Vander Jagt, D. L.; Dean, V. L.; Wilson, S. P.; Royer, R. E. "Regulation of the Glutathione S-Transferase Activity of Bilirubin Transport Protein (Ligandin) from Human Liver". *J. Biol. Chem.* **1983**, *258*, 2689–5694.
Whaley, K. J.; Sampath, D. S.; Balaram, P. "A Circular Dichroism Study of (+) Gossypol Binding to Proteins". *Biochem. Biophys. Res. Commun.* **1984**, *121*, 953–959.
Zarins, Z. M.; Cherry, J. P. "Storage Proteins of Glandless Cottonseed Flour". *J. Food Sci.* **1981**, *46*, 1855–1859, 1862.

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Factors Affecting Protein Dispersibility from Full and Defatted Egyptian Lupine Flours (*Lupinus termis*)

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Factors influencing dispersibility of lupine proteins (particle size, flour to solvent ratio, pH, temperature, time) were investigated. Moreover, the effects of four salts (NaCl, Na_2CO_3, Na_3PO_4, Na_2EDTA) in wide concentration ranges were also studied. In the range pH 3–6, protein dispersibility from the defatted lupine flour (DLF) was higher than that from the full-fat lupine flour (FLF), contrary to pH values ranging between 8 and 10. Maximum protein dispersibilities for DLF (98.82%) and FLF (97.92%) were achieved at pH 11, particle size 120 mesh, solvent to flour ratio 50:1 at room temperature for 30 min. Regarding salt dispersion, the presence of fat has appreciably affected protein dispersibility, depending on salt type, pH effect, and salt concentration.

Whereas numerous papers were published on nitrogen dispersibility of legume proteins, only a limited number of reports have appeared in recent years on lupine proteins (Blagrove and Gillespie, 1976; Ruiz and Hove, 1979; Blaicher et al., 1981; Sathe et al., 1982).

Malgarini and Hudson (1980) reported that lupine proteins from defatted flours were more than 80% soluble at pH 1.0, dropping to 50% at pH 3.5 and to 15% at pH 4.4. Other factors that influence nitrogen dispersibility from lupine flour, e.g. meal to solvent ratio, mesh size, and extraction time, were also studied by Ruiz and Hove (1979) and Sathe et al. (1982). Apart from an unusually long extraction time (28 h) used by Sathe et al. (1982), other extraction parameters were comparable to those used frequently for protein extraction from many other legume flours. Besides, Oomah and Bushuk (1983) reported that defatting lupine seed meal has influenced its protein solubility. Therefore, it was of interest to study and improve protein dispersibility from Egyptian lupine seeds in full-fat and defatted lupine flour as affected by many factors including different salts in wide ranges of concentrations.

EXPERIMENTAL SECTION

Lupine seeds (*Lupinus termis*) grown in El-Sharkia Governorate of Egypt were used in this study. Dirt and stones were removed, and seeds were decoated by hand with sharp scalpel.

Decoated seeds were ground with a hammer mill followed by an IKA Laboratory mill to pass the desired mesh sieve (40–120 mesh). Defatted lupine flour was prepared according to the method of Tsen et al. (1962), using a solvent system of hexane–chloroform (12:1, v/v). Full-fat (FLF) and defatted (DLF) flours were transferred into air-tight glass jars and kept at −20 °C until use.

Acid and Base Dispersion. Dispersion experiments were carried out on 1-g portions of FLF and DLF samples. In each dispersion experiment, the sample was dispersed in 45 mL of distilled water, the pH was adjusted to the desired value with 0.5 N HCl or 0.5 N NaOH, and the final volume was completed to 50 mL. The suspension was shaken for 60 min, and the final pH was measured. The insoluble materials were removed by centrifugation (2500*g*) for 15 min. Experiments were repeated to cover the range pH 2–11.

Factors affecting the protein dispersibility, namely particle size (40–120 mesh), solvent to flour ratio (10:1–50:1), time of extraction (15–60 min), and temperature (20–50 °C), were investigated by the same previously outlined method as well. When the effect of one dispersion parameter was studied, the other parameters were maintained constant at fixed values. Once an optimum value for a certain parameter was obtained, it was used in later experiments until all dispersion parameters were optimized.

Salt Dispersion. Four different salts, namely sodium chloride, sodium carbonate, sodium phosphate, and the disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA), were used for protein dispersion from FLF and DLF in the range of concentrations 0.005–2.0 N with the exception of Na_2EDTA, which was used in the range 0–7600 mg/L. The final pH of the protein extract, from each salt concentration, was measured. Other dispersion conditions: solvent to flour ratio, 50:1; room temperature, 20–25 °C; extraction time, 60 min. The insoluble materials were removed by centrifugation (2500*g*) for 15 min, and the supernatant was made up to 50 mL in a volumetric flask.

Analytical Methods. The total nitrogen (TN) content of flour was determined by the semimicro-Kjeldahl method (Egan et al., 1981). Nonprotein nitrogen (NPN) was estimated according to procedure of Bhatty (1973). True protein was calculated as (TN − NPN) × 5.85.

The soluble protein concentrations were determined in 1 mL of the protein extracts by the Lowry colorimetric method (Lowry et al., 1951).

Total alkaloids expressed as lupanine ($C_{15}H_{24}N_2O$) were determined for the lupine flours by the volumetric method of Blaicher et al. (1981). In this method the total alkaloids in an extract were titrated with 0.01 N *p*-toluensulfonic acid in chloroform, and the potassium salt of tetrabromophenolphthalein ethyl ester was used as an indicator.

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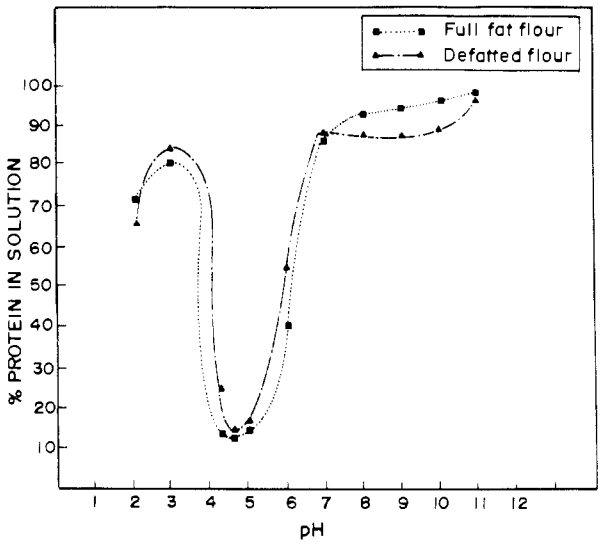

Figure 1. Extraction profiles of lupine proteins as a function of pH. Other extraction conditions: flour mesh, 120; solvent to flour ratio, 50:1; room temperature; time, 60 min.

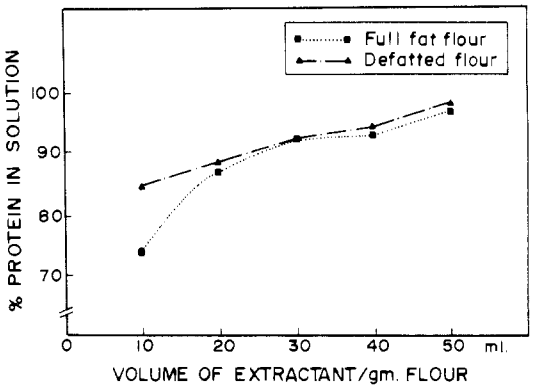

Figure 2. Effect of solvent to flour ratio on lupine protein dispersibility. Other extraction conditions: flour mesh, 120; pH 11.0; room temperature; time, 60 min.

All experiments were carried out in duplicate.

RESULTS AND DISCUSSION

Effect of pH. Figure 1 shows the dispersibility profiles of lupine protein from full-fat and defatted flours, as a function of pH. Data revealed that protein dispersibility was higher at alkaline pH values than at either neutral or acidic pH values. More than 88.0% of the lupine proteins from DLF was dispersed above pH 8.0, and a maximum of 98.3% was attained at pH 11.0. In the acidic region of the profile, a maximum protein dispersibility of 85.0% was obtained at pH 3.0 and a lower value (66.6%) at pH 2.1, with minimum protein dispersibility (14.4%) occurring at pH 4.5. Therefore, the latter pH represents the isoelectric point of majority of lupine proteins.

The lupine proteins from FLF exhibited some distinct differences from that of DLF. It was characterized by higher protein dispersibility in the range pH 8–10, apparently resulting from an emulsification effect of the fat (Na salt of fatty acids) helping protein extraction. Besides, FLF had lower protein dispersibility in the range pH 3.0–7.0, particularly at pH 4.3 and 6 when only 13.7% and 40.9% was dispersed from FLF, respectively, compared with 25.4% and 47.4%, respectively, in the cases of the defatted flour.

Effect of Solvent to Flour Ratio. Figure 2 shows protein dispersibility from FLF and DLF vs different solvent to flour ratios. Data indicated that changing this ratio from 10:1 to 20:1 had much more effect on protein dispersibility from FLF than from DLF. Therefore, the protein dispersibility increased from 73.3% at a 10:1 ratio to 78.1% at a 20:1 ratio for FLF, compared with an increase of only 84.6% to 89.1% for DLF. At lower solvent to flour ratios, protein dispersibility expectedly decreased as the mixture became too thick and extraction incomplete. However, Ruiz and Hove (1979) observed that changing the solvent to flour ratio from 10:1 to 40:1 has only slightly increased lupine protein solubility of the full-fat flour.

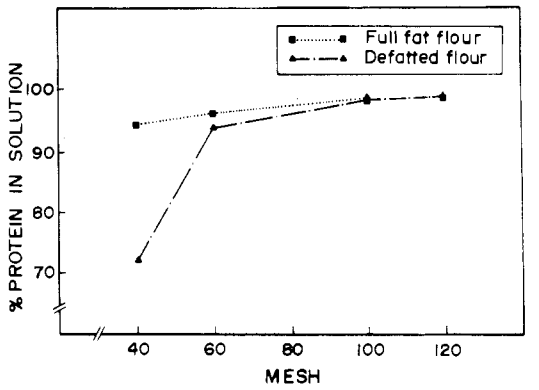

Figure 3. Effect of flour particle size on lupine protein dispersibility. Other extraction conditions: pH 11.0; solvent to flour ratio, 50:1; room temperature; time, 60 min.

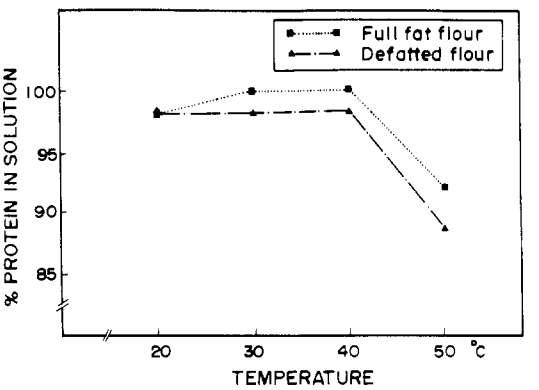

Figure 4. Lupine protein dispersibility as affected by temperature. Other extraction conditions: pH 11.0; solvent to flour ratio, 50:1; flour mesh, 120; time, 60 min.

Effect of Particle Size. The effect of flour particle size on lupine protein dispersibility is given in Figure 3 for both FLF and DLF. Data indicated that increasing the flour mesh from 40 to 60 caused a large increase in protein dispersibility, from 72.9% to 94.3% in case of DLF, but little increase was observed in case of FLF. Further increases in the flour mesh up to 120 have only caused minor changes in protein dispersibility for both flours. In accordance, Ruiz and Hove (1979) reported that protein solubility from full-fat lupine flour was increased from 55% up to 96% when their flour mesh was increased from 25 to 100.

Effect of Temperature. Dispersibility of lupine proteins as affected by temperature in the range 20–50 °C is shown in Figure 4 for both FLF and DLF. Data indicated that increasing the extraction temperature from 20 to 40 °C has only caused minor increases in protein dispersibility from both flours. However, when the temperature was increased to 50 °C, protein dispersibility dropped from 100% to 91.7% and from 98.6% to 89.2% for FLF and DLF, respectively. Generally comparable findings were reported by Flink and Christiansen (1973) and Ruiz and Hove (1979) for faba bean and lupine proteins, respectively.

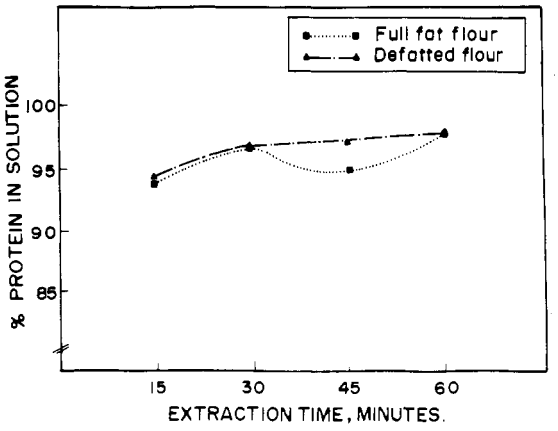

Figure 5. Effect of extraction time on lupine protein dispersibility. Other extraction conditions: flour mesh, 120; solvent to flour ratio, 50:1; pH 11.0; room temperature.

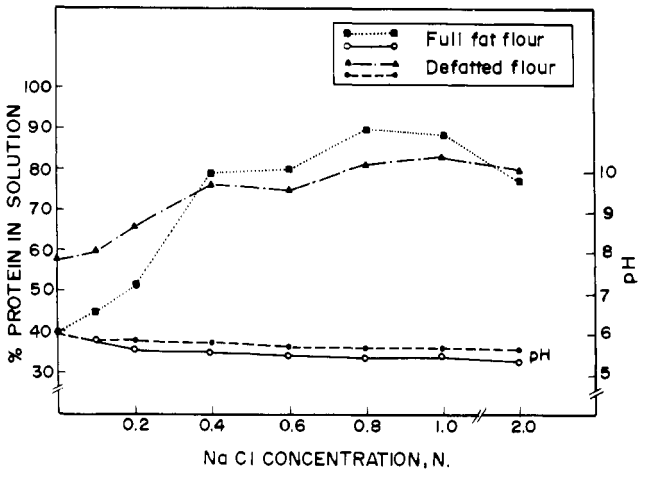

Figure 6. Lupine protein dispersibility as affected by sodium chloride concentration. Other extraction conditions as in Figure 1.

Effect of Extraction Time. Figure 5 reveals the effect of time (15–60 min) on dispersibility of lupine proteins from both FLF and DLF. There was some increase in protein dispersibility as the extraction time was increased from 15 to 30 min. These increases were from 94.0% to 97.2% and from 94.3% to 97.7% for FLF and DLF, respectively. Consequently, the bulk of the flour proteins was actually dispersed in the first 15 min of the extraction time. Data in this regard are in a good accordance with those reported for lupine flour (Ruiz and Hove, 1979).

It is noteworthy to mention that protein preparations (Mohammed, 1984) made under the present optimum extraction conditions and precipitated by different methods have contained less than 15% of the original alkaloids (1.52% of the raw lupine flour).

Salt Dispersion. *Sodium Chloride (NaCl).* The lupine protein dispersibility profile with NaCl concentration (Figure 6) for the DLF had the same general trend when compared with that of the FLF. However, the percent of dispersed proteins from the DLF in the range 0–0.38 N NaCl was higher (58.5–76.0%) than that of FLF (41.1–76.0%). This possibly resulted from the increase in globulin fraction solubility as caused by defatting (Oomah and Bushuk, 1983). However, with NaCl concentration 0.38–1.0 N, the FLF exhibited somewhat higher protein solubilities (79.9–89.4%) compared to those of the DLF (77.3–84.5%). This reversed effect for the presence of fat in FLF can be explained on the basis of increasing protein dispersibility as a result of the presence of the sodium salt

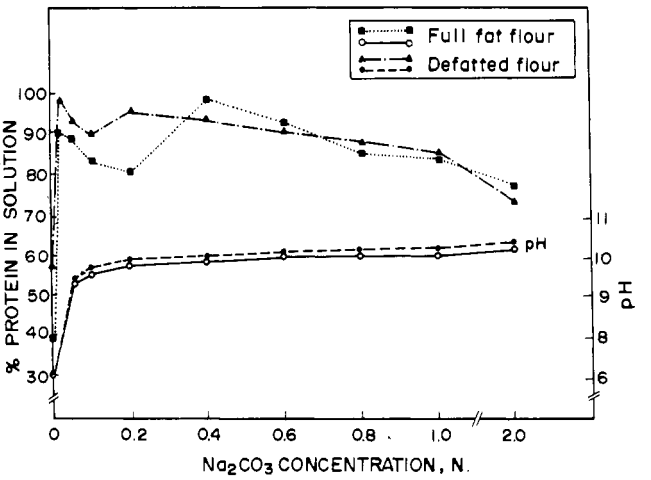

Figure 7. Effect of sodium carbonate on lupine protein dispersibility. Other extraction conditions as in Figure 1.

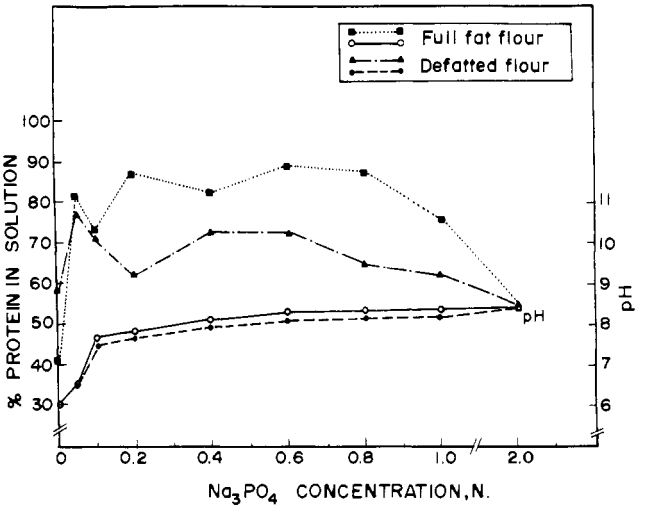

Figure 8. Effect of sodium phosphate concentration on lupine protein dispersibility. Other extraction conditions as in Figure 1.

of lupine free fatty acids, reported by Kobrehel and Bushnuk (1977). The pH of lupine protein extracts ranged from 6.0 in the absence of NaCl (control) to 5.35 at 2.0 N NaCl solution. Although these changes in pH may affect protein dispersibility during salt dispersion, the salt concentration remained the dominant factor in this respect.

Sodium Carbonate (Na_2CO_3). Figure 7 shows the dispersibility of lupine proteins from full-fat and defatted flours as a function of Na_2CO_3 concentration. A rapid increase in protein dispersibility (from both flours) was observed as the Na_2CO_3 concentration was increased from 0 to 0.005 N. At 0.005 N Na_2CO_3, the pHs were 7.05 and 6.9 and protein dispersibilities 90.4 and 90.5% for FLF and DLF, respectively. As Na_2CO_3 concentration was increased higher than 0.005 N, protein dispersibilities fluctuated mildly for both flours and reached a maximum value of 99.6% at 0.4 N and 96.7% at 0.2 N for FLF and DLF, respectively. When Na_2CO_3 concentration was further increased, protein dispersibilities declined gradually in both flours. Such a decline obviously did not result from pH effect, but it apparently resulted from a possible salting-out effect.

Sodium Phosphate (Na_3PO_4). The effect of sodium phosphate concentration on protein dispersibility from both FLF and DLF is given in Figure 8. Both the dispersion and pH profiles of both flours increased rapidly with Na_3PO_4 at low concentration and up to 0.1 N. At

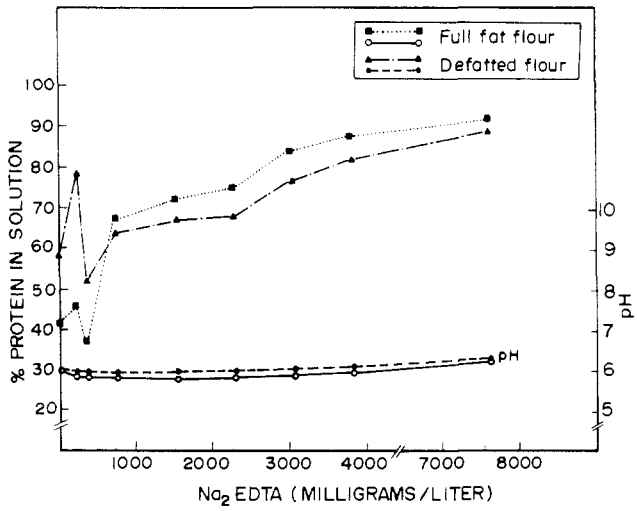

Figure 9. Lupine protein dispersibility as affected by Na_2EDTA concentration. Other extraction conditions as in Figure 1.

higher concentrations, the final pH of the protein extracts increased very slowly and the protein dispersibilities fluctuated and started to drop with different ratios at Na_3PO_4 concentrations higher than 0.6 N. However, the rate was faster for FLF at concentrations higher than 0.8 N. The dispersion of proteins was apparently the combined effects of pH and salting-in, whereas at higher concentrations the pH and salting-out effects were apparently working against each other. The general trends of the dispersion profiles with Na_3PO_4 show similarity with those with Na_2CO_3 for FLF and DLF.

Maximum protein dispersibility was 89.6% with 0.6 N Na_3PO_4 at pH 8.3 for FLF compared with a maximum value of 77.1% with 0.05 N Na_3PO_4 at pH 6.5 for DLF. Control protein dispersibilities (in the absence of salt) were 41.1% and 58.5% for the FLF and DLF, respectively.

It is noteworthy that optimum salt concentrations producing the most alkaline final pH are also those extracting the most nitrogen (Hang et al., 1970). However, the present data have revealed that alkaline salt concentrations higher than optimum values would increase pH but diminish protein dispersibility. Therefore, the correlation between pH of the salt extractant and N dispersibility cannot explain the complete extraction pattern.

Ethylenediaminetetraacetic Acid Disodium Salt (Na_2EDTA). Figure 9 shows the protein dispersibility of FLF and DLF as a function of Na_2EDTA concentration. As shown, increasing the Na_2EDTA concentration from 0 to 140 ppm of extracts led to some increase in protein dispersion from 41.1% to 46.7% and from 58.5% to 78.0% for the FLF and DLF, respectively. The further doubling of Na_2EDTA concentration caused protein dispersion to drop to 37.2% and 52.5% for FLF and DLF, respectively. Further increases in Na_2EDTA concentration up to 7600 ppm in the extractant caused gradual increases in protein dispersibilities for both flours. Besides, the FLF exhibited higher protein dispersibility at all concentrations compared with the DLF. It was clear that the final pH of the protein extracts persisted at a relatively low pH value (5.8) for the FLF up to an Na_2EDTA concentration of 1520 ppm and then started to increase slowly, reaching 6.35 at 7600 ppm. A generally similar but less pronounced change in the pH was noticed for DLF. The marked increase in dispersibility followed by a precipitous decrease and a second increase as an effect of Na_2EDTA deserves further investigation.

Registry No. NaCl, 7647-14-5; Na_2CO_3, 497-19-8; Na_2HPO_4, 7558-79-4; Na_2EDTA, 139-33-3.

LITERATURE CITED

Bhatty, R. S. *Cereal Chem.* **1973**, *50*, 329.
Blagrove, R. J.; Gillespie, J. M. *Aust. J. Plant Physiol.* **1976**, *5*, 651.
Blaicher, F. M.; Molte, R.; Muknerieo, K. D. *J. AOCS* **1981**, *58*, 761.
Egan, H.; Kirk, R. S.; Sawyer, R. *Pearson's Chemical Analysis of Foods*, 8th ed.; Churchill Livingstone: Edinburgh, London, Melbourne, New York, 1981.
Flink, J.; Christiansen, I. *J. Food Sci. Technol.* **1973**, *6*, 102.
Hang, Y. D.; Steinkraus, K. H.; Hackler, L. R. *J. Food Sci.* **1970**, *35*, 818.
Kobrehel, H.; Bushuk, W. *Cereal Chem.* **1977**, *54*, 833.
Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *139*, 265.
Malgarini, G.; Hudson, B. J. *Riv. Ital. Sostanze Grasse* **1980**, *LVII*, 378.
Mohammed, A. M. M.Sc. Thesis, University of Alexandria, Alexandria, Egypt, 1984.
Oomah, B. D.; Bushuk, W. *J. Food Sci.* **1983**, *48*, 38.
Ruiz, L. P.; Hove, L. *J. Sci. Food Agric.* **1979**, *27*, 661.
Sathe, S. K.; Deshpande, S. A.; Salunkhe, D. K. *J. Food Sci.* **1982**, *47*, 491.
Tsen, C. C.; Levi, I.; Hlynka, I. *Cereal Chem.* **1962**, *34*, 195.

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