Extraction of Proteins from Steamed Soybean Meal with Water and Aqueous 2-Mercaptoethanol

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ABSTRACT

Mildly steamed, defatted soybean meals were extracted with water or with 0.01 *M* or 0.1 *M* 2-mercaptoethanol (2-ME). The extracts were dialyzed and then analyzed for protein content and ultracentrifugal composition. Protein extractability was increased by 2-ME. Ultracentrifugation indicated that the water extracts contained disulfide polymers of the 7S and 11S proteins; in the 0.01 *M* 2-ME extracts, however, only the 7S proteins appeared to repolymerize when the reductant was removed by dialysis. In the 0.1 *M* 2-ME extracts neither the 7S nor 11S proteins repolymerized on elimination of the reducing agent. In contrast, in 0.1 *M* 2-ME extracts of unsteamed meals, both 7S and 11S proteins repolymerized when dialyzed free of 2-ME.

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Soybean proteins consist of four major ultracentrifugal fractions with sedimentation coefficients of approximately 2S, 7S, 11S, and 15S. The 7S and 11S make up about 70% of the total protein (Wolf et al 1962). These two fractions also show the ability to form disulfide-linked polymers (Nash and Wolf 1967). Such polymers are found in defatted soybean meal. The proteins in the meal are only partially extractable with water. Previously, we determined the effects of 0.01 *M* and 0.1 *M* 2-mercaptoethanol (2-ME) on protein extractability from unheated meal and found that the reducing agent increased the extractability of 7S and 11S fractions, presumably by reducing the intermolecular disulfides to yield the more soluble monomeric forms (Nash et al 1974). We have now examined the effects of the same concentrations of 2-ME on extractability of proteins from lightly steamed meal. Such steaming may occur during conditioning or desolventizing of flakes in commercial solvent extraction, particularly with flakes intended for preparation of isolates. We also determined the ultracentrifugal composition of the proteins extracted with 2-ME and the tendencies of the 7S and 11S proteins to repolymerize when the 2-ME was removed from the extracts.

MATERIALS AND METHODS

Preparation of Soybean Meal

Hexane-defatted flakes were freshly prepared from Hawkeye-63, Adams and Clark varieties. A portion of each variety sample was steamed in a preheated autoclave at 100°C (atmospheric pressure) for 4 min; unsteamed portions served as controls. All samples were ground in a Wiley-type mill to pass a 40-mesh screen.

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Extraction of Soybean Meal

Extracts were prepared and treated as described (Nash et al 1974). Briefly, treatments consisted of short-term (minimum of four days) and exhaustive dialysis (14 days). The latter assured removal of 2-ME when present and partially precipitated the proteins in order to promote disulfide polymer formation.

Protein Concentration and Ultracentrifugal Analyses

After dialysis, the extracts were adjusted in volume and analyzed for Kjeldahl nitrogen and ultracentrifugal composition as previously reported (Nash et al 1974).

Ultracentrifugal pattern areas were calculated by Pickels' method (1952) and were expressed in arbitrary area units, which resulted from a tenfold magnification of the cell at 70° phase plate angle. No corrections were made for area anomalies (Johnston and Ogston 1946).

Statistical Methods

Analyses of variance were run on data from each total protein measurement and each component area. Variations associated with extraction media, reducing agent in the analytical buffer, and meal steaming were examined for significance. Extracts from three soybean varieties were subjected to short-term and exhaustive dialysis treatments to provide replication in the experiment. All effects reported are based on statistical significance calculated at the 5% level.

RESULTS

Steamed and control meals of three soybean varieties were extracted with water, 0.01 *M*, or 0.1 *M* 2-ME, and after equilibration with phosphate buffer, were analyzed for Kjeldahl nitrogen and in the ultracentrifuge. The results indicated no differences between short-term and exhaustively dialyzed extracts of steamed meals; no protein insolubilization, change in composition, or loss of protein on exhaustive dialysis occurred,

which contrasts with the results noted with extracts of unheated meals (Nash et al 1974). We therefore averaged the results for the two dialysis treatments (Table I).

Effect of 2-ME on Protein Extractability

As it did with unheated meals (Nash et al 1974), aqueous 2-ME extracted more protein from steamed meals than did water (Table I). Water in a single 1:10 meal/water extraction solubilized 46% of the meal nitrogen (Extract 1A), and 2-ME extracted 65–68% (Extracts 2A and 3A). Extraction of control meals with water yielded 54% of the meal nitrogen, whereas with 2-ME, extraction yielded 78–80%. Ultracentrifugal analysis confirmed that aqueous 2-ME was a better extractant than water; total pattern areas were always greater for reductant extracts than for water extracts. For example, comparison of extracts 1B and 2B shows that 0.01*M* 2-ME extracted about 50% more nitrogen and 7S, 11S, and 15S fractions from steamed meal than did water.

Occurrence of Disulfide Polymers in Water Extracts

Ultracentrifugal analyses of the water extracts from steamed meals with and without 0.01*M* 2-ME in the buffer were used to detect disulfide polymers of the various fractions (Nash et al 1974). Comparison of extracts 1A and 1B (Table I) shows that, with 2-ME present, the 15S and >15S fractions decrease significantly accompanied by an increase in the 7S and 11S fractions ($P<0.10$). These shifts in areas indicate that disulfide polymers of the 7S and 11S proteins existed in the water extracts of the steamed meals. The amounts of these water-soluble polymers were estimated by taking the differences in pattern areas for the two solutions (Table II). The amounts of disulfide polymers of 7S plus 11S in the water extracts of steamed meals were similar to those in water extracts of control meals. Apparently, mild steaming of the meal, such as we used, did not insolubilize the water-soluble disulfide polymers.

Reformation of Disulfide Polymers

Increased extractability of protein from the unsteamed and steamed meals by 0.01*M* or 0.1*M* 2-ME can be attributed to cleavage of intermolecular disulfide bonds in polymers of the 7S and 11S proteins. Previously, we found that when 2-ME extracts of unsteamed meals were dialyzed to remove the reducing agent, disulfide polymers of the 7S and 11S fractions reformed (Nash et al 1974). Such reformation of disulfide polymers is evident on comparison of control meal extract 2C with 2D as well as of extract 3C with 3D (Table I). Calculated differences in the amounts of 7S and 11S fractions between these extract pairs are shown in Table II. From Table II it is also apparent that dialysis of the 2-ME extracts against buffer without reducing agent generated significantly more polymers than were extracted with water from these same meals (compare Δ area units of 7S + 11S for extracts 2D − 2C and 3D − 3C with extract 1D − 1C).

The 2-ME extracts of steamed meals that were dialyzed free of 2-ME did not behave the same as those from unsteamed (control) meals. Dialysis of the 0.01*M* 2-ME extract of steamed meal resulted in repolymerization of only the 7S fraction; the amount of 11S fraction remained unchanged (extract comparison 2B − 2A, Table II). In the 0.1*M* 2-ME extract of steamed meal, neither the 7S nor 11S fraction showed evidence of repolymerizing when the 2-ME was removed (extract comparison 3B − 3A, Table II).

DISCUSSION

Steaming defatted soybean meal decreases protein solubility, but our results show that insolubilization can be reversed at least partially by using 0.01*M* or 0.1*M* aqueous 2-ME as the extraction solvent. Nonetheless, the amount of protein extracted was always less than that extracted with the same solvents from control meals that were not steamed. Under the comparatively mild conditions of moist heating we used, the sedimentation properties of extractable proteins were comparable to those of proteins extracted from control meals.

Subtle changes were observed, however, in the behavior of the 7S and 11S proteins from steamed meals when they were extracted with 2-ME and the reducing agent was then removed by dialysis. After extraction with 0.01*M* 2-ME, the 11S fraction from steamed meals failed to repolymerize on dialysis against buffer free of reducing agent. When these meals were extracted with 0.1*M* 2-ME, both the 7S and 11S fractions apparently lost their ability to repolymerize when the reducing agent was removed. This contrasts the behavior of the 7S and 11S proteins in the 0.01 and 0.1*M* 2-ME extracts of unsteamed meals; in these extracts both proteins repolymerized when the reductant was removed. We observed earlier (Nash et al 1974) that the 7S and 11S proteins in 0.1*M* 2-ME extracts from aged meals also failed to repolymerize on dialysis. Aging or mild heating may cause conformational changes in the 7S and 11S proteins that allow 2-ME to reduce critical internal disulfide bonds and permit structural changes that prevent subsequent repolymerization.

Our findings that meal heating invokes greater sensitivity to reducing agent in the 11S protein than in the 7S protein apparently contrast studies by Koie and Djurtoft (1977). They heated soybean meal at various temperatures and moisture contents and then analyzed the meals by immunoelectrophoresis. Of 18 soybean proteins, the 11S protein was the most stable to heat, based on immunochemical analysis.

TABLE I
Effect of 2-Mercaptoethanol (2-ME) on Extractabilities and Ultracentrifugal Compositions of Proteins from Steamed and Unsteamed Soybean Meals[a]

Extractant	Treatment: Pre-extraction	Treatment: Analytical 2-ME in Buffer	Extract Number	Nitrogen Conc. (mg/ml)	Meal Nitrogen Extracted (%)	Ultracentrifugal Areas: 2S	7S	11S	15S	>15S
Water	Meal steaming	−	1A	2.15	45.7	5.59	5.36	8.55	3.09	4.37
	Meal steaming	+	1B	2.14	45.3	5.86	8.00	9.16	1.39	2.18
	Control	−	1C	2.56	54.2	7.64	7.03	6.91	2.29	4.49
	Control	+	1D	2.55	53.9	8.00	8.97	7.69	1.11	2.70
0.01*M* 2-ME	Meal steaming	−	2A	3.08	65.4	5.42	8.76	14.64	5.16	5.05
	Meal steaming	+	2B	3.16	66.9	6.14	12.31	14.73	2.43	3.55
	Control	−	2C	3.67	77.5	7.55	10.22	11.41	4.59	6.70
	Control	+	2D	3.63	76.7	8.04	13.34	13.01	2.14	4.75
0.1*M* 2-ME	Meal steaming	−	3A	3.19	67.7	5.92	12.02	14.32	2.92	4.06
	Meal steaming	+	3B	3.15	66.8	6.13	12.22	14.59	2.52	3.57
	Control	−	3C	3.79	80.0	7.23	10.60	13.32	4.56	7.27
	Control	+	3D	3.78	79.9	7.78	13.14	14.80	2.50	5.16
LSD[b], 95% level				0.10	2.1	0.42	0.65	0.62	0.49	0.75

[a] Values are means of duplicate analyses of extracts from three soybean varieties.
[b] Least significant difference for comparisons of means at the same extraction level.

Disulfide cleaving reagents have been used to solubilize proteins from steamed meals with extensive denaturation (Shibasaki et al 1969, Cooke et al 1970). Our results indicate that use of a reducing agent is also advantageous in extraction of proteins from flakes that have received minimal heat treatment, such as are used to prepare protein isolates. Increased extraction of protein is the major advantage of adding a reducing agent to the extractant, but decreased molecular sizes of the proteins are other possible benefits.

TABLE II
Extent of Disulfide Polymer Formation of 7S and 11S Proteins in Extracts

Extraction Solvent	Meal Treatment	Extract Comparison[a]	Disulfide Polymers in Δ Area Units		
			7S	11S	7S + 11S
H_2O	Steaming	1B − 1A	2.64	0.61	3.25
H_2O	None	1D − 1C	1.94	0.78	2.72
0.01 *M* 2-ME[b]	Steaming	2B − 2A	3.55	0.09	3.64
0.01 *M* 2-ME	None	2D − 2C	3.12	1.60	4.72
0.1 *M* 2-ME	Steaming	3B − 3A	0.20	0.27	0.47
0.1 *M* 2-ME	None	3D − 3C	2.54	1.48	4.02

[a] See Table I for identities of extracts.
[b] 2-ME = 2-mercaptoethanol.

LITERATURE CITED

COOKE, R. R., HUNTER, J. E., and MITCHELL, R. W. 1970. Making soya flour functional in prepared culinary mixes. U.S. Patent 3,542,562.

JOHNSTON, J. P., and OGSTON, A. G. 1946. A boundary anomaly found in the ultracentrifugal sendimentation of mixtures. Trans. Faraday Soc. 42:789.

KOIE, B., and DJURTOFT, R. 1977. Changes in the immunochemical response of soybean proteins as a result of heat treatment. Ann. Nutr. Aliment. 31:183.

NASH, A. M., KWOLEK, W. F., and WOLF, W. J. 1974. Extraction of soybean proteins with aqueous 2-mercaptoethanol. Cereal Chem. 51:220.

NASH, A. M., and WOLF, W. J. 1967. Solubility and ultracentrifugal studies on soybean globulins. Cereal Chem. 44:183.

PICKELS, E. G. 1952. Ultracentrifugation. Methods Med. Res. 5:107.

SHIBASAKI, K., OKUBO, K., and ONO, T. 1969. Food chemical studies on soybean proteins. V. On the insoluble protein components of defatted soybean heated by steaming. Nippon Shokuhin Kogyo Gakkai-Shi 16:22.

WOLF, W. J., BABCOCK, G. E., and SMITH, A. K. 1962. Purification and stability studies of the 11S component of soybean proteins. Arch. Biochem. Biophys. 99:265.

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