Nitrogen Solubility of Cereals and Legumes Subjected to Micronization

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Six cereals (wheat, barley, rye, triticale, millet, and wildrice) with moisture contents of 7–13% and six legumes (green pea, yellow pea, lentil, black bean, kidney bean, and pinto bean) with moisture contents of 8–10% were micronized to surface temperatures of 115 and 140 °C, respectively. Compared to nontreated grain/seed, micronized samples showed lower nitrogen solubilities in water at pH 6.0, 0.5 M NaCl, and 70% ethanol, indicating denaturation of albumins and globulins and, to a lesser extent, prolamins for some cereals such as barley and triticale. Results of extracting insoluble nitrogen with 0.5% sodium dodecyl sulfate and 0.6% 2-mercaptoethanol in borate buffer (pH 10) suggested that micronization induced hydrophobic aggregation in legume proteins and both hydrophobic and disulfide bonds in cereals. Increasing grain moisture content and micronization temperature progressively reduced nitrogen solubility in cereals and legumes.

Keywords: *Nitrogen solubility; protein; micronization; cereals; legumes*

INTRODUCTION

Micronization, or infrared radiation, involving short-time exposure of a material to electromagnetic radiation at a wavelength of 1.8–3.4 μm, is a continuous dry-heating process to precook grains before eventual use in human food, animal feed, or malting/brewing/distilling. Studies on wheat and barley (South and Ross, 1993) showed that protein solubilities were reduced by micronization. Similar results were obtained on sorghum (Shiau and Yang, 1982), soybean (Metussin et al., 1992), and pea and canola (McCurdy, 1992). Micronization reduced trypsin inhibitor activity and hemagglutinin in field bean (Melcion and Valdebouze, 1977) and improved the digestibility of protein in soy milk (Mettusin et al., 1992). Animal studies demonstrated improvements in nutritive values of cereal, legume, and oilseed meals after micronization (Shiau and Yang, 1982; Savage and Clark, 1988).

Protein solubility is an important functional property that affects the utilization and nutritional value of cereal grain and legume seeds (Sosulski, 1977). Protein solubility determined by nitrogen solubility is also used for classification of plant proteins. Heat processing generally causes denaturation of proteins, resulting in reduced solubilities. Heat-induced denaturation of proteins may vary from aggregation of polypeptide chains due to the formation of hydrophobic and disulfide bonds (Wall et al., 1975) to pyrolysis of proteins (Hansen et al., 1975). The extent of protein denaturation is dictated by moisture content, heating temperature, and time (Hansen et al., 1975; Neucere and Cherry, 1982; Nakai

and Li-Chen, 1989). Little is known about how micronization affects proteins in a wide range of cereals and legumes. The objectives of this study were to investigate nitrogen solubilities in a variety of cereals and legumes subjected to micronization and to assess the effects of grain moisture content prior to micronization and micronization temperature on solubility fractions of plant proteins.

MATERIALS AND METHODS

Cereals and Legumes. Hard red spring wheat, two-rowed hull-less barley, winter rye, triticale, green field pea, yellow field pea, and lentil were obtained from the Department of Plant Sciences, University of Saskatchewan (Saskatoon, SK). Wildrice, millet, kidney bean, black bean, and pinto bean were from local commercial suppliers. The control and micronized grain/seed was ground into meals with an UDY sample mill (UDY Corp., Fort Collins, CO) to pass a 0.5-mm screen. Moisture and total nitrogen contents were determined according to American Association of Cereal Chemists (1995) Approved Methods 44-15A and 46-11A, respectively.

Micronization. A laboratory-scale, propane-fired micronization system equipped with two ceramic infrared burners set ~17 cm above the 100-cm table (Micronizing Co.) was used to treat intact grains and seeds (Figure 1). During operation, grain/seed was fed onto the vibratory conveyor via a vibrating feeder. The vibration of the conveyor turned the grain/seed frequently as it passed beneath the infrared burner, thus ensuring uniform exposure of grain/seed to infrared energy. The cereal grains were heated to a surface temperature of 115 °C and the legumes to 140 °C. The hull-less barley and pinto beans were subjected to detailed studies at a range of moisture contents and micronizing temperatures. Barley grain with an original moisture content of 13.3% was tempered at room temperature (≈23 °C, RT) for 16 h to obtain final moisture contents of 19.3 or 25.9%. Similarly, pinto bean seeds with an original moisture content of 8.8% were tempered to 15.1 or 18.2% moisture contents. The barley grain was then micronized to surface temperatures of 115, 135, or 150 °C and the pinto bean seeds to 125, 140, or 150 °C. The treated grain/seed was cooled with air immediately after micronization. The

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Table 1. Moisture, Total Nitrogen Contents, and Nitrogen Solubility of Cereals Subjected to Micronization (115 °C Surface Temperature)

grain	treatment	moisture (% as-is)	total N (% db)	nitrogen solubility (% total N)					
				pH 6.0	0.5 M NaCl	70% EtOH	0.5% SDS	0.5% SAS/0.6% MCE	total
rye	control	6.6	1.86	37.6	13.5	25.1	17.1	5.0	98.3
	treated	6.2	1.81	32.6	13.1	26.4	22.7	7.0	101.8
		*a	ns*a*	*	ns	ns	*	ns	ns
triticale	control	10.3	2.31	24.7	11.9	31.8	18.5	13.0	99.9
	treated	8.8	2.27	18.5	7.5	27.5	21.8	25.5	100.8
		*	ns	*	*	*	ns	*	ns
wheat	control	9.0	2.66	18.4	11.7	35.7	14.9	21.3	102.0
	treated	8.1	2.68	14.9	8.2	34.4	17.9	10.0	85.4
		*	ns	*	*	ns	ns	*	*
barley	control	13.3	1.81	16.8	9.8	37.2	20.3	17.4	101.5
	treated	8.0	1.78	7.8	6.5	20.6	22.7	40.9	98.5
		*	ns	*	*	*	ns	*	ns
millet	control	10.4	2.06	5.8	4.0	3.1	63.9	9.0	85.8
	treated	8.5	2.04	4.4	4.5	3.2	64.8	9.6	86.5
		*	ns	ns	ns	ns	ns	ns	ns
wildrice	control	9.5	2.28	4.4	2.2	1.3	34.6	25.8	68.3
	treated	8.1	2.26	3.5	2.8	1.5	36.7	23.5	68.0
		*	ns	ns	ns	ns	ns	ns	ns

[a] *, ns = means of treated samples were significantly or not significantly different from those of controls at the 0.01 level of probability based on analysis of variance (moisture and total nitrogen) or *t* test (nitrogen solubility).
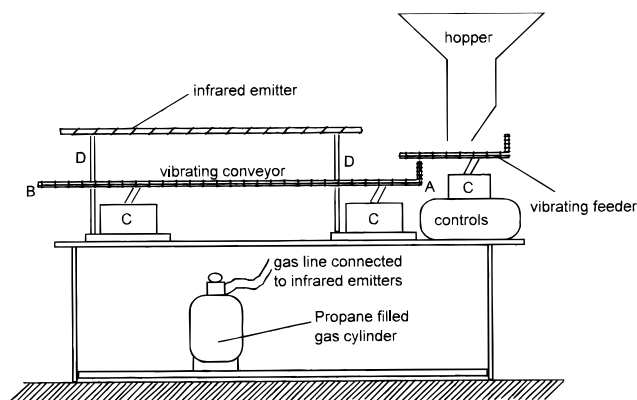

Figure 1. Schematic diagram of the laboratory-scale micronization system: A–B, vibrating conveyor; C, magnetic vibrator; D, stand supporting infrared emitters and side plates.

grain/seed was ground, and their moisture and total nitrogen contents were determined.

Solubility Fractionation. The classic Osborne (1895) procedure was used to prepare solubility fractions by consecutive extraction with deionized water at pH 6.0, 0.5 M NaCl, and 70% (v/v) ethanol. Meal samples (1.00 g) were extracted with 25 mL of solvent in a 50-mL beaker with moderate mixing at RT for 30 min, followed by centrifugation at 5000*g* for 10 min at 20 °C. The extraction was repeated on the sedimented samples with 25 mL of the same solvent. After centrifugation, the corresponding supernatants of the two extractions were combined and made up to 50.0 mL with the same solvent, and 5.00 mL of the combined soluble fractions was used for nitrogen determination (AACC Method 46-23). To investigate the nature of micronization-induced denaturation of proteins, residues after this successive extraction were further extracted twice with 25 mL of borate buffer (pH 10.0, 0.025 M) containing 0.5% (w/v) sodium dodecyl sulfate (SDS) followed by two extractions with 25 mL of the same buffer containing 0.5% (w/v) SDS and 0.6% (w/v) 2-mercaptoethanol (MCE) (Wall et al., 1975). Nitrogen in these fractions was determined as described above.

Solubility Curve. AACC (1995) Method 46-23 was used for determination of nitrogen solubilities in water at pH 2, 4, 6, 8, 10, and 12, except that extractions were conducted using 0.5 g of sample (weighed to 0.1 mg) and 80.0 mL of deionized water with moderate mixing at RT for 30 min after pH adjustment. Hydrochloric acid (HCl, 1 and 0.1 N) and NaOH

(1 and 0.1 N) were used for pH adjustment; the amount of HCl or NaOH used was recorded for correction of total solvent volumes.

Statistical Analysis. Micronization was performed in duplicate on each sample, and moisture and total protein contents were determined twice for each of the duplicates. The duplicate samples were combined, and nitrogen solubility was determined in duplicate for the combined samples. Data were subjected to analysis of variance (ANOVA) or *t* test using Minitab statistical software (Minitab Inc., State College, PA).

RESULTS AND DISCUSSION

Effect of Micronization on Moisture Content and Nitrogen Solubilities in Osborne Extraction. Micronization is a short-time and high-temperature dry treatment that generally results in moisture loss. After micronization treatment, moisture content was reduced by 6 (rye) to 40% (barley) in the cereals heated to 115 °C (Table 1) and by 7 (green pea) to 39% (pinto beans) in the legumes heated to 140 °C (Table 2). For cereals, moisture loss after micronization was significantly correlated ($p < 0.1$) to the original grain moisture contents, whereas no correlation between moisture loss and original moisture content was found for legumes. This was probably attributed to the differences in original moisture content, grain size, composition, and structure between cereals and legumes.

Total nitrogen contents were 1.8–2.7% for the cereals and 3.8–4.4% for the legumes, respectively equivalent to 10–15 and 22–25% protein contents (N × 5.7) (Tables 1 and 2). As expected, micronization had little effect on total nitrogen content for either cereals or legumes.

The solubility fraction extracted with water at pH 6.0, attributed to albumins and non-protein nitrogen (Nikokyris and Kandylis, 1997), varied from 4 to 38% for nontreated cereals (Table 1) and from 24 to 27% for nontreated legumes (Table 2). Nontreated legumes showed high (48–61%) nitrogen solubilities extracted in 0.5 M NaCl, indicating their high levels of globulins (Sosulski, 1977). The NaCl-soluble nitrogen was quite low for the nontreated cereals, being only 2–14% (Table 1). The ethanol-soluble fraction, namely prolamins, was only 2–3% for the legumes (Table 2) and for millet and wildrice (Table 1). About 32–37% of ethanol-soluble

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Table 2. Moisture, Total Nitrogen Contents, and Nitrogen Solubility of Legumes Subjected to Micronization (140 °C Surface Temperature)

seed	treatment	moisture (% as-is)	total N (% db)	nitrogen solubility (% total N)					
				pH 6.0	0.5 M NaCl	70% EtOH	0.5% SDS	0.5% SDS/0.6% MCE	total
green pea	control	9.8	3.96	24.2	61.2	2.6	11.4	1.4	100.8
	treated	9.1	4.07	14.2	25.6	2.7	47.9	1.6	92.0
		*[a]	ns[a]	*	*	ns	*	ns	*
yellow pea	control	10.4	3.95	25.9	57.3	2.7	13.2	1.5	100.6
	treated	8.8	4.11	17.9	20.9	2.7	56.1	1.4	99.0
		*	ns	*	*	ns	*	ns	ns
kidney bean	control	8.4	4.38	26.9	54.2	3.3	13.3	1.3	99.0
	treated	7.3	4.35	23.7	48.3	3.0	24.6	1.3	100.9
		*	ns	*	*	ns	*	ns	ns
black bean	control	8.4	4.05	24.9	52.7	2.6	15.6	2.4	98.2
	treated	7.0	3.96	15.8	40.1	2.8	40.4	2.3	101.4
		*	ns	*	*	ns	*	ns	ns
lentil	control	8.1	4.18	23.7	53.2	2.7	11.2	1.4	92.2
	treated	5.7	4.19	16.3	48.7	2.7	28.2	1.3	97.3
		*	ns	*	*	ns	*	ns	ns
pinto bean	control	8.8	3.75	24.7	47.9	2.6	13.0	1.7	89.9
	treated	5.4	3.73	17.4	34.4	2.9	26.4	1.4	82.5
		*	ns	*	*	ns	*	ns	*

[a] *, ns = means of treated samples were significantly or not significantly different from those of controls at the 0.01 level of probability based on analysis of variance (moisture and total nitrogen) or *t* test (nitrogen solubility).

nitrogen was detected in triticale, wheat, and barley. The proportions of solubility fractions for the cereals and legumes were generally in agreement with values reported previously (Sosulski, 1977; Lasztity, 1984; Nikokyris and Kandylis, 1997) except for millet. Compared to other cereals, wildrice and millet have received scant attention particularly in regard to nitrogen solubility. Low nitrogen solubilities in wildrice may be attributed to its high glutelin contents. Wang et al. (1978) reported that wildrice had a high proportion of glutelins and was low in prolamins compared to wheat, corn, barley, and oat. Another possible explanation for the low solubilities observed in wildrice is that commercial wildrice is often processed by field fermentation and parching where the grain is dried to ~135 °C at 40−45% moisture content (Oelke et al., 1997). In our additional experiments, freshly harvested and air-dried wildrice showed higher nitrogen solubilities in Osborne extraction than the commercial sample (41 vs 8%). Data in Table 1 and Figure 2 demonstrated that micronization had little additional effect on nitrogen solubility for commercial wildrice. Low nitrogen solubilities in millet obtained after Osborne fractionation could also be attributed to preprocessing treatments or storage time, which were unknown to us, because high values of albumins (3−22%), globulins (3−25%), and prolamins (6−39%) were reported for a variety of millets (Hulse et al., 1980). However, Lorenz and Dilsaver (1980) reported only 5−15% nitrogen solubilities for millet flours compared to 23−67% solubilities for a wheat flour at pH 2−10. In their study, heating the millet flour at 80 °C for 1 h had little effect on nitrogen solubility. In our study, micronization also showed negligible effect on Osborne solubilities (Table 1). Therefore, a varietal variation may also account for low nitrogen solubilities in millet.

Micronization reduced nitrogen solubility in water at pH 6.0 by 13−54% for cereals (Table 1) and by 12−41% for legumes (Table 2), indicating denaturation of albumins. Compared to control samples, nitrogen solubility in 0.5 M NaCl was reduced by 9−64% for legumes (Table 2) and by 30−37% for barley, wheat, and triticale after micronization (Table 1). For rye, however, nitrogen solubility in 0.5 M NaCl was not affected by micronization. Nitrogen solubility in 70% ethanol was

not affected by micronization for all legumes and cereals except for triticale and barley (Tables 1 and 2). The significant reduction in ethanol-soluble nitrogen of micronized barley and triticale (Table 2) suggested that denaturation of prolamins occurred in these grains. It is generally recognized that albumins and globulins are more sensitive to heat than prolamins and glutelins. When a heat treatment is applied to albumins and globulins, their structures are altered so that the protein molecules are unfolded and hydrophobic sites are exposed, resulting in reduced solubility (Nakai and Li-Chen, 1989). Shiau and Yang (1982), working on sorghum, reported that micronization reduced protein solubility extracted in 0.5 M NaCl solution to greater extent than those extracted in 2-propanol and MCE or in borate buffer plus NaCl and MCE. Similar results were reported for wheat and barley (South and Ross, 1993).

Nitrogen Solubilities with SDS and MCE. Heat-induced denaturation in plant proteins may involve aggregation of polypeptide chains through either hydrophobic conformation or disulfide bonding or both. Such denatured proteins should be solubilized by dissociating and reducing agents such as SDS and MCE. Extracting the residues (after Osborne fractionation) with 0.5% SDS in pH 10 borate buffer yielded 15−65% solubility for cereals and 11−56% solubility for legumes (Tables 1 and 2). Further extracting the residues with 0.6% MCE in addition to 0.5% SDS in pH 10 borate buffer resulted in 5−40% additional solubility for cereals but only 1−2% additional solubility for the legumes (Tables 1 and 2), indicating the significance of intermolecular disulfide bonding in cereal proteins. Nitrogen solubility with SDS exceeded 60% in millet and 35% in wildrice, whereas nitrogen solubility with MCE was only 9% for millet and 24−26% for wildrice (Table 1). These results indicated that the insolubility of millet protein was primarily due to the formation of hydrophobic bonds, whereas both hydrophobic and disulfide bonds were responsible for the insolubility of proteins in wildrice. Micronization showed little effect on nitrogen solubilities with either SDS or MCE in millet and wildrice (Table 1).

Wall et al. (1975) reported that drying corn grain with heated air reduced nitrogen solubilities due to the
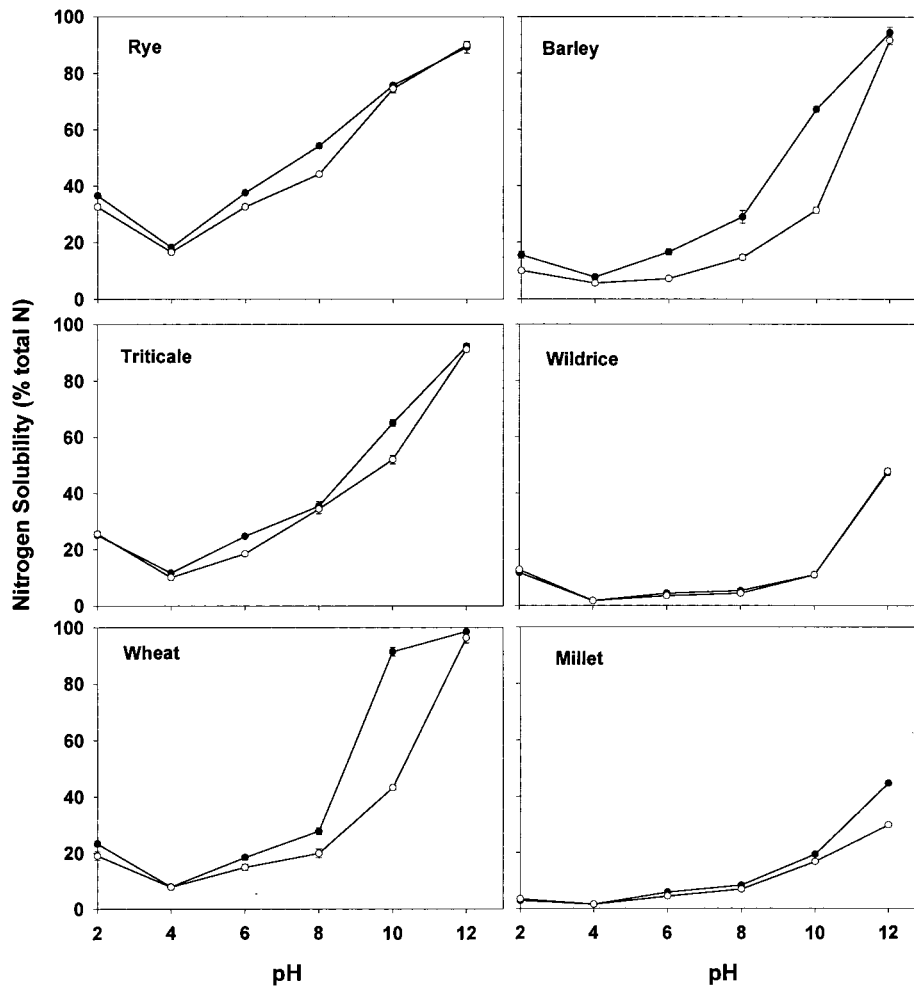
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Figure 2. Nitrogen solubility curves of nontreated (●) and micronized (○) cereals. Vertical bars indicate standard errors. Grains were micronized to 115 °C surface temperature at as-is moisture level (Table 1).

formation of hydrophobic and disulfide bonds. Data shown in Tables 1 and 2 demonstrated that hydrophobic aggregation was the primary form of denatured albumins and globulins in legumes, whereas the nature of denaturation in cereal proteins was dependent on the type of grain. For example, micronized rye showed significantly higher solubility in 0.5% SDS borate buffer than the control, indicating the formation of hydrophobic bonds in rye grain after micronization. On the other hand, micronized triticale and barley showed significantly higher nitrogen solubility in MCE than control samples (Table 1), suggesting additional intermolecular disulfide bonds induced by micronization. For wheat, however, micronization reduced nitrogen solubility with MCE, resulting in lower total solubility than the control (Table 1). More severe structural changes, which were not investigated in this study, may have occurred in wheat protein after micronization.

Nitrogen Solubility Curves after Micronization. Nitrogen solubility curves as a function of extraction pH are presented in Figures 2 and 3. Because grain/seed proteins have an isoelectric point of pH 3.5—4.5 (Sosulski, 1977), both cereals and legumes used in this study showed the lowest solubility at pH 4. Solubility at this pH, largely attributed to non-protein nitrogen and/or free amino acids, varied from 1 to 2% (millet and wild rice) to 18% (rye); most grain/seed was in the range of 5—8%. This was in agreement with the findings of

Nikokyris and Kandylis (1997). Micronization had little effect on nitrogen solubility at pH 4.0 for all cereals and legumes.

In general, all cereals showed similar solubility patterns and so did the legumes. At acid pH (pH 2.0), cereals showed lower solubility than legumes. Micronization had little effect on acid solubility for the cereals (Figure 2) but significantly reduced solubility at pH 2.0 for the legumes (Figure 3). Compared to cereals, legume proteins have high levels of acidic and basic amino acids, giving high solubility at pH above or below their isoelectric points (Sosulski, 1977). Reduced solubility at pH 2.0 for the legumes suggested that micronization reduced hydrophilicity of legume proteins, probably due to unfolding of protein molecules. Neucere and Cherry (1982) reported that peanut globulin underwent the formation of unordered structures after heating.

Increasing extraction pH greater than pH 4 resulted in progressive increase in nitrogen solubility for both cereals (Figure 2) and legumes (Figure 3). The increase was more rapid for legumes than for cereals, again due to a higher level of ionic amino acids in legume proteins. For nontreated legumes, >80% of nitrogen was solubilized at pH 8.0, whereas only <30% solubility was obtained at the same pH for cereals with the exception of rye. Compared to other cereals, rye had the highest nitrogen solubilities in water at pH 2—6 (Figure 2) and in 0.5 M NaCl (Table 1). Chen and Bushuk (1970) reported higher albumin contents in rye than in wheat
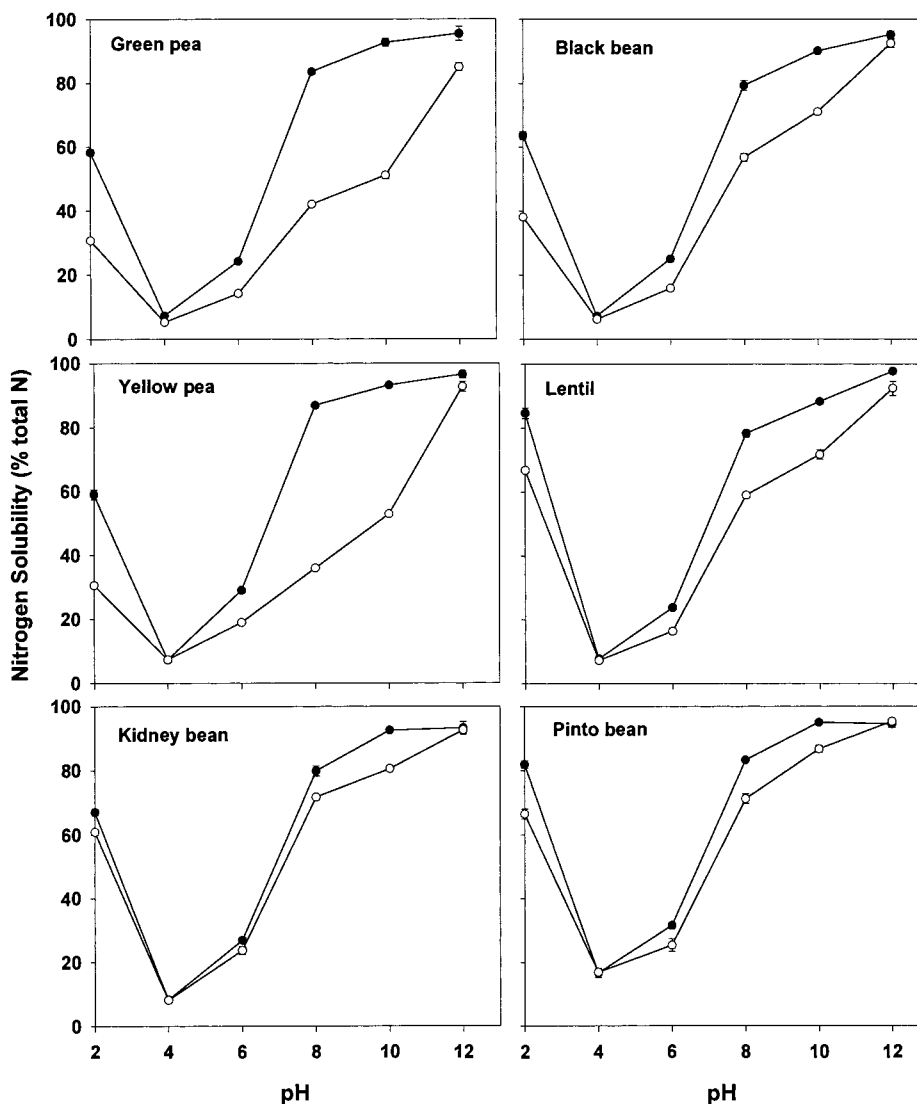
Figure 3. Nitrogen solubility curves of nontreated (●) and micronized (○) legumes. Vertical bars indicate standard errors. Grains were micronized to 140 °C surface temperature at as-is moisture level (Table 2).

and triticale. Micronization reduced nitrogen solubility mainly at pH 6–10 for both cereals and legumes, probably due to heat-induced structure changes as discussed above. For most cereals and legumes, nitrogen solubility at pH 12 was not affected by micronization, suggesting denatured proteins were extractable in alkali. However, nitrogen solubility at pH 12 was reduced by micronization for green pea and millet (Figures 2 and 3).

Effects of Moisture and Micronizing Temperature on Nitrogen Solubility. Hull-less barley with a moisture content of 13.3% was tempered to 19.2 or 26.5% moisture and subsequently micronized to a surface temperature of 115, 135, or 150 °C. Similarly, pinto bean seed adjusted to 8.8, 15.1, or 18.2% moisture was micronized to 125, 140, and 150 °C. Barley showed high correlations ($p < 0.05$) between initial grain moisture level and moisture loss after micronization and between temperature and percent moisture loss. The same relationships were also found for pinto bean, but the correlations were not significant (Table 3).

When barley grain was micronized at 115 °C and 13% moisture content, nitrogen solubility in water at pH 6 was reduced from 17 to 8% (Figure 4), a value that approached the nitrogen solubility at pH 4 (Figure 6)

Table 3. Effects of Initial Moisture Content and Temperature on Final Moisture Contents of Hull-less Barley and Pinto Beans Subjected to Micronization

temp (°C)	initial moisture (% as-is)	final moisture (% as-is)
	Hull-less Barley	
control	13.3	13.3
115	13.3	8.0
115	19.2	8.9
115	26.5	10.4
135	13.3	7.3
150	13.3	5.4
LSD[a] ($p < 0.01$)		1.9
	Pinto Beans	
control	8.8	8.8
125	8.8	5.5
125	15.1	7.7
125	18.2	10.3
140	8.8	5.4
150	8.8	4.2
LSD ($p < 0.01$)		1.8

[a] Least significant differences based on analysis of variance (ANOVA).

and remained relatively constant at higher moisture contents or higher micronization temperatures (Figure 4). These results suggested that most albumins in
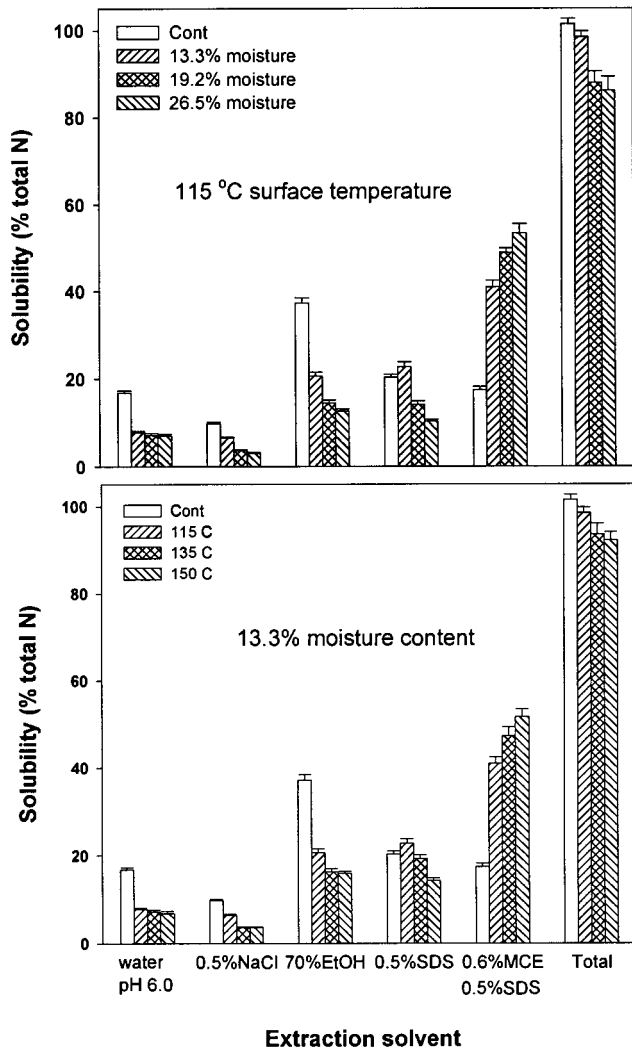
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Figure 4. Nitrogen solubilities of hull-less barley extracted with various solvents. Barley grain was tempered to different moisture contents prior to micronization to 115 °C surface temperature (top) or micronized to various surface temperatures at a moisture level of 13.3% (bottom). Vertical bars indicate standard errors.

Figure 5. Nitrogen solubilities of pinto beans extracted with various solvents. Pinto beans were tempered to different moisture contents prior to micronization to 125 °C surface temperature (top) or micronized to various surface temperatures at a moisture level of 8.8% (bottom). Vertical bars indicate standard errors.

barley grain were readily denatured under the relatively milder micronization condition. Increasing grain moisture or micronization temperature progressively reduced nitrogen solubilities of barley grain in 0.5 M NaCl and in 70% ethanol (Figure 4). Nitrogen solubility with 0.5% SDS in borate buffer after Osborne extraction showed a slight increase at the mildest (13.3% moisture and 115 °C surface temperature) micronization condition and then decreased particularly with the increase in grain moisture. In contrast, solubility with the addition of 0.6% MCE (after Osborne fractionation and extraction with 0.5% SDS) increased correspondingly with the decrease in Osborne solubilities (Figure 4). These results clearly demonstrated that micronization induced intermolecular disulfide bonding in barley protein and that the extent of denaturation was manipulated by both grain moisture and the time of exposure of the grain to infrared heating. Nitrogen solubility curves (Figure 6) showed that most barley proteins were readily denatured at 115 °C and 13% moisture; however, the denatured proteins could be solubilized in alkali. Increasing micronization temperature, particularly at high moisture levels, would result in severe damage to
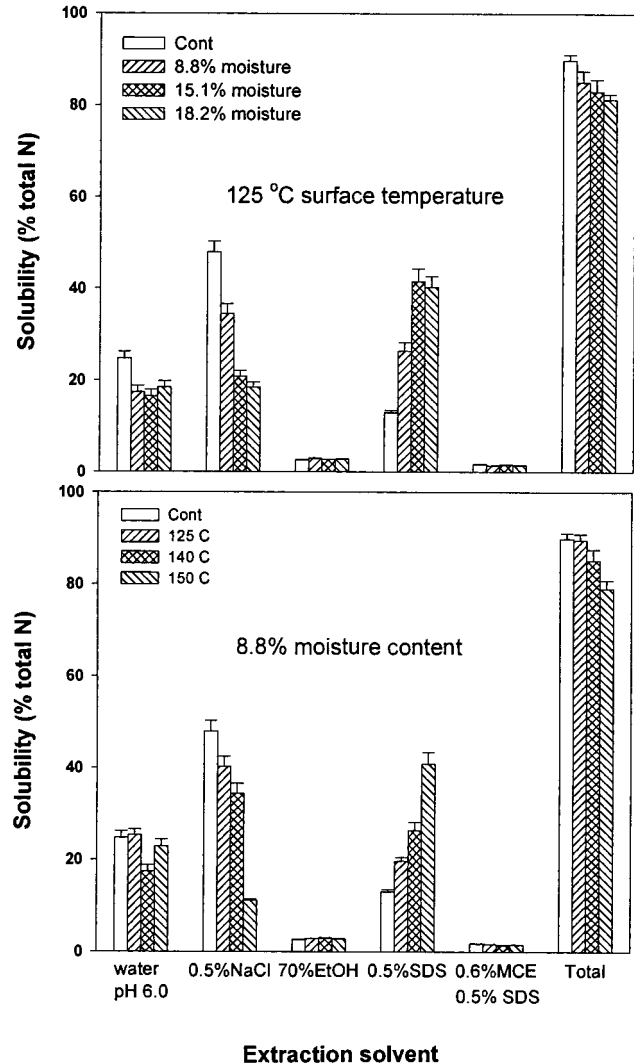
the proteins as they became insoluble after five consecutive extractions (Figure 4) or at pH 12 (Figure 6).

Figure 5 shows nitrogen solubility extracted with various solvents for pinto beans after micronization at various levels of moisture content and temperature. Similar to barley, micronization reduced solubility in water at pH 6 and in 0.5 M NaCl for pinto bean. However, nitrogen solubility in 70% ethanol was not affected by micronization irrespective of temperature or moisture content. Increasing moisture to 15% at 125 °C or increasing temperature to 140 °C resulted in additional reduction of nitrogen solubility in water at pH 6. However, micronization at 150 °C with 9% moisture or at 125 °C with 18% moisture resulted in slight increases in nitrogen solubility in water. It is well-known that excessive heating, particularly with high moisture contents, may induce disruption of the insoluble aggregates (Newcere and Cherry, 1982) or pyrolysis of proteins (Hansen et al., 1975), resulting in relatively high nitrogen solubility. For pinto beans, the increased nitrogen solubility in water at pH 6 was indicative of protein breakdown at high temperature and high moisture level. Nitrogen solubility in 0.5 M
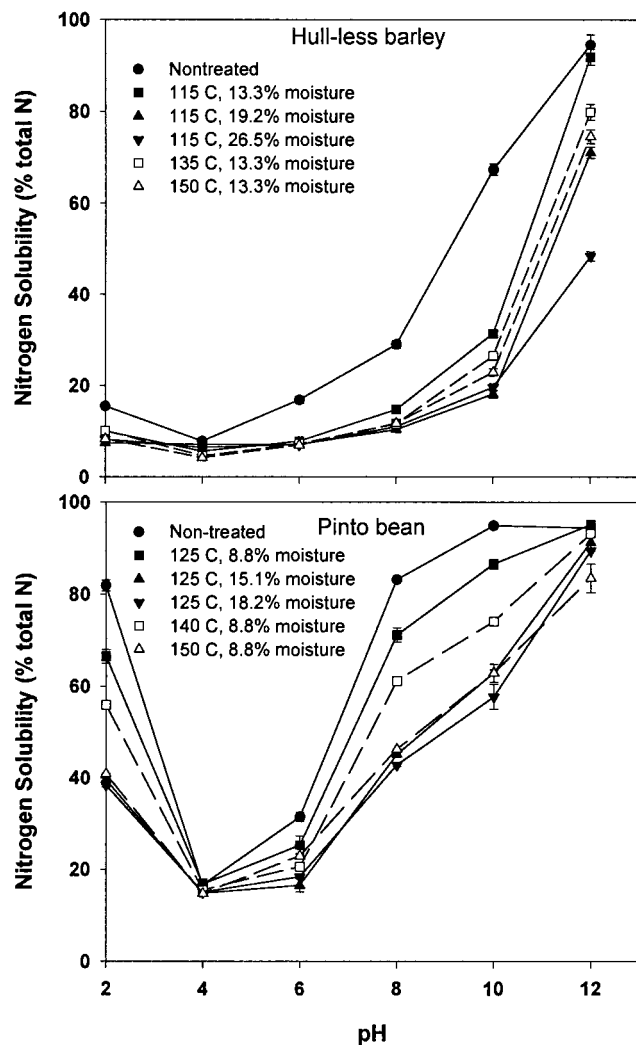
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Figure 6. Nitrogen solubility curves of hull-less barley and pinto beans micronized to various surface temperature at various moisture contents. Vertical bars indicate standard errors.

NaCl was progressively reduced by micronization with either increasing temperature or increasing moisture content. The denaturation of saline-soluble proteins was mainly due to hydrophobic aggregation, as a significant ($p < 0.01$) negative correlation was found between nitrogen solubilities extracted with 0.5% NaCl and those with 0.5% SDS in borate buffer. Reduced total nitrogen solubility at the increased moisture level and temperature indicated more stable conformations of polypeptide chains after micronization. Nitrogen solubility curves also showed that nitrogen solubilities at pH 2 and 6−12 were gradually reduced as micronization temperature and moisture content increased (Figure 6). Unlike barley, pinto bean showed high (>80%) nitrogen solubility at pH 12, even at the highest micronization temperature and moisture level.

Conclusion. Micronization is a short-time and high-temperature dry treatment for precooking cereals and legumes. The present study indicated that micronization resulted in protein denaturation of cereals and legumes, and the denaturation was more pronounced in water- and salt-soluble proteins (albumins and globulins). For some cereals such as barley and triticale the denaturation also occurred to prolamins. Micronization-induced denaturation in legumes was mainly due to hydrophobic aggregation of polypeptide chains. For cereals, both hydrophobic and disulfide bonds were formed after micronization. Like other heat treatments, moisture content and the time of exposure of the grain/seed to infrared heating had significant effects on protein solubility in cereals and legumes. At normal moisture level (10−14% for most cereals and legumes) and micronizing temperatures up to 115 °C for cereals and 140 °C for legumes, protein denaturation was mild. However, micronization at high moisture content, for example, 26% for barley, or high temperature, for example, 150 °C for pinto beans, may result in severe damage to proteins. Results of wildrice and millet suggested limited effect of micronization on nitrogen solubility of preprocessed cereals.

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