MILK PROTEINS

Effects of Heat Treatment on Serum Proteins

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The effects of heat on the proteins of milk serum are of importance in relation to cooked flavor, antioxygenic properties, and impairment of coagulation of the casein. The milk serum proteins are denatured by heat treatments in the range 60° to 80° C. for times up to 2 hours. Two important manifestations of denaturation are decreased solubility in acid and salt solutions and increased activity of sulfhydryl groups, the latter largely confined to the β-lactoglobulin fraction. Individual lots of mixed milk differ in content of serum proteins and in the response of these proteins to heat treatment; precipitation tests are consequently not precise indexes of the heat treatment a sample has received. Many of the sulfhydryl groups of β-lactoglobulin are activated by heat treatment to such an extent that they are oxidizable by atmospheric oxygen. Denaturation of β-lactoglobulin as measured by sulfhydryl activity follows first-order kinetics and has an activation energy of about 80,000 calories per mole. The activation of sulfhydryls parallels the decrease in solubility. The activated —SH groups may function as antioxidants in dairy products. The deleterious action of unheated milk on the consistency of wheat flour doughs and on the loaf volume of bread is not associated with sulfhydryl groups, as was formerly postulated.

THE effect of heat on the serum proteins of milk constitutes a very important problem in dairy chemistry. From the practical standpoint dairy chemists are interested in the changes produced in these proteins by heat in relation to the properties and utility of manufactured milk products. The wide range of practical interest is illustrated by the fact that such heat-produced phenomena as cooked flavor, development of antioxygenic properties, impairment of clotting of the casein in cottage cheese manufacture, inhibition of gelation of evaporated milk on sterilization and improvement in baking quality of nonfat dry milk solids have all been attributed, in whole or in part, to effects on the serum proteins. On the more fundamental side, there is considerable interest in studying the basic effects of heat on milk serum proteins, not only to furnish a background for interpreting phenomena of practical interest, but also to extend the knowledge of protein chemistry.

This paper reviews the effect of heat on milk serum proteins, with particular emphasis on recent work with which the author has been associated at the University of Minnesota.

Milk Serum Proteins

Milk contains a number of protein entities, although only recently has much progress been made in isolating and identifying them (*22*). Quantitatively the most prominent group of milk proteins is the caseins, which are precipitable by acid or the enzyme rennin. Those proteins remaining in solution after removal of the caseins from milk comprise about 0.6 to 0.7% of the milk and are designated "serum proteins" or "whey proteins." This group constitutes the subject of the present paper.

Classically, the serum proteins were often separated into a "lactoglobulin" fraction insoluble in ammonium sulfate at half saturation and a "lactalbumin" fraction soluble at this concentration, or by some similar means of separation by salts. More recently, these fractions have been partially resolved into more homogeneous components. Thus three relatively homogeneous proteins have been crystallized from lactalbumin: (1) "β-lactoglobulin," amounting to about 50% of the total serum protein, (2) an albumin, identical to the albumin of bovine blood, and constituting about 5% of the total serum protein, and (3) "α-lactalbumin," constituting about 12% of the serum protein. Workers at the Eastern Regional Research Laboratory of the U. S. Department of Agriculture have been particularly active in isolating and characterizing these proteins (*7, 22, 24*). The lactoglobulin fraction has also been partially resolved into some more homogeneous components, but none of them has been crystallized to date (*27*). Both lactalbumin and lactoglobulin contain components that have not yet been isolated in pure form.

The serum proteins, unlike the caseins, are heat-denaturable; evidently they exist as particles with specific configurations which are disrupted by heat treatment, with the result that the characteristic properties of the proteins are altered. Among the manifestations of heat denaturation of the milk serum proteins are decrease in solubility at pH 4.7 or in concentrated salt solutions and increase in activity of various groups, of which the sulfhydryl group has been most widely studied. Recent studies at the University of Minnesota have been concerned with these two manifestations of the effects of heat. Some experiments have dealt with the

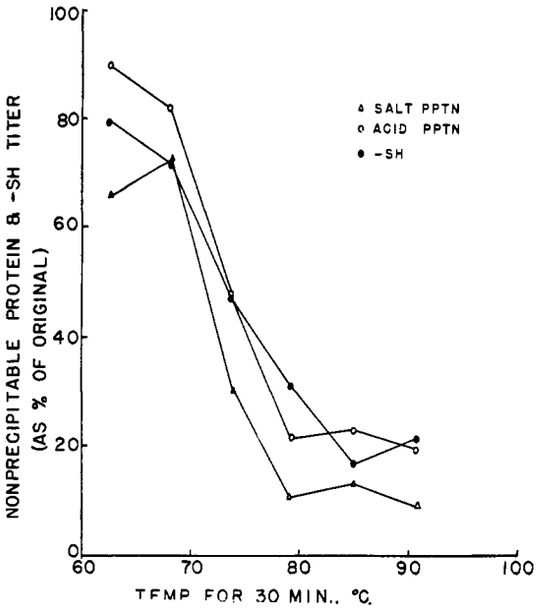


Figure 1. Heat denaturation of milk serum proteins in skim milk

Measured by precipitability and sulfhydryl activity. Data of Larson *et al.* (*21*)

proteins in their natural environment in skim milk, others with the isolated mixture of serum proteins in suitably buffered media, and still others with crystalline β-lactoglobulin in buffered media.

Solubility Changes

In raw milk, precipitation of casein by adjusting the pH to 4.7 leaves the serum proteins in solution. Heat treatment renders them acid-precipitable, so that they come down with the casein at pH 4.7 to an extent dependent on the severity of the heating. A maximum of about 80% of the serum proteins is rendered acid-precipitable.

A similar situation holds in the case of precipitation of the casein by saturation with sodium chloride, but the protein content of sodium chloride filtrates is always lower than that of acid filtrates. Evidently sodium chloride precipitates a serum protein fraction that acid does not. These relationships are shown in Figure 1, which represents an experiment (*21*) in which skim milk was heated for 30 minutes at various temperatures. (Figure 1 also contains data on sulfhydryl groups.) This figure shows that for any given sample sodium chloride precipitates more protein than acid and that a maximum of about 80% of the serum protein is rendered acid-precipitable by heat treatment. Thus it seems that sodium chloride precipitates a serum protein fraction that acid does not, and that this fraction may be related to or identical with that which is not rendered acid-precipitable by heating. The identity of the protein fraction that is not rendered acid-precipitable by heating has not been established definitely. Rowland (*26*) proposed that it be called the "proteose-peptone" fraction because its properties resemble those of proteoses and peptones. More recently Ashworth and Krueger (*1*) have suggested that it merely represents certain casein particles that escape acid precipitation.

It is of interest to know whether the proteins of the serum protein mixture are individually rendered precipitable by heat treatment or whether their precipitability is due to their interaction with casein or among themselves. The possibility of interaction with casein seems to be precluded by experiments (*4, 25*) in which heated skim milk was centrifuged at high speed and the casein particles were thrown out, leaving the serum proteins in solution. These experiments also demonstrate that heat treatment does not cause aggregation of the serum proteins to particles of sufficient size to be centrifuged out with the casein. Rather the serum proteins seem merely to be rendered coprecipitable with the casein.

The possibility that heat treatment causes some interaction among the various serum proteins themselves has been investigated by means of electrophoretic patterns (*16*), for which dispersions of the entire serum protein mixture in phosphate buffer, pH 6.9, $\mu = 0.1$, have been employed. Briggs and Hull (*2*) had previously demonstrated that if β-lactoglobulin is heated in this medium it is transformed by a two-step process into a denatured form of higher mobility than the native protein. Figure 2 shows patterns obtained on β-lactoglobulin heated for 30 minutes at various temperatures; these confirm the finding of Briggs and Hull (*2*). Figure 3 shows patterns obtained on the unfractionated serum protein mixture after similar heat treatments. The transformation of β-lactoglobulin to the fast form is again evident, but also a tendency is shown for all the components to migrate as a single peak. This fact suggests the possibility of interaction among the several components, although it does not necessarily prove that it occurs.

The rate of denaturation of the serum proteins in skim milk at various temperatures has been studied; for the most part the sodium chloride precipitation method for analysis (*10*) has been used. The rate of denaturation increases regularly as the temperature is raised from 68° to 80° C. (Figure 4). These data do not lend themselves to an exact treatment for determination of the order of reaction and energy of activation, largely because the serum proteins consist of a mixture rather than a single entity, but a very useful relationship is obtained by plotting temperature against log of time for a given extent of denaturation. Such plots are similar to the thermal death time curves used by bacteriologists and result

Figure 2. Effect of heat treatment on electrophoretic pattern of β-lactoglobulin in phosphate buffer

Heated for 30 minutes at various temperatures pH 6.9, $\mu = 0.1$, 2 hours, 4° C., 10% protein

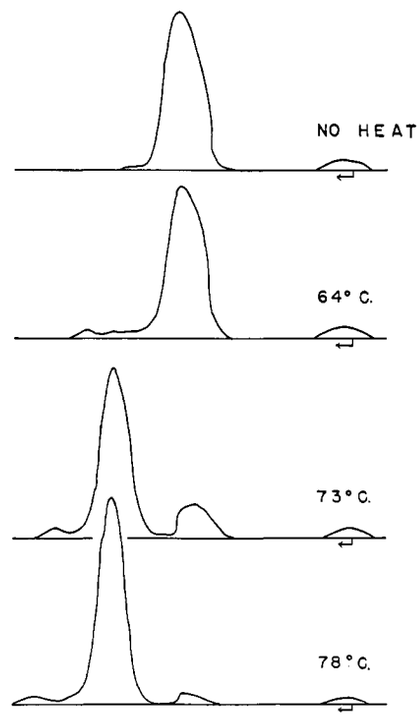
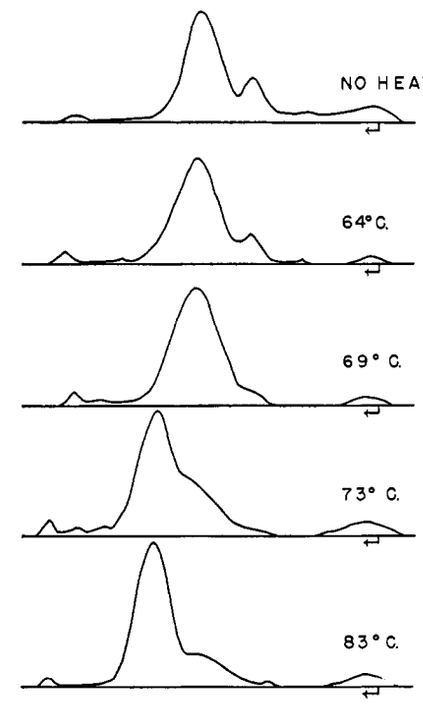


Figure 3. Effect of heat treatment on electrophoretic pattern of milk serum protein mixture in phosphate buffer

Heated for 30 minutes at various temperatures pH 6.9, $\mu = 0.1$, 2 hours, 4° C., 10% protein



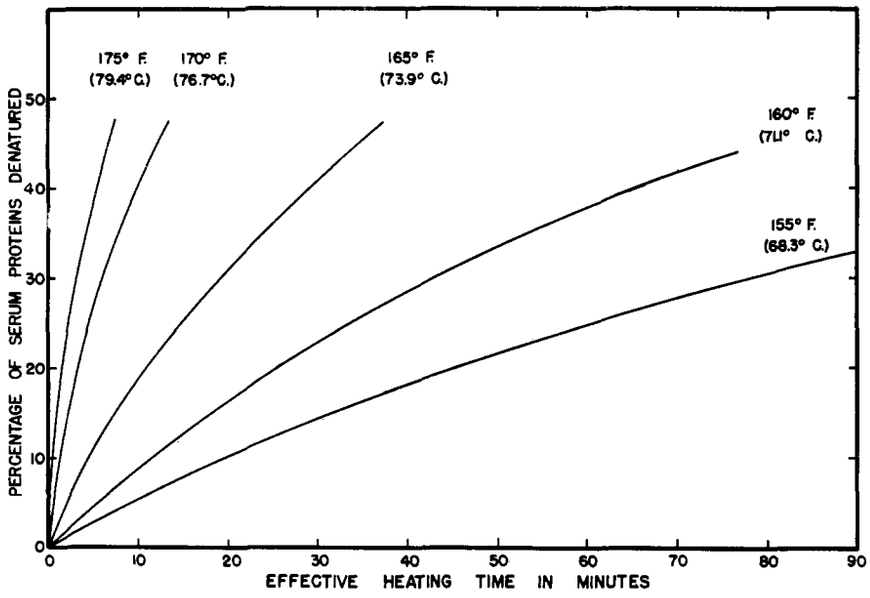


Figure 4. Heat denaturation of milk serum proteins in skim milk

As measured by precipitability with saturated NaCl. Data of Harland *et al.* (*10*)

in straight lines at least over short temperature ranges. In Figure 5 the data from Figure 4 have been plotted in this fashion to show the equivalent time-temperature treatments required for denaturations of 5 to 40%. Also on Figure 5 is plotted a line representing normal pasteurization treatments (63° C. for 30 minutes or 71.1° C. for 15 seconds). Obviously, the denaturation of the serum proteins requires more drastic heat treatments than normal pasteurization.

The determination of undenatured—i.e., nonprecipitable with acid or salt—serum protein has often been suggested as a means of assessing the heat treatment which a sample of unknown history may have received. This would be satisfactory if all lots of milk contained the same concentration of serum protein and if the percentage denaturation by a given treatment were always the same. Unfortunately milk fulfills neither of these requirements. Both the quantity of serum protein and the response to heat treatment vary among lots of milk. In order to obtain some information on these points, 80 samples of fresh, raw mixed milk were collected from 10 regions in the United States at three different seasons of the year (winter, summer, and autumn); these were analyzed for total serum protein and for the per cent denaturation by a treatment at 74° C. for 30 minutes (*11*). Figure 6 shows that the variability in response to this heat treatment is considerable, ranging all the way from 32 to 59%. On plotting the percentage denaturation against serum protein content of the original raw milk (Figure 7), it is evident that there is considerable variability in serum protein content among the original samples (0.6 to 0.9 mg. of nitrogen per milliliter) and that there is some correlation between serum protein content and response to heat treatment. A higher percentage denaturation occurs, in general, in the samples with higher serum protein contents. Obviously, other factors enter in also, because the correlation is far from perfect—i.e., the points exhibit considerable scatter from a linear relationship.

The question arises as to whether the variations in serum protein content in the raw milk and the susceptibility to heat denaturation follow any seasonal or regional pattern. Seasonal variations appear to be small. Samples taken in June averaged only about 4% higher in serum protein content than those taken in the other seasons. Susceptibility to heat denaturation differed little with season. Regional variations seem to reflect differences in the predominant breed of dairy cattle, the serum protein content being higher in regions in which Jerseys and Guernseys predominate. Of course, these regional variations in content are reflected to some extent in differences in susceptibility to heat denaturation. It was considered possible that differences in response to heating might be the result of differences in proportions of the several proteins in the serum protein mixture. To check this possibility, electrophoretic patterns were made under standard conditions (pH 6.85, μ = 0.1, phosphate buffer, 2 hours at 4° C.) on the serum proteins from a representative cross section of the 80 lots of milk in the survey. These patterns failed to reveal any characteristic differences in the composition of the serum proteins that could be associated with the response to heat treatment.

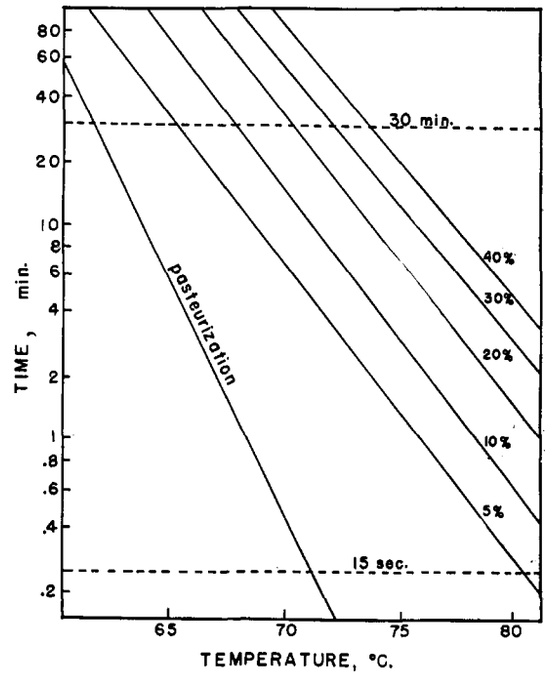
Activity of Sulfhydryl Groups

The activity of sulfhydryl groups has been employed widely as an index of protein denaturation and several methods have been proposed for determining them either qualitatively or quantitatively. The quantitative reagents are usually grouped as oxidizing (such as iodine, *o*-iodosobenzoate, thiamine disulfide or ferricyanide), alkylating (such as iodoacetate or iodoacetamide), and mercaptide-forming (such as mercury, silver, or *p*-chloromercuribenzoate) reagents (*23*). The various available reagents do not necessarily yield identical results for the sulfhydryl content of a given native protein. Evidently the —SH groups in proteins are of graded reactivity, with different amounts available to the various reagents. Denaturation usually produces an increase in reactivity of these groups. In the author's studies on milk serum proteins, and in particular on β-lactoglobulin, the oxidizing agents thiamine disulfide and *o*-iodosobenzoate and the alkylating agent iodoacetamide have been employed.

Thiamine disulfide is a very weak oxidant, but it is quantitatively reduced by cysteine to form thiamine, which can be estimated fluorometrically. Thiamine disulfide does not react at all with native β-lactoglobulin or milk serum protein, but upon denaturation by heat treatment some of the —SH groups become active enough to reduce this reagent. Figure 8 shows the increase in reactivity produced by heating skim milk at vari-

Figure 5. Relation of time and temperature of heating to denaturation of milk serum protein

Data of Harland *et al.* (*10*)



ous temperatures for periods up to 2 hours (*9*). The groups thus activated are susceptible to oxidation by atmospheric oxygen. This is illustrated by the fact that the curves for the higher temperatures in Figure 8 rise to a peak and then fall, and more particularly by the fact, also shown in Figure 8, that a high titer of active groups is preserved by heating in an atmosphere of nitrogen. Thiamine disulfide is a valuable reagent for measuring very active sulfhydryl groups. Results obtained with it correspond rather closely to those secured by the classical nitroprusside test, but they are somewhat more easily put on a quantitative basis.

o-Iodosobenzoate is a rather strong —SH oxidant. It was introduced by Hellerman *et al.* (*13*, *14*), who showed that at a pH near 7 it oxidizes the —SH groups of cysteine and glutathione quantitatively to the disulfide form. It also oxidizes the —SH groups of proteins denatured with guanidine. As outlined by Hellerman, the method involves adding an excess of *o*-iodosobenzoate, allowing it to react, and determining the unreacted excess iodometrically—i.e., adding acid and iodide and titrating the liberated iodine. Hellerman employed the yellow color of the iodine-protein reaction product as a visual end point. In the studies of the author and his associates, however (*18*, *19*), the method was modified by using two platinum electrodes in a technique similar to the so-called dead-stop method for detecting the end point amperometrically. Application of this method to native β-lactoglobulin (*17–19*) indicated a cysteine content of 1.30% calculated on the basis of oxidation of —SH to disulfide. [This result is virtually identical to those reported by Christensen (*3*) on the basis of reaction of urea-denatured β-lactoglobulin with ferricyanide and by Zweig and Block (*28*), who titrated with silver. It is, however, higher than other results in the literature such as 0.55% cysteine with porphyrindin on guanidine-denatured protein (*8*), 0.57% cysteine with silver on alcohol-denatured protein (*15*), and 0.60 and 0.85% cysteine with *p*-chloromercuribenzoate and iodoacetamide, respectively, on guanidine-denatured protein (*5*).]

This is essentially four —SH groups per mole of protein, if the molecular weight be taken as 36,000. Exactly the same result was obtained for the guanidine-denatured protein. Furthermore, it was shown that if *o*-iodosobenzoate is added to the native protein at pH 7 and then dialyzed out, the original —SH content is retained. Thus in the native protein oxidation evidently is not performed by the *o*-iodosobenzoate at all, but rather by the iodine liberated in the process of determining the excess *o*-iodosobenzoate. In denatured protein the oxidation is due partly to *o*-iodosobenzoate and partly to iodine. It is considered that the *o*-iodosobenzoate–iodine method detects all of the —SH in the protein.

The —SH content of the entire milk serum protein mixture, as determined with iodosobenzoate-iodine, is about one half that of β-lactoglobulin but exhibits considerable variability (*19*). As electrophoretic patterns show that components having the mobility of β-lactoglobulin comprise at least 50% of the serum proteins, it is probable that β-lactoglobulin is the principal sulfhydryl-containing protein of the mixture and that the variations in sulfhydryl content of serum protein samples reflect, in part at least, differences in the relative amount of β-lactoglobulin present. Of the other purified proteins that have been prepared from milk serum, eu- and pseudoglobulin and α-lactalbumin are devoid of —SH groups (*16*, *20*). If the albumin of Polis *et al.* (*24*) is indeed identical with bovine blood serum albumin, its —SH content is probably of the order of 0.2% cysteine, and since it comprises only about 5% of the serum protein its contribution to the total sulfhydryl content of the mixture is negligible.

On heat treatment the —SH groups of β-lactoglobulin are activated, some of them to the extent that they are oxidized by atmospheric oxygen if the heat treatment is conducted in air. The net result is a loss in —SH titer as determined by the

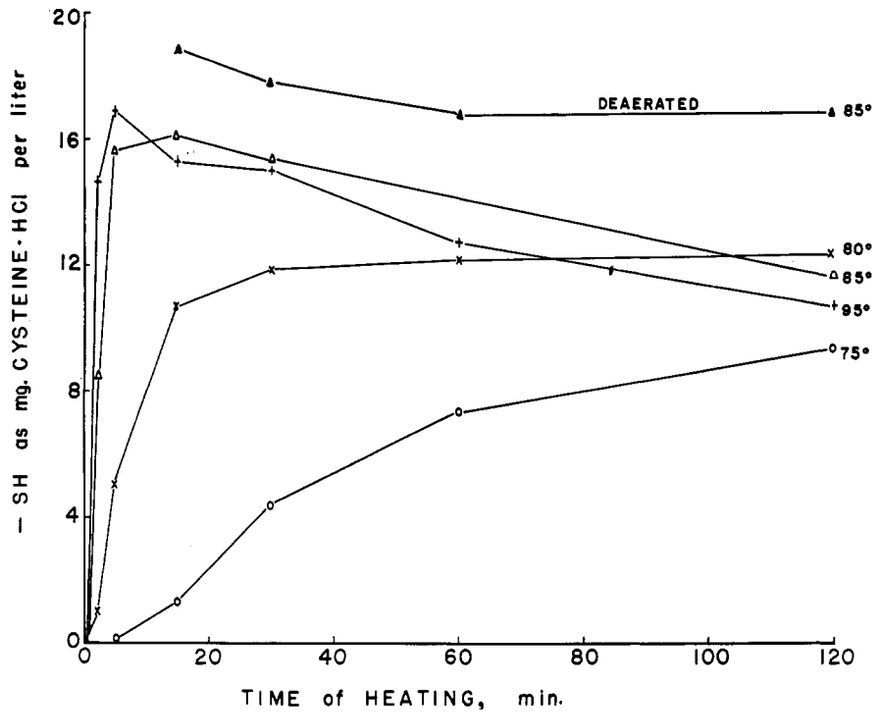


Figure 8. Activation of sulfhydryl groups by heating skim milk

As measured by thiamine disulfide. Data of Harland *et al.* (9)

Figure 6. Variation in extent of denaturation on heating

80 samples of mixed milk heated at 74° C. for 30 minutes. Data of Harland *et al.* (12)

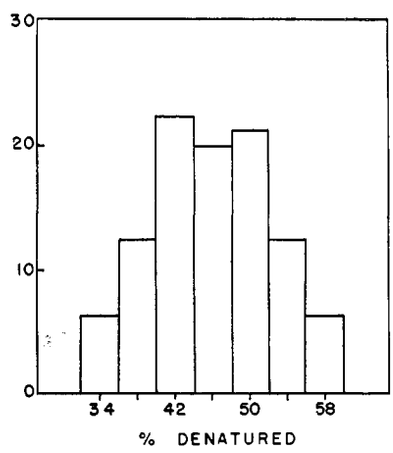
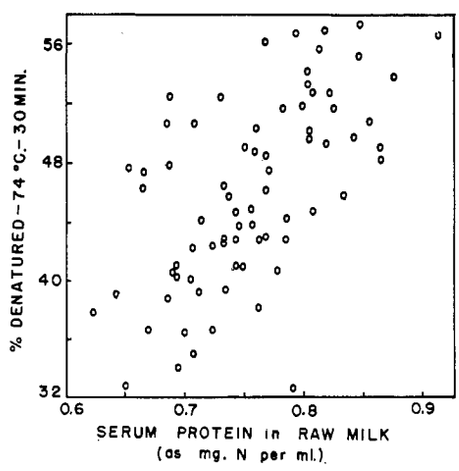


Figure 7. Relation of serum protein content to denaturability of serum protein

In 80 samples of mixed milk. Data of Harland *et al.* (12)



iodosobenzoate-iodine method. This point is illustrated in Table I (*19*), which also shows the protective action of a nitrogen atmosphere. The relative changes in reactivity to thiamine disulfide and to iodosobenzoate-iodine, produced by heating skim milk for 30 minutes at various temperatures, are shown in Figure 9 (*21*). Some of these determinations were made shortly after heating in air and others 24 hours later. It is evident that the total —SH content is decreased by oxidation during heating but that a considerable number of groups reactive enough to reduce thiamine disulfide remain at the end of heating.

Table I. Effect of Heat Treatment on o-Iodosobenzoate Reducing Capacity of Milk, Milk Serum Proteins, and Crystalline β-Lactoglobulin[a]

Temperature for 30 Min., ° C.	Reducing Capacity as Cysteine, %: Reconstituted skim milk[b]	Serum proteins[c]	β-Lactoglobulin[c]
Heated in nitrogen, titrated immediately on cooling			
Control	0.58	0.69	1.30
78	..	0.65[d]	1.03–1.10[d]
Heated in air, titrated shortly after cooling			
64	..	0.59	1.15
69	0.37	0.50	..
73	..	0.32	0.72
78	0.20	0.28	0.63
83	..	0.17	..
97	0.22	0.20	0.43

[a] Data of Larson and Jenness (*19*).
[b] Freeze-dried unheated milk reconstituted in amount of 10 g./100 ml. Ascorbic acid content of this milk was negligible. Cysteine percentages calculated on basis of serum protein in unheated control.
[c] In phosphate buffer at pH 6.9, $\mu = 0.1$.
[d] Greater precautions to exclude air were taken in case of milk serum protein sol than for β-lactoglobulin sol.

The activated —SH groups gradually decrease by oxidation if the protein sample is held at 5° C. after heating. The ability to reduce thiamine disulfide is lost entirely and the capacity to reduce iodosobenzoate-iodine drops to a level determined by the extent of heating. This loss in iodosobenzoate-iodine reducing capacity is depicted in Figure 10 (*17*). The initial drop on heating is followed by a slow decline which is virtually completed in 48 hours. Figure 10 also shows that iodoacetamide, which does not react at all with the native protein, can alkylate the majority of the heat-activated —SH groups if added immediately after heat treatment. Furthermore, the presence of catalytic amounts of copper during heating causes the loss to be virtually complete during the heating process itself. Incidentally, it was recently found (*16*) that the decrease on holding after heating (but not the decrease during heating) can be prevented by the presence of the chelating agent ethylenediamine tetraacetate (Versene). This would seem to suggest that heavy metal catalysis is involved in the oxidation of —SH groups after heating.

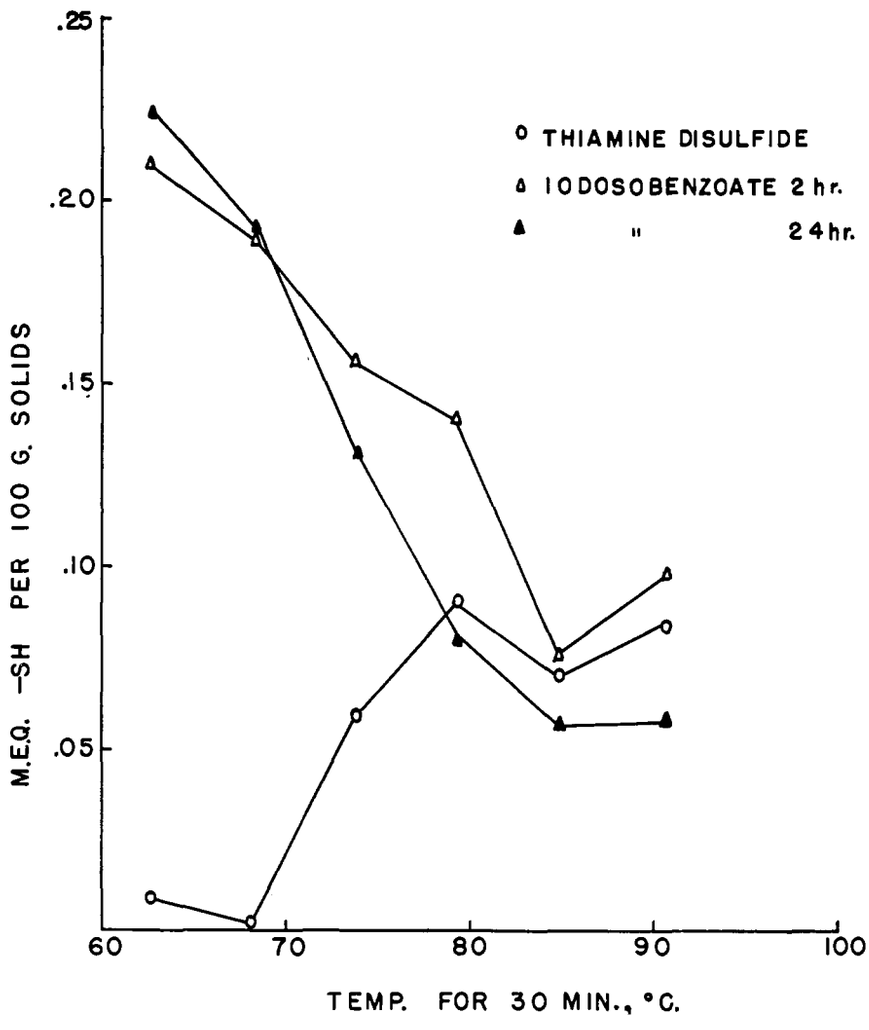
As mentioned above, the level of —SH reached on oxidation after heating is a function of the extent of activation. Thus it is possible to follow the kinetics of the reaction and to determine the energy of activation by applying a series of heat treatments and determining the —SH titer after 48 hours when oxidation of the activated groups is complete. Results of such an experiment (*17*) on β-lactoglobulin at pH 6.9 are given in Figure 11, showing the rate of activation at various temperatures. The activation reaction follows the kinetics of a first-order reaction at least up to the point of activation of half of the —SH groups. Upon plotting the log of the rate constant against the reciprocal of the absolute temperature, the expected linear relation between log K and $1/T$ was obtained. From the slope of this plot, the apparent energy of activation was calculated by means of the Arrhenius equation to be about 80,000 cal. per mole. This is within the range reported for denaturation of other proteins as measured by other criteria. Unfortunately, comparative data for denaturation of β-lactoglobulin as measured by properties other than sulfhydryl activity are not available and sulfhydryl activity has not been used in kinetic studies of the denaturation of other proteins.

The activation of —SH groups occurs over the same range of conditions required to render the serum proteins precipitable. This is illustrated by Figure 1 (*21*), in which is plotted the percentage undenatured as measured by precipitation and by —SH titer for skim milk heated for 30 minutes at various temperatures. However, the kinetics of denaturation of these proteins needs much more study to ascertain the extent of parallelism that exists between the various manifestations of the reaction.

Since heat-activated protein sulfhydryl groups are readily oxidized in liquid systems, it would be expected that they might

Figure 9. Changes in sulfhydryl activity produced by heating skim milk

Data of Larson et al. (21)



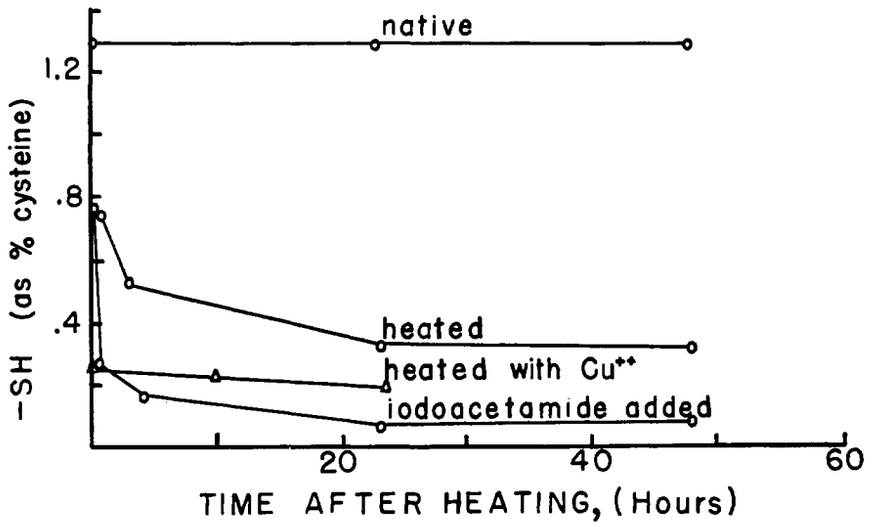


Figure 10. Loss of titratable sulfhydryl groups after heating β-lactoglobulin

Influence of iodoacetamide added after heating and copper (1 p.p.m.) added before heating. Data of Larson and Jenness (17)

function as antioxidants, protecting the fat in fluid dairy products against oxidation. This is generally held to be true, although it has never been proved unequivocally. It is known that fluid milk so heated as to have a high content of very active —SH groups (thiamine disulfide—reducing) has increased resistance to fat oxidation. Likewise dry whole milk prepared with a high heat treatment and containing a high level of very active —SH groups is resistant to oxidation (*12*), but in this case the —SH groups are not oxidized during storage; the level remains constant in dry products. Much more needs to be learned about the mechanisms of these antioxygenic effects in fluid and dry milk products.

For many years it has been known that unheated milk cannot be incorporated into bread satisfactorily, because it softens the dough and depresses the loaf volume. This effect is largely overcome by heat treatment. In fact, it has been shown that the heat-labile factor(s) reside(s) in the serum protein fraction and that its inactivation by heat parallels the denaturation of these proteins (*21*). It was thought for some time that the deleterious effect on bread resulted from a reducing action of —SH groups on gluten, as such compounds as glutathione and cysteine are known to produce similar results. However, it has recently been shown (*20*) that β-lactoglobulin, which carries most, if not all, of the —SH groups of the serum proteins, does not depress the loaf volume of bread. Furthermore, recent studies (*6*) have shown that the deleterious factor resides in the classical lactoglobulin fraction precipitated by half saturation with ammonium sulfate. Thus at present the problem is to isolate and identify this component. The essential point for the present discussion, however, is the fact that the effect of serum proteins on bread probably does not involve —SH groups, as was formerly thought to be the case.

Acknowledgment

The work reported herein represents the joint efforts of several individuals, who are or have been interested in one or more aspects of the problem. The author wishes to express his gratitude to his colleagues, H. A. Harland and S. T. Coulter of the Department of Dairy Husbandry and W. F. Geddes of the Department of Agricultural Biochemistry, with whom he has been associated in this work. Very valuable contributions have been made by the author's former graduate students, R. A. Larsen, B. L. Larson, and A. L. Gordon.

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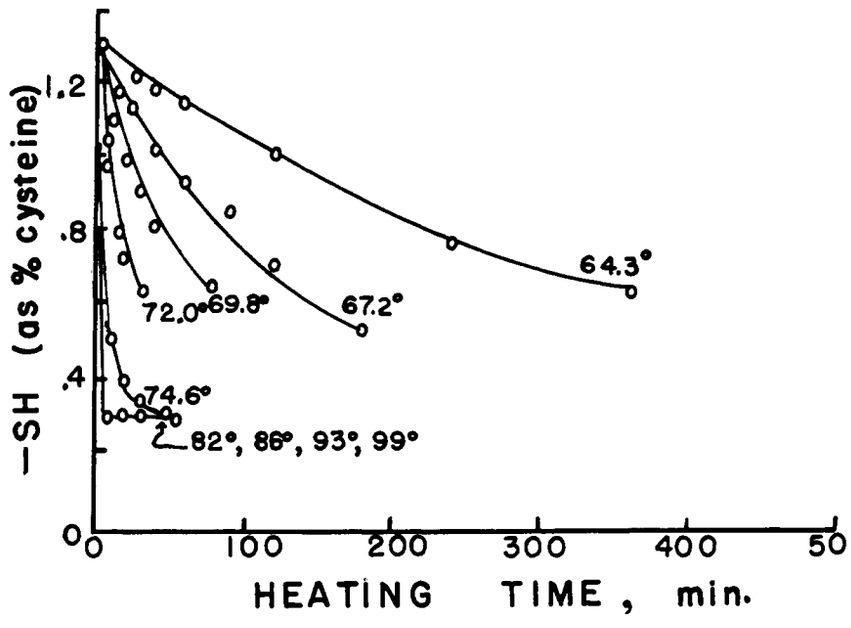
Figure 11. Influence of temperature of heating on loss of titratable sulfhydryl groups of β-lactoglobulin in phosphate buffer

pH 6.9, $\mu = 0.1$. Data of Larson and Jenness (17)



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ANTIOXIDANTS

New Developments for Food Use

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The use of antioxidants in fats and in foods containing fats has increased greatly during the past 10 years. In addition to preventing loss from impairment of palatability, antioxidants conserve the nutritive value of foods, and thus are making an important contribution to better nutrition and to conservation of our food supply. Several substances isolated from natural sources have been found effective in increasing the keeping time of fats as measured by the active oxygen method, but none of them have the important property of "carry-through." Two synthetic antioxidants, butylated hydroxyanisole and 2,6-*tert*-di-butyl *p*-cresol, have excellent carry-through properties. Butylated hydroxyanisole is now used extensively in lard and other edible fats, breakfast cereals, fried corn crisps, potato chips, and nuts, and to treat paraffin-coated wrappers and paper wrappers used in food containers. It is estimated that currently at least 50% of the federally inspected lard produced in this country is stabilized with this antioxidant.

THE USE OF ANTIOXIDANTS to retard rancidity in food fats and foods containing fats is making an important contribution to better nutrition and conservation of our food supply. The development of rancidity in foods lowers the palatability and impairs the nutritive value. Precise data are not available on the amount of food lost owing to rancidity, but it is known to be a considerable amount and experience shows that the use of antioxidants has materially reduced this loss. Rancidity in foods impairs the nutritive value by reducing the content of carotene, various fat-soluble vitamins, and essential fatty acids. There is evidence to show that destruction of these nutrients begins before rancidity can be detected organoleptically. Thus in addition to prevention of loss through impairment of palatability, the use of antioxidants is important in conserving the nutritive value of foods. Rancidity is not confined to foods of high fat content. It may occur in cereals and other foods of relatively low fat content, and under conditions of storage which retard or prevent microbial spoilage, such as refrigeration, dehydration, and salting.

The use of antioxidants in foods, cosmetics, rubber, gasoline and other petroleum products, vitamins, insecticides, and dyes has increased greatly during the past 10 years. During this period about 600 United States patents on antioxidants have been issued.

In 1947 Lundberg (*38*) published a comprehensive review of the antioxidants then in use and proposed for use in fats and foods. Since that time a number of other substances have been proposed and put in use. This paper reviews briefly some of the more recent developments in antioxidants in foods.

Antioxidants from Natural Sources

A number of substances isolated from natural sources have been found to have antioxidant properties and have been proposed for commercial use. Some of these products could be made available in large quantities at low prices. Others probably could not be produced economically. Although some of these naturally occurring substances are effective in increasing the keeping time of fats as measured by the active oxygen method, none have been shown to have the important property of carry-through. [The term "carry-through" is used to denote the effect of the antioxidant in retarding development of rancidity in foods made with fat, as, for example, pastry, crackers, and potato chips.]

Norconidendron was reported by Fisher *et al.* (*18*) in 1947 to be an effective antioxidant for certain vegetable oils. This substance was prepared from conidendron isolated from sulfite waste liquor of the western hemlock. Norconidendron has since been resolved into two isomeric substances, α- and β-conidendrol. Mack and Bickford (*40*) found these substances effective as antioxidants for fats, oils, and candies, as measured by the active oxygen method. No data were reported to show whether the effectiveness carries through into the foods made with the fats. Toxicity studies are being conducted on the conidendrols.

Dihydroquercetin, isolated from the bark of the Douglas fir and Jeffrey pine, was reported by Kurth and Chan (*36*) to be effective as an antioxidant for lard, butter oil, and cottonseed oil. It imparts no color, odor, or taste to fats and is believed to be nontoxic. The authors have found in their laboratories that it is about as effective as propyl