Effect of Physical and Chemical Processing Factors on the Redispersibility of Dried Soy Milk Proteins[1]

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ABSTRACT

Heating conditions before drying of soy milk (SM) had a large effect on the redispersibility of SM proteins after drying. Redispersibility decreased rapidly to a minimum at several min. of heating at either 100° or 120°C. and then increased gradually at 100° and rapidly at 120°C. Higher pH or lower concentration of SM during heating increased the redispersibility after drying. The redispersibility was also greatly increased by decreasing the concentration of the heated SM, from which the SM was dried. The use of emulsifiers or EDTA had a slight effect on protein redispersibility. However, more effective reagents were disulfide bond-splitting reagents, such as Na_2SO_3, cysteine, 2-mercaptoethanol, and H_2O_2. The reducing reagents showed similar patterns in their relations between the added concentration and the redispersibility. For cysteine, e.g., there was a fairly large and sharp increase in redispersibility at 0.001M; then it dropped abruptly, later rising again to a maximum at 0.050M. The combined effect of cysteine and emulsifiers was additive, but the effect of cysteine was decreased by the addition of EDTA. It is concluded that the disulfide bonds play an important role in the insolubilization of SM protein during the drying.

Soy milk (SM) has been given considerable attention as an economical high-protein food suitable for overcoming the malnutrition of infants in developing countries. Recently, bottled SM drinks have become commercially available in limited quantity in some areas. An alternative, of supplying SM as a dry powder, might have advantages of convenience and economy, because of easier transportation and preservation along with centralized large-scale production.

One of the most important qualities desired for powdered SM is a high degree of redispersibility in water. In the manufacture of the powdered product, the milk must be heated in order to inactivate antinutritive factors and avoid off-flavors (1,2). However, when the powdered SM is prepared from the heated solution, large amounts of the proteins are insolubilized during drying, even freeze-drying (1).

To obtain basic knowledge to aid in solving this problem, various physical and chemical processing factors which influence redispersibility after drying were systematically investigated.

MATERIALS AND METHODS

Soybeans

The soybeans used were Harosoy 63, which contain 5.83% total nitrogen, 19.7% crude fat, and 3.90% water.

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Soy Milk

Whole soybeans were soaked in water overnight, rinsed, ground in a Reitz disintegrator Model No. RA-4-53, with the addition of tap water so that the total amount of water was tenfold the original soybean weight, and filtered on a filter press. All the processes were carried out at room temperature. The raw SM prepared thus was freeze-dried, stored at −40°C., and used as the starting material throughout this work.

Heating and Drying of Soy Milk

The heating and drying of samples was carried out as follows, unless described otherwise. Freeze-dried SM was dissolved into distilled water and, if necessary, chemical reagents were added to it. Samples (15 ml.) of the resultant SM were put into 20-ml. ampoules, sealed, and then immersed in a water or oil bath. No shaking was done during the incubation, unless described otherwise. After a given time the milk was cooled quickly, and 10 ml. was transferred into a 250-ml. beaker and dried in a 50°C. constant-temperature room for 16.5 hr. This drying procedure was adopted as a convenient, reproducible method suitable for handling a large number of samples.

Measurement of Redispersibility of Dried Soy Milk

To the beaker containing the dried SM, water was added at the rate of 77 ml. per g. of SM solids. After being shaken for 2.5 hr. at room temperature on a 170-r.p.m. rotary shaker, the liquid was filtered through milk filter paper (Agway Co., No. 87-0440). The total nitrogen of the filtrate was measured by the semimicro Kjeldahl method.

Addition of Chemical Reagents

Chemical reagents were dissolved in water and the pH was adjusted to 6.6–6.7 by HCl or NaOH and then added to SM before heating. The nitrogen from Na-ethylenediaminetetraacetate (EDTA) was corrected by a blank test (EDTA + water). In the case of cysteine, two blank tests were carried out. One was for the nitrogen from cysteine (cysteine HCl + water) and the other was for the NaCl produced by neutralization of cysteine-HCl (NaCl + water), because the redispersibility decreased in the presence of NaCl. The concentration of H_2O_2 was determined by a $KMnO_4$ titration. The emulsifier preparations with different ratings for hydrophile-lyophile balance (HLB) (3) were made by mixing a dispersion of Span 60 and a solution of Tween 60 in various ratios.

RESULTS

Effect of Temperature and Time of Heating before Drying

The heat-treatment of soy materials is necessary in order to destroy naturally occurring antinutritive factors. This beneficial effect of heating can be achieved by a variety of times and temperatures of heating, keeping in mind that excessive heat-treatment decreases the nutritional value (1,2,4). It is important, therefore, to examine the effects of various heating temperatures and time on redispersibility of dried milk to obtain a product combining high nutritional value and high redispersibility. However, since SM coagulated under some heating conditions (Fig.

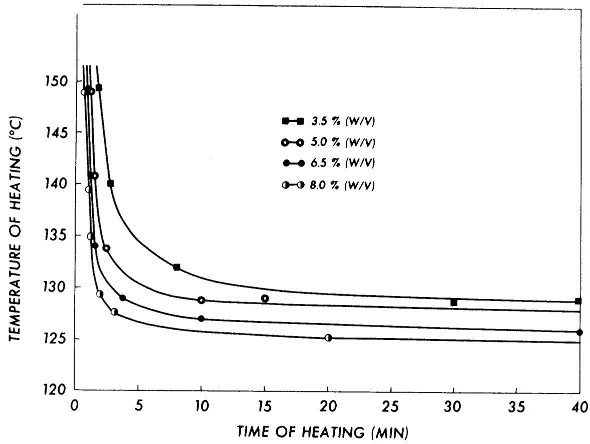


Fig. 1. Coagulation curves at various concentrations of soy milk (SM) by heat-treatments. SM was sealed in a 13 by 100-mm. test tube and shaken for 4 min. at 25 r.p.m. in an oil bath.

1), the range in which such tests could be run was restricted. There were limiting temperatures below which coagulation did not occur, regardless of the heating time, and these limits were higher with decreased concentration of SM. For instance, the limiting temperatures for the milks with 3.5, 5.0, 6.5, and 8.0% solids were about 130°, 129°, 127°, and 126°C., respectively. Above these temperatures, coagulation occurred after a certain heating time. These coagulation times decreased with elevation of temperature and with increases in concentration of SM. Further, it was found that preliminary heating of SM below the limiting temperature inhibited the coagulation which otherwise occurs in subsequent treatment above the limiting temperature. For example, the 6.5%-solids SM coagulated after 3 min. of heating at 130°C., but, when the milk was given preliminary treatment for 60 min. at 100°C., coagulation did not occur even after 50-min. heating periods at 130°C. This means that the limiting curves shown in Fig. 1 would be shifted to the right by a preliminary heating of SM below the limiting temperatures.

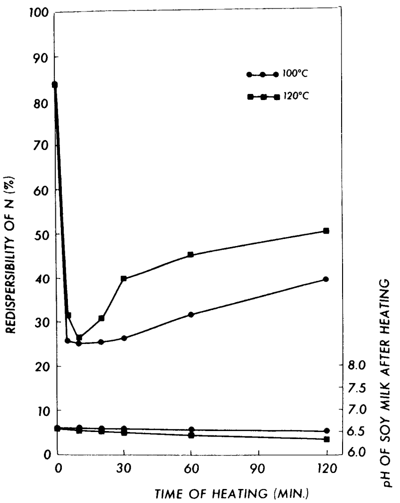
Since SM coagulated at high temperatures, experiments on the effects of the heating temperature and time on the redispersibility were carried out under the time and temperature conditions below the limiting curves. Redispersibility decreased rapidly to a minimum at 10 min. of heating (Fig. 2) and then increased gradually at 100°C. heating and more rapidly at 120°C. Beyond a point in heating time the redispersibility increased with a prolongation of the heating time. The pH of SM after heating decreased with higher temperatures and with longer heating time, but the degree of the decrease was very small (Figs. 2 and 3).

Effect of pH

Redispersibility increased markedly with higher pH (Figs. 3 and 4).

Effect of Percent Solids during Heating and Drying

Figure 5 shows the effect of the percent solids of SM during heating and drying on redispersibility after drying. Soy milk solutions having the solid concentrations



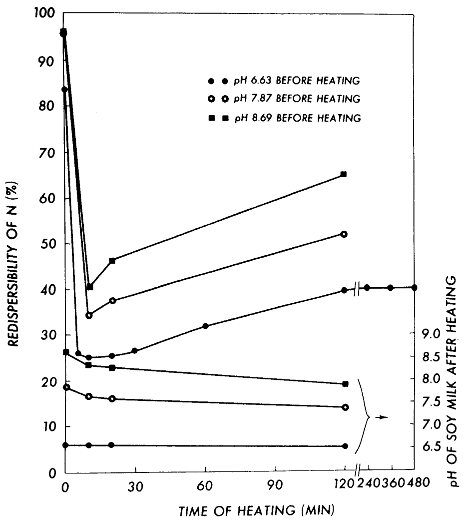


Fig. 2 (left). Effect of heating temperature and time before drying on the redispersibility of dried SM proteins. The concentration of solids in the milk before drying was 6.5% (w./v.).

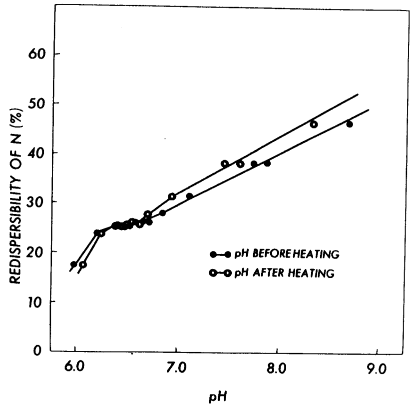
Fig. 3 (right). Effect of pH and heating time before drying on redispersibility of dried SM proteins. The concentration of soy solids in the milk before drying was 6.5% (w./v.) and heating temperature was 100°C.

shown in the horizontal axis of this figure were heated and then diluted to 6.50% (w./v.) in the right side and to 2.17% (w./v.) in the left side. Portions (10 ml.) of the resultant SM were dried and the redispersibility was measured. Redispersibility increased markedly with lowered solids concentrations of SM both during heating or just prior to drying; this suggests that intermolecular interactions of the proteins during both heating and drying are related to redispersibility after drying.

Effect of Disulfide-Splitting Reagents

It is known that the major soybean protein molecules have more than two free SH groups per one molecule and can polymerize through the formation of intermolecular disulfide bonds when precipitated at the isoelectric point (5,6). To examine whether disulfide bonds are responsible for the insolubilization of dried SM proteins, various concentrations of disulfide bond-splitting reagents were added to SM just prior to heating and drying. The results (Figs. 6 and 7) show that the Na_2SO_3, cysteine, and 2-mercaptoethanol greatly increased redispersibility of SM after drying. However, it should be noted that the redispersibility shows some complicated but characteristic patterns with respect to the levels of the reagents used, and these patterns are similar for all the reducing reagents. That is, there is a maximum of redispersibility for each curve at very low concentrations of the reagents, beyond which the redispersibility decreased rapidly to a minimum value lower than the redispersibility without addition of reagents. At still higher reagent levels, redispersibility increased again to higher levels than before.

Since H_2O_2 is an oxidative disulfide bond-splitting reagent, its effect was



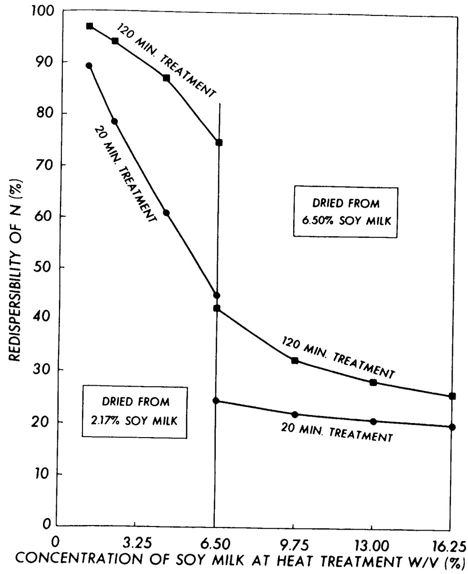


Fig. 4 (left). Effect of pH on the redispersibility of dried SM proteins. SM was heated at 100°C. for 20 min.

Fig. 5 (right). Effect of milk solids concentration during heating prior to drying, and during drying, on the redispersibility of the dried SM proteins. Temperature of heating was 100°C. Dilutions to the solids concentrations indicated in the boxes were made after heating and prior to drying.

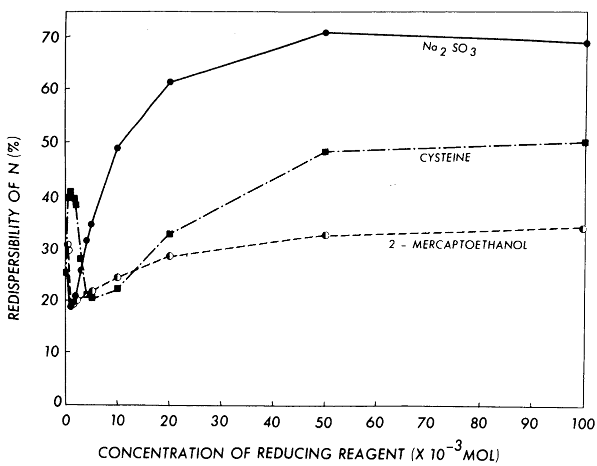


Fig. 6. Effect of reducing agents on redispersibility of dried SM proteins. The 6.5%-solids SM was heated at 100°C. for 20 min. after addition of the reagents, then dried.

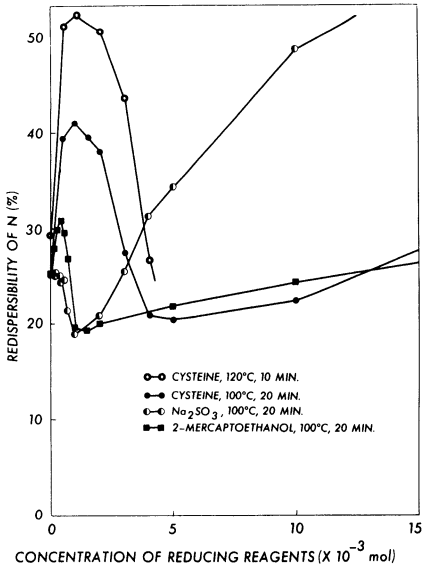


Fig. 7. Effect of reducing reagents at a low level on the redispersibility of dried SM proteins. Concentration of SM solids was 6.5% (w./v.).

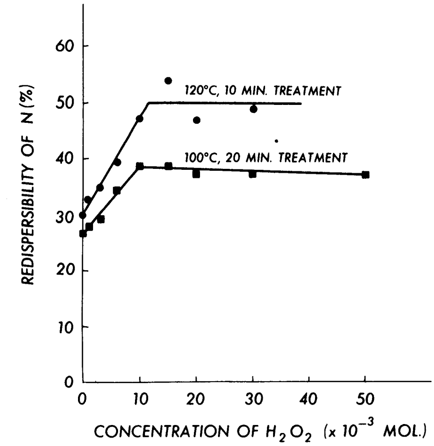
examined. Protein redispersibility increased markedly as a result of its addition (Fig. 8). Thus the increase in redispersibility by the addition of various kinds of disulfide bond-splitting reagents showed that disulfide bonds were responsible for part of the insolubilization of SM protein during drying.

Effect of Emulsifier (Span 60 + Tween 60)

There was the possibility that a part of the insolubilization brought about by drying was due to the presence of nonpolar groups on the protein molecules. If such was the case, the use of emulsifiers capable of complexing with the nonpolar groups should increase the redispersibility of the dried product. Therefore, the effects of emulsifier concentration and HLB value were examined. Redispersibility increased by the addition of emulsifier to reach a maximum at a concentration around 0.6% (Fig. 9). The effective range of the HLB value was found to be from 7 to 1 (Fig. 10). The increase in redispersibility by emulsifiers was independent of cysteine (Fig. 10). Generally, the degree of increase brought about by emulsifiers was not large.

Effect of Chelating Agent

Under certain conditions, some proteins are considered to polymerize through the intermolecular cross-linkage mediated by multivalent metals (7). To ascertain whether SM proteins are insolubilized by such a mechanism during drying, the effect of addition of EDTA was examined. As shown in Fig. 11, redispersibility of dried SM was increased by addition of EDTA, reaching a maximum at 4×10^{-5}M concentration. However, the extent of this increase was quite small. Furthermore, addition of EDTA and cysteine gave results inferior to those of cysteine alone.



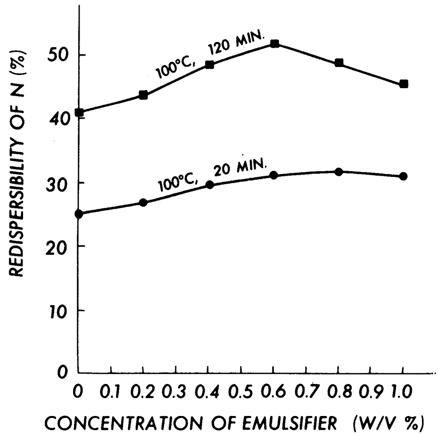


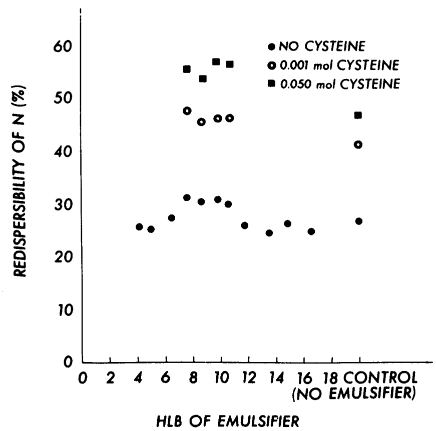
Fig. 8 (left). Effect of hydroperoxide on redispersibility of dried SM proteins. The 6.5%-solids SM was heated after the addition of hydroperoxide.

Fig. 9 (right). Effect of emulsifier concentration on redispersibility of dried SM proteins. The emulsifier (HLB 7.5) was prepared from Span 60 and Tween 60 and added to SM before heating.

These results indicate that metals in SM were not important factors in the insolubilization of the SM protein during drying.

DISCUSSION

The insolubilization of SM proteins during drying can be ascribed to an



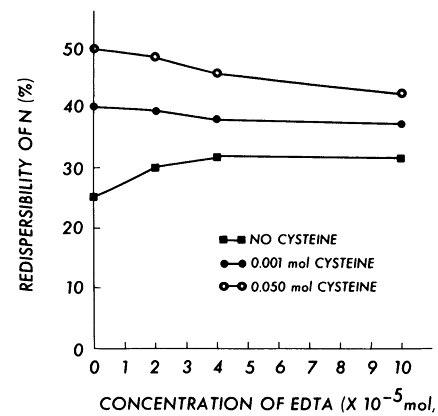


Fig. 10 (left). Effect of emulsifier HLB and combinations with cysteine on redispersibility of dried SM proteins. Emulsifiers were prepared from Span 60 and Tween 60 and added to a final concentration of 0.6% (w./v.) in the milk. Heat-treatment of SM was done at 100°C. for 20 min. after addition of reagents.

Fig. 11 (right). Effect of EDTA and its combination with cysteine on redispersibility of dried SM proteins. (Heat-treatment at 100°C. for 20 min. after addition of reagents.)

interaction among protein molecules which might also be thought of as colloidal particles. The protein concentration of SM becomes very high during the drying process and as a result the average distance between protein molecules becomes small. If, at this time, binding sites are located on the surfaces of the molecules or colloidal particles, interactions occur very easily, aggregating the proteins. The formation of intermolecular disulfide bonds is one of the more usual interactions that occur between proteins. It is evident from Fig. 6 that these bonds play an important role in the insolubilization of the SM proteins during drying, in spite of the very complicated curves which the disulfide bond-splitting reagents showed. Most native soybean protein molecules have a compact three-dimensional structure, in which various amino acid residues are buried (8). The decrease in redispersibility by heat-treatment of SM before drying can be considered largely as the result of SS polymerization among the SH groups exposed on the outside of the molecules as a result of heat-denaturation and conformational change. These mechanisms will be discussed in detail and quantitatively in a later paper.

Of the disulfide bond-splitting reagents used here, cysteine may be most promising for practical use, because cysteine gave the highest increase in redispersibility at a low level of addition. Further, cysteine is a partial nutritional substitute for methionine, which is the limiting essential amino acid in soy milk.

Literature Cited

1. VAN BUREN, J. P., STEINKRAUS, K. H., HACKLER, L. R., EL RAWI, I., and HAND, D. B. Heat effects on soy milk. Indices of protein quality in dried soy milk. Agr. Food Chem. 12: 524 (1964).
2. HACKLER, L. R., VAN BUREN, J. P., STEINKRAUS, K. H., EL RAWI, I., and HAND, D. B. Effect of heat treatment on nutritive value of soy milk protein fed to weanling rats. J. Food Sci. 30: 723 (1965).
3. BECHER, P. Emulsions: Theory and practice (2nd ed.), p. 232. American Chemical Society Monograph Series. Reinhold: New York (1966).
4. HACKLER, L. R., and STILLINGS, B. R. Amino acid composition of heat-processed soymilk and its correlation with nutritive value. Cereal Chem. 44: 70 (1967).
5. WOLF, W. J., and SMITH, A. K. Food uses and properties of soybean protein. Food Technol. 15(5): 12 (1965).
6. BRIGGS, D. R., and WOLF, W. J. Studies on the cold-insoluble fraction of the water extractable soybean proteins. I. Polymerization of the 11S component through reactions of sulfhydryl groups to form disulfide bonds. Arch. Biochem. Biophys. 72: 127 (1957).
7. BJORKSTEN, J. Cross linkages in protein chemistry. Adv. Protein Chem. 6: 343 (1951).
8. FUKUSHIMA, D. Internal structure of 7S and 11S globulin molecules in soybean proteins. Cereal Chem. 45: 203 (1968).

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