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Note

Occurrence of Sauce Separation in White Sauce with Added Bovine Serum Albumin

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Extensive sauce separation occurred in a white sauce containing bovine serum albumin (BSA) upon heating at 90°C; however, this occurred only slightly in the case where the BSA contained sulfhydryl groups which were modified with *N*-methylmaleimide (NMM) (modified BSA). SDS-PAGE analysis confirmed that BSA homopolymers were formed through disulfide bonds during heating and that α- and β-casein were not involved in the polymer formation during heating. Although the surface hydrophobicity decreased significantly upon heating in the case of BSA solution or the white sauce containing either BSA or the modified BSA, there was no effect of NMM modification on the decrease in surface hydrophobicity, indicating that the degrees of aggregation of both denatured BSAs were similar. On the other hand, the surface hydrophobicity did not change on heating in the case of white sauce alone. These results suggest that the network-formation of denatured BSA through disulfide bonds is responsible for the extensive sauce separation, while the aggregates of denatured BSA due only to noncovalent interactions cause only slight sauce separation.

Keywords: white sauce, sauce separation, disulfide bond, bovine serum albumin, vacuum cooking, surface hydrophobicity

Vacuum cooking has been used as a new cooking or food processing method, since Georges Pralus devised this method in 1966 (Waki, 1989; Editorial Staff, 1989, 1992). This procedure is as follows. All raw materials to be vacuum cooked are packed in a plastic film bag, which is evacuated to increase heat conduction with the air removed by vacuum packing. The sample is then heated at a relatively low temperature (below 100°C) and stored at 0 to 3°C till being served (Goto *et al.*, 1995). The vacuum cooking is different from the ordinary retort pouch cooking, because the mixing of all raw materials before heating and the existence of the evacuation process are not found in the processing procedure for retort pouch foods.

When we made a cream stew with chicken using vacuum cooking, the sauce aggregated and separated from the stew, so that a clear supernatant and a white precipitate were generated (Goto & Nishimura, 1995), meaning that the commercial value of the cream stew was lost. We examined the cause of this phenomenon and found that this extensive sauce separation depended not on the evacuation procedure but on the sarcoplasmic protein liberation from the meat during heating. We showed that the extensive sauce separation also occurred on adding over 0.4% of other soluble proteins such as bovine serum albumin (BSA) or ovalbumin instead of chicken meat (Goto & Nishimura, 1995).

Because the sarcoplasmic protein consists of several kinds of proteins, the system is complex. Thus, in this study, we investigated further the extensive sauce separation using BSA instead of meat to simplify the system.

Materials and Methods

Materials Butter and wheat flour were bought at a local market. The chemicals used were of reagent grade from Nacalai Tesque, Ltd. (Kyoto), and BSA (A-4503, Lot. 64H0248) was obtained from Sigma Chemical Co. (St. Louis).

Preparation of the white sauce The white sauce, containing butter, wheat flour and milk (in a ratio of 15, 15 and 170, respectively), was prepared according to the method described in the previous paper (Goto & Nishimura, 1995).

Sauce separation with BSA To investigate the influence of sulfhydryl groups on the extensive sauce separation with BSA, the following four kinds of samples were prepared: 0.4% BSA solution, 0.4% BSA solution with added 0.36 mM *N*-methylmaleimide (NMM) (modified BSA), a mixture containing 33% white sauce and 0.4% BSA, and a mixture with 33% white sauce and 0.4% modified BSA. These samples were placed into plastic film bags (Asahikasei Ltd., Tokyo) and heated at 90°C for 10 min. Changes in the appearance of each sample were observed. NMM was used to modify the sulfhydryl groups.

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) analysis Before and after heating at 90°C, 0.1 ml of each sample was mixed with 0.9 ml of 0.125 M Tris-HCl buffer at pH 6.8 containing 4% SDS and 20% glycerol. After 2-mercaptoethanol (2-ME) was added to a part of each mixture to a final concentration of 10%, they were heated at 100°C for 3 min and electrophoresed by SDS-PAGE on a slab gel at 20 mA for 1–2 h according to Laemmli's method (Laemmli, 1970).

Measurement of surface hydrophobicity The white

sauce was diluted with distilled water 1500 times to obtain a diluted white sauce. BSA was solubilized in distilled water (BSA solution) or added to the diluted white sauce to a final concentration of 0.004% in the absence or presence of 3.6 μM NMM. The BSA solutions or the mixtures containing the diluted white sauce and BSA were heated at 90°C for 5 min. The diluted white sauce alone was also heated. The surface hydrophobicity of each sample was determined using 8-anilino-1-naphthalenesulfonic acid (ANS) according to the method of Niwa *et al.* (1986) before and after heating. To the sample (5 ml) 1 ml of 0.04% ANS was added. After the mixture was allowed to stand for 10 min, the fluorescence was measured with a Shimadzu RF-500 fluorescence spectrophotometer at an excitation of 365 nm and an emission of 470 nm. The surface hydrophobicity is expressed by regarding the fluorescence of the diluted white sauce before heating as 10.

Unless otherwise stated, at least duplicates were independently assessed in each experiment, and the average±standard deviation was calculated for the experiment of surface hydrophobicity. Statistical differences were evaluated using the Student's *t*-test.

Results and Discussion

The appearance of the sauce separation is shown in Fig. 1. Although both BSA (Photo A) and the modified BSA solutions (Photo B) before heating were transparent, the formation of large aggregates was clearly observed in the BSA solution after heating (Photo C). On the other hand, not aggregation but turbidity was observed in the modified BSA solution (Photo D). A similar tendency of the effects of modification of the sulfhydryl groups by NMM was observed in the case of the mixture of white sauce and BSA (Photos E and F). Extensive sauce separation occurred in the sample consisting of BSA and white sauce after heating (Photo G) but occurred only slightly in that sample containing the modified BSA (Photo H).

The changes in proteins in the four types of samples during heating were examined by SDS-PAGE analysis (Fig. 2). Although there was no difference in the pattern between BSA (lane A) and the modified BSA solutions (lane B), the band of BSA disappeared upon heating in the case of BSA solution (lane C). Adding 2-ME restored the band of BSA (lane K), indicating that BSA polymerized through disulfide bonds during heating. On the other hand, the band of BSA did not change during heating in the case of the modified BSA solution (lane D). These results suggest that the extensive aggregation observed in Fig. 1-Photo C was due to the formation of BSA polymers through disulfide bonds. Similar changes in the BSA band on heating were observed in the case of the mixtures of white sauce and BSA (lanes E, F, G and H, and M, N, O and P). The band of BSA disappeared in the case of a mixture of white sauce and BSA, while there was no significant change in the bands of α- and β-casein (lane G). This may indicate that heteropolymers were not formed during heating through disulfide bonds between BSA and milk proteins derived from white sauce.

The surface hydrophobicity of white sauce, BSA solutions, and the mixture of white sauce and BSAs was measured

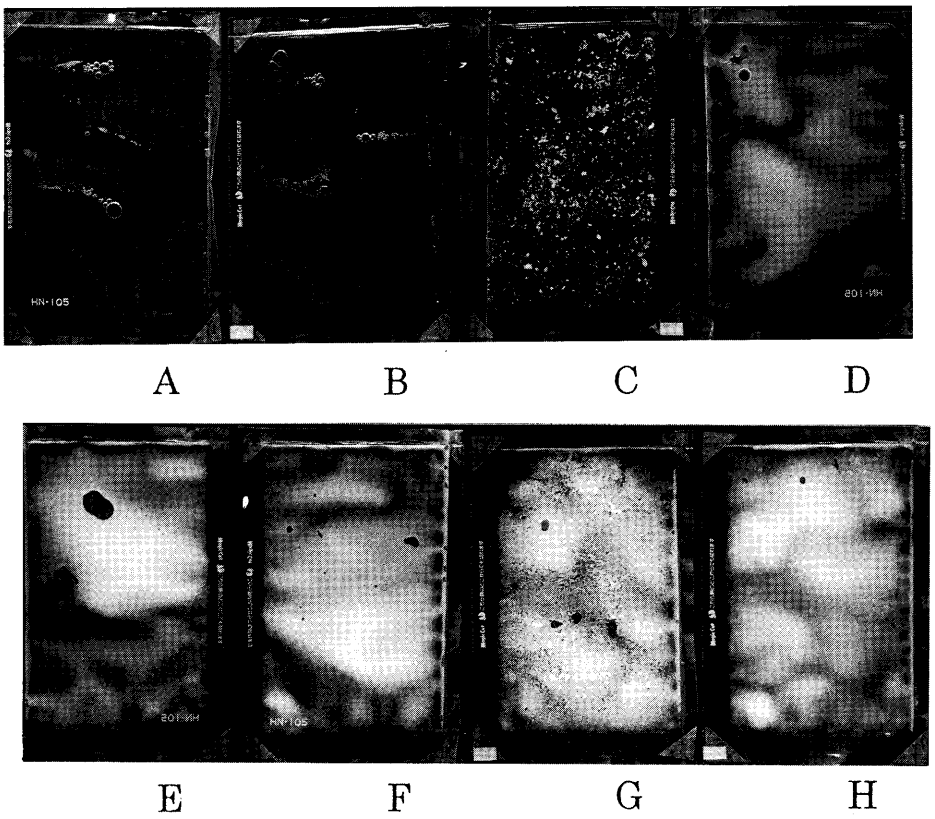

Fig. 1. Influence of BSA on sauce separation by heating in white sauce. A, 0.4% BSA solution; B, 0.4% modified BSA solution; E, mixture of 0.4% BSA and 33% white sauce; F, mixture of 0.4% modified BSA and 33% white sauce. The samples A, B, E and F were heated at 90°C for 10 min to give C, D, G and H, respectively.

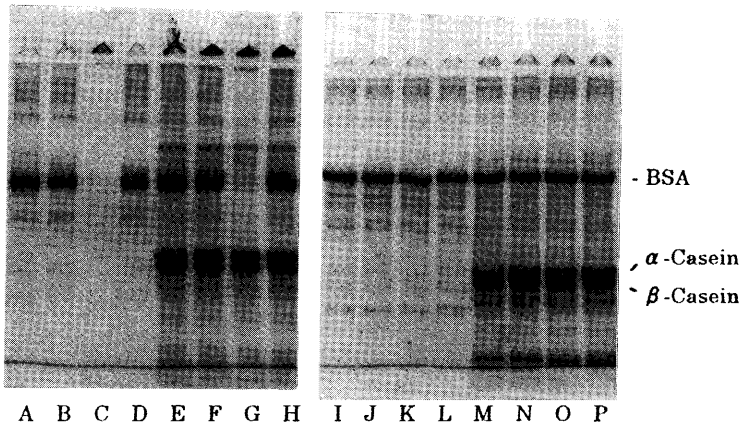

Fig. 2. SDS-PAGE analysis of changes in proteins on heating in BSA solutions and mixtures of white sauce and BSA. The samples of A–H shown in Fig. 1 were subjected to SDS-PAGE analysis. The samples of A–H contained added 2-ME, which gives I–P, respectively and were then subjected to SDS-PAGE.

Table 1. Influence of heating on surface hydrophobicity of diluted white sauce, the BSA solutions (non-modified or NMM-modified), or the mixture with diluted white sauce and BSA (non-modified or NMM-modified).

Heating time (min)	NMM	Surface hydrophobicity		
		White sauce	BSA	Mixture
0	Absence	10.01±0.03[a]	25.08±3.42[a]	29.23±1.96[a]
	Presence		22.25±3.10[a]	28.81±2.56[a]
5	Absence	10.01±0.47[a]	19.25±3.11[b]	23.40±1.17[b]
	Presence		17.58±1.51[b]	22.56±2.10[b]

Values are the mean±SD (n=4). The surface hydrophobicity is expressed by relative values as described in the text. Means within the same column followed by different superscripts were significantly different (p<0.01).

before and after heating for 5 min (Table 1). The surface hydrophobicity of white sauce did not change, while the BSA solutions and the mixtures showed a significant decrease in the surface hydrophobicity. This indicates that the denaturation of BSA and the subsequent aggregation occurred upon heating and that the degree of aggregation of BSA was not affected by NMM modification.

From the results obtained here, the phenomenon of the extensive sauce separation with BSA may be explained as follows: the casein micelles and oil droplets derived from white sauce are caught in and confined by the aggregate of denatured BSA formed through disulfide bonds during heating, so that they accumulate and then the sauce separates severely. Similarly, the aggregates of denatured modified BSA by noncovalent interactions cause slight sauce separation. This sauce separation seems to be low enough to be free of commercial problems. Inomata and Kawamura (1982) reported that the clarifying effects of egg white on preparation of consommé soup depended on the adsorption of soluble proteins on the aggregates of egg white formed during heating. Considering this, we can assume that casein micelles and oil droplets might not only be caught in but also adsorbed on the aggregates of denatured BSA in this system, and thereby the extensive sauce separation occurs. In order to investigate this possibility, the mixture containing BSA and white sauce before heating was centrifuged at 3000 rpm×20 min to obtain the cream, and SDS-PAGE analysis of the cream was carried out. We could not find the existence of BSA in the cream (data not shown). Furthermore, the aggregates of BSA formed by heating were added to the white sauce, and the mixture was then heated at 90°C for 10 min. However, the extensive sauce separation did not occur (data not shown). These results suggest that the adsorption of casein micelles and oil droplets on the aggregates of BSA does not occur in this system. Accordingly, the extensive sauce separation is likely to occur only when casein micelles and oil droplets are caught and confined during the formation of BSA polymers by heating.

Although it is not clear whether the extensive sauce separation by the sarcoplasmic proteins derived from the chicken meat occurs according to this mechanism, we observed that some sarcoplasmic proteins formed aggregates through disulfide bonds during heating (data not shown). This observation together with the results shown here suggests that such the sarcoplasmic proteins as BSA cause the sauce separation in this system when making the cream stew using vacuum cooking.

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References

Editorial Staff (1989). Vacuum cooking method. *Syoku no Kagaku*, No. 134, 41–43 (in Japanese).

Editorial Staff (1992). Vacuum cooked food and the present state of food processing maker. *Shokuhinkougyou,* No. 7, 50–51 (in Japanese).

Goto, M. and Nishimura, K. (1995). Sauce separation in cream stew during vacuum cooking. *J. Home Econ. Jpn.*, **46**, 1159–1165.

Goto, M., Hashimoto, K. and Yamada, K. (1995). Difference of ascorbic acid content among some vegetables, texture in chicken and pork, and sensory evaluation score in some dishes between vacuum and ordinary cooking. *Nippon Shokuhin Kagaku Kogaku Kaishi*, **42**, 50–54 (in Japanese).

Inomata, M. and Kawamura, F. (1982). Clarifying effects of egg white on preparation of consommé soup (part 2). *Chori Kagaku,* **15**, 100–103 (in Japanese).

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriopage T4. *Nature*, **227**, 680–685.

Niwa, E., Kohda, S., Kanoh, S. and Nakayama, T. (1986). Exposure of hydrophobic amino acid residues from actomyosin on freezing-reconfirmation by fluorometry. *Nippon Suisan Gakkaishi*, **52**, 1039–1042 (in Japanese).

Waki, M. (1989). Vacuum cooking method. *Chori Kagaku*, **22**, 190–195 (in Japanese).