that, even at the same concentration level, the chemicals employed are not equivalent as antimicrobial agents.

MATERIALS AND METHODS

The corn used in this experiment was freshly harvested high-moisture corn (28% moisture level) that was stored at 0 °C until used. Samples (400 g) were placed in 2-L Erlenmeyer flasks and brought to ambient temperature. The various preservatives were then added (Table I) to the corn on a weight of active preserving agent to weight of corn basis. The flasks were sealed and kept at ambient

Table I. Quantity of Preservative Used to Reach the Desired Concentration on 400 Grams of High-Moisture Corn[a]

preservative	concentration of preservative			
	0.1 %	0.2 %	0.5 %	1.0 %
acetic acid	0.4[b]	0.8	2.0	4.0
ACS ammonium hydroxide	1.33	2.66	6.67	13.34
formaldehyde	1.16	2.32	5.80	11.60
MBP	0.4	0.8	2.0	4.0
propionic acid	0.4	0.8	2.0	4.0
sulfur dioxide	0.4	0.8	2.0	4.0

[a] The concentrations were calculated from the "wet" weight of the corn. [b] Values are in grams.

Table II. Protocol Followed for Preserved, High-Moisture Corn Fermentations

step 1: add 162.4 g of treated corn to 560 mL of distilled water in a 1-L Erlenmeyer flask
adjust the pH to 6.2
add 0.32 mL of Taka-therm α-amylase
heat to 90 °C with stirring
maintain at 90 °C for 1 h
step 2: cool by adding 150.4 mL of distilled water
reduce temperature to 60 °C
adjust pH to 4.0
add 1.2 mL of Diazyme L-100
maintain at 60 °C for 2 h
step 3: cool to 32 °C
adjust pH to 5.0
add yeast inoculum, 1% v/v
allow to ferment for 3 days at 32 °C

Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604.