Thermal Denaturation of Soy Proteins as Related to Their Dye-Binding Characteristics and Functionality

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The binding of Cresol Red and of Acid Orange 10 (in the absence and presence of urea) to unheated and progressively heated, defatted soy meal was compared with their NSI values, urease activities, *in vitro* digestibilities, unreactive lysine contents, and foaming and emulsifying capacities. These results suggested that increased amounts of Cresol Red and of Acid Orange 10 (in the presence of urea) bound to the heated samples were due to the progressive exposure of hydrophobic residues caused by thermal denaturation. High statistical correlations were obtained between dye-binding, the duration of heating, and functional properties. Our results indicate that dye-binding has potential for predicting certain functional properties as well as for monitoring thermal denaturation of soy proteins.

KEY WORDS: Dye-binding, functionality, soy proteins, thermal denaturation.

Different organic dyes have been used for centuries for dyeing wool and other fabric materials, and the nature of the interaction between dyes and proteins has been studied intensively since the end of the nineteenth century (1). Also, these techniques have been used to estimate the quantity and to evaluate the nutritional quality of food proteins (2–4). Various sulphonated azo dyes, including Cresol Red (*o*-cresolsulfon phthalein) have also been commonly used to estimate the nutritional quality of soy and rapeseed meals (5). Cresol Red has also been used to evaluate the nutritional quality of soy meal and has been shown to be well correlated with data from biological trials with chicks (6).

The empirical nature of these techniques has resulted in controversial and even conflicting results, thus limiting their wide spread application in the food industry. By understanding dye-binding mechanism(s), perhaps it will become possible to develop a method for monitoring thermal denaturation of soy proteins for routine quality control.

The present work is concerned with investigating correlations between Cresol Red binding and other well-accepted (or practiced) methods for monitoring denaturation of soy proteins (e.g., Nitrogen Solubility Index Test, Urease Activity Test, unreactive lysine measurement, *in vitro* digestibility measurement, foaming and emulsifying property measurements). These studies should indicate the potential for the Cresol Red binding to measure the extent of protein denaturation. In addition, the binding (with or without addition of urea) of Acid Orange 10 was studied to investigate the mechanisms of the interaction between this azo dye and soy proteins.

EXPERIMENTAL PROCEDURES

Dyestuffs. Purified Acid Orange 10 (C.I. Constitution Number 16230) was obtained by recrystallizing Standacol Orange G (Williams Ltd., Hounslow, U.K.) from aqueous ethanol (water:ethanol = 1:9, v,v). Water-soluble Cresol Red from BDH Ltd. (Poole, U.K.) was used as supplied.

Heat treatment of defatted soy meal. Cracked whole soybeans were provided by British Arkady Ltd. (Manchester, U.K.). They were ground in a laboratory mill to pass 80 U.S. mesh and defatted by exhaustive elution with petroleum ether (b.p. 40–60°C) at room temperature over a period of several weeks. Subsequent desolventizing was conducted at room temperature. The crude fat content of the final product was below 1% (d.b.).

The defatted soy meal was tempered with water in a food processor to make its final moisture content 25%. Approximately 35 g of tempered soy meal were obtained from the bulk, tempered meal by automatic sampling on a Sample Divider (Model PTZ, GlenMills, NJ), and were put into aluminum trays (185 mm × 120 mm). The trays, covered with lids and wrapped with aluminum foil, were steamed at atmospheric pressure for different periods of time (i.e., 10, 20, 30, 40, 60, 90, 120 and 150 min). The samples were put into a freezer immediately after heat treatment and subsequently freeze-dried, reground and stored at room temperature in air-tight bottles.

Analytical methods and experimental procedures. Ash, moisture and crude fat contents were determined by the Association of Official Analytical Chemists (AOAC) Methods 7.009, 14.004 and 14.008, respectively (7). Table 1 shows the proximate composition of the defatted soy meal. The content of carbohydrate was calculated by difference. Protein (N × 6.25) and unreactive lysine were determined as described by Lin *et al.* (8). Digestibility *in vitro* was measured by the method of Hahn *et al.* (9). Nitrogen Solubility Index and urease activity were determined by American Oil Chemists' Society (AOCS) Standard Methods Ba 11-65 and Ba 9-58, respectively (10).

Cresol Red binding was determined as described by Frolich (5), and Acid Orange 10 binding (in the absence or presence of urea) was determined as follows. A weighed portion (1.00 g) of unheated or heated, defatted soy meal was mixed in a 100-mL plastic bottle with 50 mL of 5 mM Acid Orange 10 in 0.2 M citric acid working solution with the desired urea concentration. The bottle was shaken at 25°C ± 2°C for 24 hr. the optical density of the filtered unadsorbed dye was measured, after proper dilution with distilled water, at 470 nm on a Perkin-Elmer PE-552 Spectrophotometer. The dye-binding capacity was expressed as mM dye bound per 100 g sample, which was the

TABLE 1

Proximate Composition of Defatted Soy Meal

Component	% (d.b.)
Protein (N × 6.25)	54.7
Crude fat	1.0
Ash	6.4
Carbohydrate	38.9

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difference in dye concentrations of the working solution and that of the filtrate after reaction.

Foaming capacity was measured as follows: A weighed portion of soy meal was added to 250 mL water to make the final protein concentration of the dispersion 1% (m/v). The sample was allowed to hydrate for 30 min and then whipped in a Kenwood food mixer for 30 seconds at Speed Mark 3, and then 120 seconds at Speed Mark 6. Foaming capacity was expressed as the height of the foam in the mixing container.

The procedure employed for emulsifying capacity was substantially that of Pearce and Kinsella (11) and Yamauchi and Shibasaki (12). A weighed portion (containing 0.12 g protein) of soy meal was dispersed and hydrated for at least 30 min in 3 mL of 0.1 M potassium phosphate buffer (pH 8.0) in a 15-mL Universal glass bottle. After 3 mL of refined peanut oil had been added, the dispersion was blended by using an 'Ultra-turrax' blender (Janke & Kunkel, Kika-Werk, U.K.) at the maximum speed (ca. 20,000 rpm) for 60 seconds, with the bottle being cooled by immersion in an ice-water bath. The emulsion was diluted with 10 mL of buffer and mixed by inverting the bottle gently three times; then 0.05 mL of the diluted emulsion was further diluted with 10 mL additional buffer. With reference to the buffer as the blank, the absorbancies of duplicate aliquots of each diluted emulsion were measured at 500 nm using a Perkin-Elmer PE-552 spectrophotometer. The emulsifying capacity was expressed as turbidity/g meal (on a dry basis).

Statistical analysis. Correlation coefficients between the results of all the above tests were obtained usine the "MINITAB RELEASE" 5.1.3 program (Minitab Inc., PA).

RESULTS AND DISCUSSION

All data are reported on a dry matter basis and are the means of four replicates, except those for TIA, unreactive lysine measurement, and *in vitro* digestibility for which five replicates, duplicates and triplicates were carried out, respectively.

Nitrogen Solubility Index (NSI). Unheated soy meal was found to have a NSI of 88% (Fig. 1), which rapidly fell to 18% after heating for 60 min, and then fell more slowly to a value of 5% after heating for 150 min.

The high NSI value of the unheated soy meal indicates that native soy proteins have a rather high water-dispersibility, most likely due to the presence of hydrophilic regions at the molecular surface. The NSI value of 88% is in keeping with data reported in the literature (13,14).

The mechanisms responsible for insolubilization of soy proteins by heat treatment have been discussed by Fukushima and Buren (15,16). Intermolecular disulphide bonding polymerization and hydrophobic interaction are the two main factors causing insolubilization of soymilk proteins during heating and drying. Also, there is good correlation between hydrophobicity and protein insolubility (17). The decline of NSI values with increased heating time in our study was attributed to progressive exposure of hydrophobic regions at the molecular surface of the proteins of soy meal. In addition, aggregation effects (such as those due to sulphydryl groups) would have occurred.

Urease activity. Figure 2 shows the urease activity data

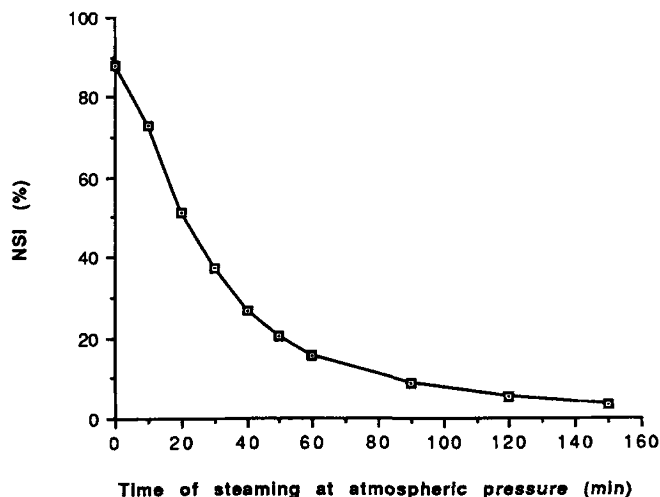

FIG. 1. The effect of thermal treatment on Nitrogen Solubility Index (NSI) of defatted soy meal.

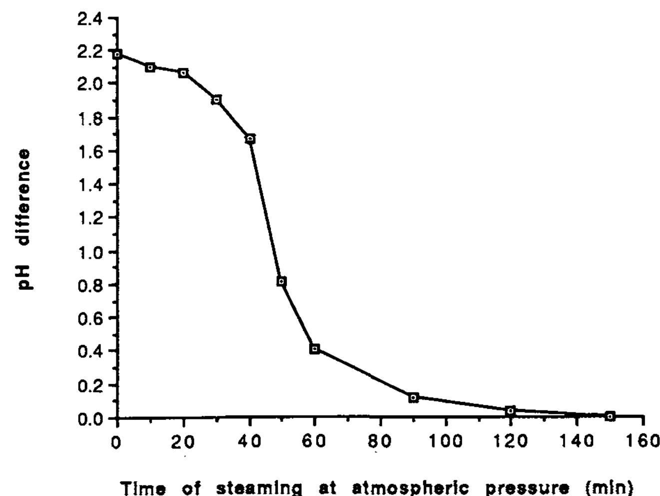

FIG. 2. The effect of thermal treatment on urease activity of defatted soy meal.

for the series of heated soy meals. The unheated soy meal gave a pH difference of 2.18; this decreased slightly (to 2.06) with the sample heated for 20 min, and then dropped considerably (to a negligible pH difference) with samples heated up to 90 min. The urease activity of soy meal was completely inactivated after heating for 150 min.

The Urease Activity Test has been often used as an indication of adequate heat treatment of soybean products. Differences of pH in the range of 0.02-0.30 indicate sufficient exposure of soybean to heat, differences of pH greater than 0.3 indicate insufficient exposure, and pH differences less than 0.02 indicate over-heating (18). This test can indicate progressive denaturation of moderately heated soy meal. However, comparison of Figures 1 and 2 suggests that Urease Activity Test is not particularly sensitive in monitoring the denaturation of large amounts of storage proteins in slightly heated or excessively heated soy meals. Changes in NSI occurred in these samples without appreciable changes in urease activity.

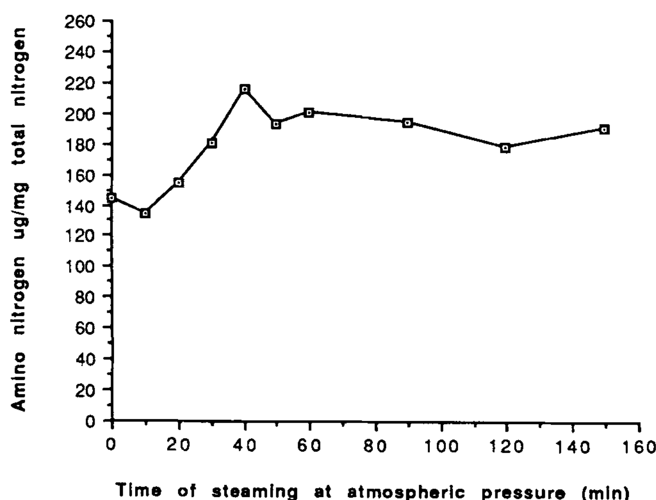

FIG. 3. The effect of thermal treatment on *in vitro* digestibility of defatted soy meal.

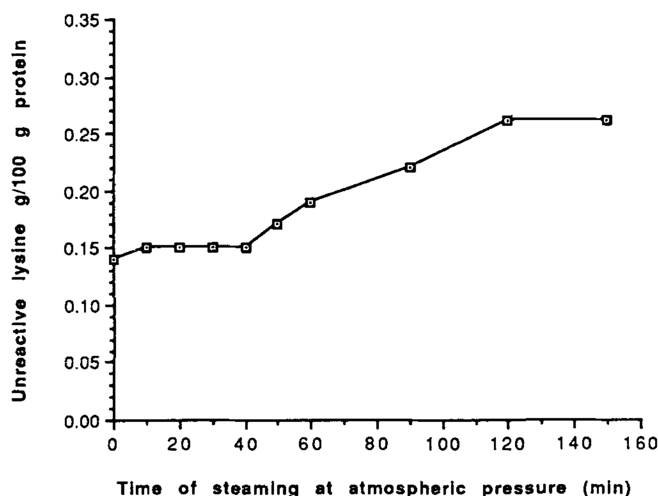

FIG. 4. The effect of thermal treatment on unreactive lysine content of defatted soy meal.

The sensitivity to heat treatment of urease differs from that of storage soy proteins, such as 7-S and 11-S globulins.

In vitro digestibility by pronase. Unheated soy meal was found to have rather poor digestibility (144.8 μg amino nitrogen/g total nitrogen). This was gradually improved by heat treatment up to 40 min (Fig. 3) where maximum digestibility by pronase was obtained (216.4 μg amino nitrogen/g total nitrogen). The digestibilities of samples heated for more than 40 min then fell slowly as heating time increased, reaching 178.4 μg amino nitrogen/g total nitrogen after heating for 120 min. These results are in accord with other reports which relate declining protein digestibility to excessive heating (19).

The effect of heating soy meal on the digestibility of its proteins by proteases is two-fold. Heat inactivates protease inhibitors and causes conformational changes of the proteins. The latter effect is important because peptide bonds become more accessible to enzymes. Native soybean proteins are resistant to hydrolysis by proteases owing to their fairly compact structures in which most of the peptide bonds are buried inside the molecules (20,21). Consequently, if the closely packed peptide chains are partially unfolded upon denaturation, hydrolysis would be facilitated and digestibility improved.

However, excessive heating causes a reduction in digestibility. Thus, changes in digestibility are not necessarily proportional to the degree of protein denaturation. With prolonged heating, the conformations of the proteins may refold to give new, enzyme-resistant structures, possibly stabilized by S-S polymerization. Consequently, the usefulness of this test as a general method to monitor the denaturation of soy proteins, or to characterize various functional properties, is certainly in doubt.

Unreactive lysine residues. Unreactive lysine residues of soy meals increased as the heating time increased (Fig. 4). The unreactive lysine content of unheated soy meal was found to be 0.14 g/100 g crude protein, which remained unchanged for samples heated up to 40 min. It then increased gradually as heating time increased, eventually reaching 0.26 g/100 g crude protein after heating for 120 min.

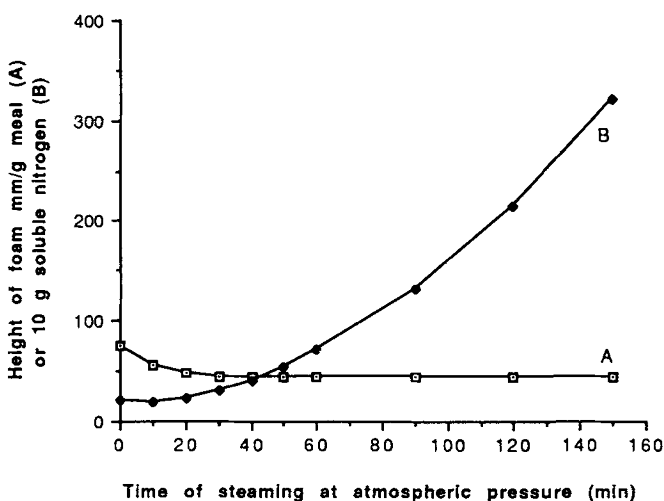

FIG. 5. The effect of thermal treatment on foaming property of defatted soy meal.

These observations indicate that a large amount of lysine in the unheated soy meal was unreactive. Perhaps the ε-amino groups were involved in intra- and intermolecular cross-linking, and/or in reactions with other food constituents. During heat treatment, the ε-amino groups of the reactive lysine may react with reducing sugars (Maillard reaction). Alternatively, dehydroalanine (a decomposition product of cystine or serine) may react with the free amino groups of lysine to form lysinoalanine. A third possible reaction is that between the amide groups of glutamine and free amino groups of lysine to form a peptide-type linkage with the release of ammonia. Any one, or all of these reactions, could cause significant loss of reative lysine as shown in Figure 4. The changes in the unreactive lysine contents of the progressively heated soy meals (Fig. 4) do not reflect the changes in their NSI values (Fig. 1).

Foaming properties. Fig. 5A shows the effect of heat treatment on foaming capacity of defatted soy meal [expressed as height (mm) of foam per g meal (d.b.) after

whipping]. The decline in foaming capacity reflects the decline in NSI values shown in Figure 1. The unheated soy meal had a foaming capacity of 74.8 mm/g, which then decreased sharply to 45 mm/g as heating time increased up to 30 min; it then remained constant with further heating. The unheated soy meal had a corrected foaming capacity of 20.4 mm/10 g soluble nitrogen, which increased progressively with increased heating time and eventually reached 321.4 mm/10 g soluble nitrogen after heating for 150 min. A difference in foam types from unheated and heated samples was also observed. Foams formed by the latter were more viscous and had much smaller bubbles than those obtained from the former.

Foaming capacity and stability depend on the ability of the protein to migrate rapidly to the interface, reorient, and form a viscous film without excessive aggregation or coagulation and the ability of the film to possess intermolecular cohesiveness and a certain degree of elasticity to permit localized contact deformation (22). Also proteins, being surfactants, lower interfacial tension between gas and water and, consequently, facilitated deformation of liquid and expansion against its surface tension. In this respect, Horiuchi *et al.* (23) have related foam stabilities to surface hydrophobicities of protein molecules. This implies that protein molecules with high surface hydrophobicities and low surface tensions readily locate at the interface and resist migration back into the aqueous phase. Hence, molecules are more concentrated at the interface and, consequently, more stable foams are obtained.

Soy proteins dissociate upon heating (24,25). Thus, heating 11-S protein solution at 100°C for 30 min results in the formation of precipitates and a 3-4-S soluble fraction, which consists of the dissociated subunits (26). Therefore, if soluble subunits or dissociated products were formed during heat treatment of the soy meal, and if they had high surface hydrophobicity, due to thermal unfolding, they would have better foaming capacity than either the original unheated soy meal (less hydrophobic regions at the surface), or the heat-denatured insoluble proteins (incapable of forming a liquid protein film to encapsulate gas droplets). Curve B (Fig. 5) supports the above hypothesis in that the corrected foaming capacity is proportional to heating time. If the proposed model concerning increased protein surface hydrophobicity is correct, it suggests that a better foaming agent can be obtained from soy proteins if both high dispersibility and surface hydrophobicity can be retained simultaneously.

Emulsifying properties. In Figure 6, the emulsifying capacity (expressed as turbidity/g soy meal) of the unheated soy meal was found to be 1.16/g meal (Curve A). It decreased gradually as heating time increased. The emulsifying capacity of soy meal heated for 150 min was 0.12/g meal.

However, unheated soy meal was found to have a corrected emulsifying capacity of 14.9 (expressed as turbidity/10 g soluble nitrogen). After heating the meal for 10 min, it decreased slightly to 12.4/10 g soluble nitrogen; then, as the heating time increased up to 40 min, it increased to 20.2/10 g soluble nitrogen, where it dropped again (Curve B). Subsequently, after heating for 50 min and more, the emulsifying capacity increased as the heating time increased. The soy meal heated for 150 min had a corrected emulsifying capacity of 41.3/10 g soluble nitrogen.

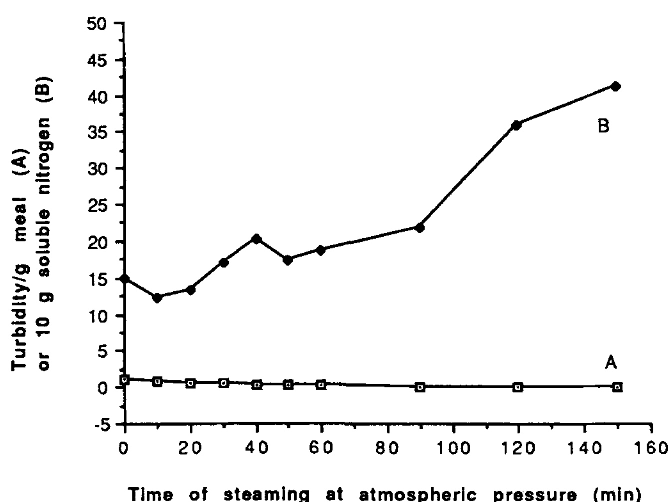

FIG. 6. The effect of thermal treatment on emulsifying property of defatted soy meal.

Protein with high relative hydrophobicity tends to be surface active. However, to form a stable emulsion, not only should protein molecules be able to migrate to the interface between oil and aqueous phases, but they should also be able to partially unfold in order to rearrange their hydrophillic and hydrophobic residues (27, 28).

Although soluble native soy proteins can migrate to the interface relatively rapidly, the unfolding of these molecules is limited due to their compactly folded conformation. Therefore, they tend to move back into the aqueous phase. Furthermore, even though denatured globulins have hydrophobic regions exposed at the surface, they tend to associate with each other and precipitate before they are able to move to the interface. The data presented in Figure 6 are in accord with this model, in that they show that the emulsifying capacity of the unheated soy meal is relatively low, and that it progressively decreases with heating. This is in keeping with the decrease of NSI values with progressive heat treatment (Fig. 1).

Data presented for the corrected emulsifying capacity are for those protein molecules remaining in the dispersed form. As discussed previously, the soluble subunits formed during heat treatment may have both high surface hydrophobicity and solubility. Consequently, they are able to migrate to the interface to form a stable emulsion.

Acid Orange 10 binding characteristics. The Acid Orange 10 adsorption isotherm for unheated defatted soy meal gave an increasing straight line and had no limiting value of Acid Orange 10 binding to soy proteins (data not shown). This behavior could be described as Freundlich adsorption isotherm and indicates the involvement of the secondary binding phenomenon (1,29).

The effect of urea concentration on the dye-binding capacities of unheated and extremely heated (i.e., 150 min) soy meals was examined (Fig. 7). The total amount of dye bound by both samples decreased substantially as urea concentration increased [e.g., 22.3 and 22.1 mM/100 g unheated and heated soy meal, respectively, in absence of urea; 1.62 and 3.41 mM/100 g unheated and heated soy meal, respectively, in the presence of urea, 40% (m/v)]. In addition, even when only 5% urea (m/v) was added, a

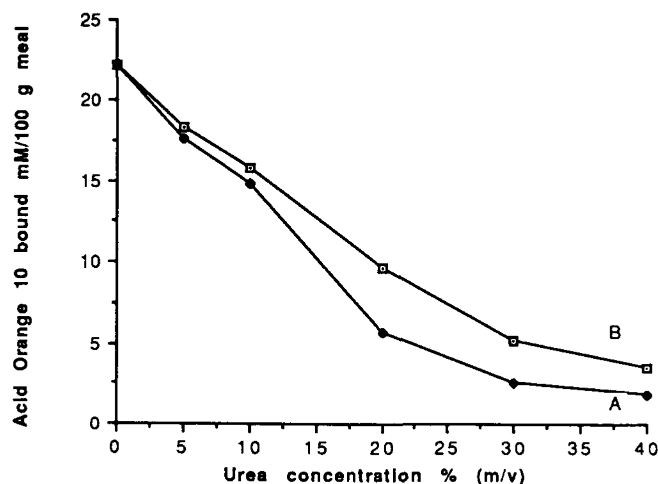

FIG. 7. The effect of urea concentration on the binding of Acid Orange 10 by unheated (A) and heated (B) soy meal.

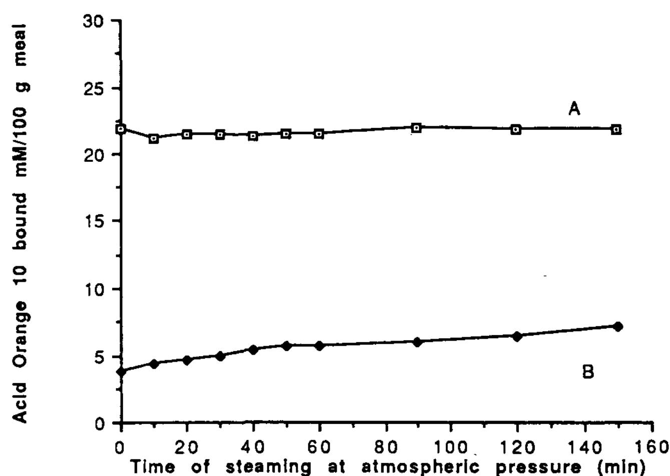

FIG. 8. The effect of thermal treatment on the binding of Acid Orange 10 by defatted soy meal [in the absence (A) or presence (B) of 20% urea, m/v].

significant difference in dye-binding capacities of the unheated and extremely heated soy meals was observed. This difference increased as urea concentration increased up to 20%, and then decreased as urea concentration increased up to 40%.

Heating soy meal probably caused exposure of hydrophobic regions and other chemically reactive sites. Also, the interactions between acid azo dyes and proteins are mainly electrostatic and hydrophobic association. Therefore, heated soy meal would bind more dye than the unheated one. However, this was not the case when no urea was added (Fig. 7). Acid Orange 10 (in the absence of urea) can denature protein and cause conformational changes under conditions favorable for hydrophobic interactions and, by doing so, affect maximum binding to protein, irrespective of whether it has been heated. Due to thermal denaturation, more basic groups and hydrophobic regions of the proteins of the heated soy meals are exposed at the molecular surface than those of unheated soy meal. Also, when reacting with the dye under conditions favoring hydrophobic interaction (i.e., in the absence of urea), thermally denatured protein molecules can further unfold, as induced by the hydrophobicity of the dye. Consequently, more basic groups and hydrophobic regions (in addition to those exposed by the thermal treatment), become accessible to the dye. Similarly, unheated soy proteins are also denatured and unfolded by the dye so as to have the same amounts of reactive sites accessible for dye-binding as those of heated samples. Therefore, there is no difference in the dye-binding capacities between the unheated and heated soy proteins, because of denaturation being taken to completion by reaction with dye.

The effect of urea on dye binding is in keeping with its use as a hydrophobic bond disruption reagent due to its ability to destroy the "normal" structure of water by breaking hydrogen bonds (29). Thus, when the reaction between Acid Orange 10 and soy proteins takes place in the presence of urea, the driving force for hydrophobic interaction is reduced. Consequently, urea (at about 4 M) inhibits further denaturation of soy proteins, which would have been otherwise induced by the hydrophobicity of the dye.

Because of the resistance of proteins to conformational change, dye-induced denaturation to allow binding to less accessible sites is more readily affected by urea than binding to exposed sites. Hence, although total dye binding progressively falls as urea concentration increases, binding to less unfolded proteins (i.e., "less heated") is affected to a greater extent than binding to more unfolded proteins (i.e., "more heated"). However, since dye binding continues to fall as hydrophobic interactions become further reduced, this difference tends to diminish. In this experiment, for example, a urea concentration of 20% (m/v) gave the largest difference in dye-binding capacities between unheated and extremely heated soy meals. While urea becomes effective in inhibiting hydrophobic interactions at concentrations greater than about 4 M (30), higher concentrations (e.g., 8 M) can cause protein denaturation, which could reduce differences in dye-binding capacities when 40% (m/v) urea was added.

The above explanation is further supported by the results for the effect of urea addition (20%, m/v) on the Acid Orange 10 binding (in 0.2 M citric acid) (Fig. 8). When dye binding occurred in the absence of urea, there was no difference in the dye-bindinng capacities of unheated and heated samples (e.g., 21.9 and 21.7 mM/100 g for unheated and soy meal heated for 150 min, respectively). However, when urea (20%, m/v) was added, not only were the amounts of dye bound by soy meals drastically reduced (e.g., 3.70 and 7.11 mM/100 g for unheated and soy meal heated for 150 min, respectively), the dye-binding capacities of progressively heated samples increased as the heating time increased. The added urea prevented dye-binding to less accessible (internal) sites, but progressive unfolding of protein molecules caused by thermal denaturation increased the number of exposed sites in the heated samples.

Cresol Red binding characteristics. The results for Cresol Red binding by unheated and heated soy meals are shown in Figure 9. The unheated soy meal bound 2.02 mg Cresol Red/g soy meal, which increased progressively as heating time increased and eventually reached 4.17 mg/g meal after heating for 150 min.

Increased dye binding upon heating has been observed

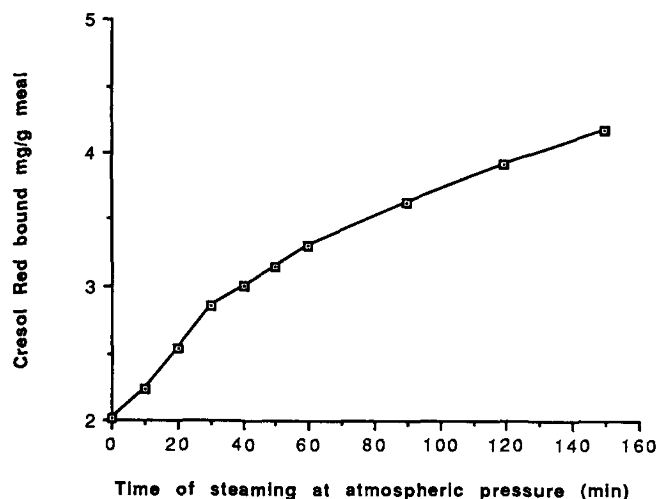

FIG. 9. The effect of thermal treatment on the binding of Cresol Red by defatted soy meal.

for samples of soy meal and rapeseed meal (5,6). Hydrophobic rather than electrostatic associations may be involved in the binding of Cresol Red to proteins. This mechanism is supported by this study, because the amounts of Cresol Red bound are of the same order as those for Acid Orange 10 binding in the presence of 20% urea, (m/v) (0.005 vs 0.037 mM/g unheated soy meal, and 0.011 vs 0.071 mM/g soy meal heated for 150 min, for Cresol Red and Acid Orange 10, respectively). Consequently, as in the case of Acid Orange 10 binding (in the presence of urea), Cresol Red binding will not induce further denaturation of soy proteins.

The results for Cresol Red binding by the complete range of heated soy meals (Fig. 9) support the above hypothesis. There is a significant difference in dye-binding capacities of the unheated and heated soy meals and, furthermore, the amounts of Cresol Red bound by the heated samples increased with increased heating time. As discussed before (Acid Orange 10 binding in the presence of urea), the basis of this progressive increase in binding is due to the progressive exposure of hydrophobic regions caused by thermal denaturation. Therefore, the amount of Cresol Red bound by the soy proteins actually reflects the extent of denaturation affected by the heat treatment.

Statistical analysis. Correlation coefficients between the results for all the tests are shown in Table 2. The results for the Cresol Red binding test are highly correlated to those for the Urease Activity Test (r = −0.934); the Nitrogen Solubility Index Test (r = −0.952); Acid Orange 10 binding in the presence of 20% urea, (m/v) (r = 0.991); emulsifying capacity (r = −0.942); and heating time (r = 0.969). They are fairly well correlated to unreactive lysine (r = 0.916); corrected foaming capacity (r = 0.871). The results for Acid Orange 10 binding [in the presence of 20% urea, (m/v)] are well correlated with the results for NSI test (r = −0.949); emulsifying property measurement (r = −0.949); and heating time (r = 0.958). They are fairly well correlated to the results of corrected foaming and emulsifying capacities (r = 0.867 and 0.856, respectively).

Acid Orange 10 binding (in the absence of urea) and *in vitro* digestibility were poorly correlated to heating time (r = 0.389 and 0.496, respectively). However, unreactive lysine was highly correlated to heating time (r = 0.972).

While the results for foaming and emulsifying property measurements have either poor or fairly good correlations to heating time (r = −0.550 and −0.847, respectively), those of corrected foaming and emulsifying capacities are well correlated to heating time (r = 0.965 and 0.946, respectively).

The hypothesis that the binding of Cresol Red to proteins is by weak hydrophobic interaction has been supported by the present work with soy proteins. Since the amounts of the dye bound by soy proteins are thought to be related to the extent of the progressive exposure of hydrophobic regions caused by thermal denaturation, the Cresol Red binding test can be used as an indication of this change. Furthermore, this test, in relation to the other methods evaluated, is superior with respect to its simplicity, precision and sensitivity to protein denaturation (Table 3). Consequently, the indications are that it should be very useful as a quality control method in the food industry. In addition, the high correlations of this

TABLE 2

Correlation Matrix for Dye-Binding and Functional Properties of Heat Treated Soy Proteins

	1	2	3	4	5	6	7	8	9	10	11
2	0.991										
3	−0.952	−0.949									
4	−0.934	−0.918	0.884								
5	0.665	0.669	−0.811	−0.581							
6	0.916	0.889	−0.770	−0.915	0.345						
7	0.284	0.194	−0.114	−0.386	−0.055	0.468					
8	−0.704	−0.734	0.847	0.557	−0.709	−0.442	0.339				
9	0.875	0.867	−0.688	−0.809	0.313	0.947	0.452	−0.362			
10	−0.942	−0.949	0.988	0.889	−0.747	−0.782	−0.051	0.872	−0.687		
11	0.871	0.856	−0.697	−0.767	0.378	0.918	0.402	−0.374	0.973	−0.676	
12	0.969	0.958	−0.849	−0.912	0.496	0.972	0.389	−0.550	0.965	−0.847	0.946

1. Cresol Red binding capacity.
2. Acid Orange 10 binding capacity (in the presence of 20% urea, m/v).
3. Nitrogen Solubility Index.
4. Urease activity.
5. *In vitro* digestibility.
6. Unreactive lysine content.
7. Acid Orange 10 binding capacity (in the absence of urea).
8. Foaming capacity.
9. Corrected foaming capacity.
10. Emulsifying capacity.
11. Corrected emulsifying capacity.
12. Heating time.

TABLE 3

Standard Deviations and Coefficients of Variation of Results of Several Methods

Methods	No. of replicates	S.D.[a]	C.V. (%)[b]
Cresol Red binding	10	0.02[d]	0.06
Acid Orange 10 binding[c]	10	1.46[e]	9.4
Urease Activity Test	10	0.02[f]	1.0
NSI test	8	2.48[g]	2.8

[a]Standard deviation.
[b]Coefficient of variation.
[c]In the presence of 20% urea, m/v.
[d]mg/g soy meal.
[e]mM/100 g soy meal.
[f]pH difference.
[g]Percentage

method with those of other well-accepted methods (e.g., NSI test and Urease Activity Test), suggest its usefulness for predicting certain functional properties of soy products.

The hypothesis that the binding of Acid Orange 10 by proteins in 0.2 M citric acid through electrostatic and hydrophobic attractions has been further supported by the present work with soy proteins. The addition of urea to the reaction mixture inhibited the hydrophobic interaction between the dye and soy proteins, and significantly reduced the amount of dye bound to proteins. This may be due to its effect of destructuring water. Consequently, this addition eliminates the possibilities of dye-induced denaturation of proteins. Therefore, the difference between the dye-binding capacities (in the presence of urea) of the unheated and progressively heated soy meals reflects the extent of soy protein denaturation.

The correlations of heating time with the results for foaming and emulsifying property measurements ($r = -0.550$ and -0.847, respectively) are substantially different from those of heating time with corrected foaming and emulsifying capacities ($r = 0.965$ and 0.946, respectively). Because the latter results were calculated on the basis of soluble nitrogen, food components of the defatted soy meal, other than soy proteins, may also be involved in foam and emulsion formation. The physicochemical properties of carbohydrates can also be profoundly affected by heating. Regardless of the reasons for the differences, foaming properties are more affected than emulsifying properties, which may indicate that the mechanisms involved in these two functionalities may be different from one another, even though they are both derived from the surfactancy of protein.

Heating was found to improve *in vitro* digestibility of soy proteins. However, the increase in digestibility was not in proportion to the progressive exposure of hydrophobic regions caused by thermal denaturation (i.e., the extent of denaturation). This is probably due to new enzyme-resistant structures being formed during the thermal unfolding of the soy protein molecules.

Unreactive lysine determination and *in vitro* digestibility are useful for ascertaining the effect of thermal treatment on nutritional quality. However, it has been shown that it is difficult, if not impossible, to monitor the progress of protein denaturation, or to predict functional properties, by means of these methods.

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