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Changes in Functional Properties of Casein by Different Chemical Modifications

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Abstract

Casein was chemically modified with acetic, succinic, and maleic anhydride and changes in functional properties were evaluated as affected by the degree of modification. Chemical modification resulted in casein with unique functional properties depending upon the type of anhydride used and the degree of modification. It was possible to control heat coagulation, calcium precipitability, foaming and emulsion capacity and stability. At pH 4.5 heat coagulation was 0% in the case of 74.1% acetylated casein; on the contrary, succinylation and maleylation resulted in highly heat sensitive protein. Foaming properties were improved markedly by succinylation and maleylation at pH 4.5. However, emulsifying properties were enhanced only by maleylation.

Key words: casein, functionality, acetylation, maleylation, succinylation, chemical modification

INTRODUCTION

The physicochemical and functional properties of proteins can be altered by physical, chemical and enzymatic treatment. These treatments include heating, pH adjustment, hydrolysis and covalent attachment of other constituents. The deliberate modification of food proteins to change their properties has become increasingly popular research area(1-3). The purposes of the modifications are several(4). Modification may be a means of eliminating the undesirable toxic and/or antinutritional properties of some proteins or other constituents; increasing or decreasing the solubility of proteins, especially by adding or eliminating charged groups; changing the functional properties of proteins; improving the nutritional properties by the covalent attachment of limiting essential amino acids; protecting the protein against processing-induced modification, such as the Maillard reaction.

Casein, the most frequently pursued protein by both consumers and food scientists, frequently fails to meet functional and organoleptic properties as related to processed food formulation(5). To solve the drawback of casein, various modification methods were reported(6-11). In spite of these efforts, most researches were carried out in different approaches and experimental methods, which has made the published results hard to compare. Kinsella et al.(12) also pointed out the need for standard methods for quantifying functional properties of proteins.

Therefore, we employed three kinds of chemical modifier to evaluate the functionality changes of casein at different degrees of modification. Two different pH models were compared to elucidate the effect of modification in biological(pH 7.0) and food system(pH 4.5). For chemally modified casein the following functional properties were evaluated: heat coagulation, calcium precipitability, foaming capacity and stability, emulsion capacity and stability.

MATERIALS AND METHODS

Materials

Casein milk(Junsei Chemical Co., Japan lot No. 7E1203) was suspended in 0.3M sodium phosphate buffer(pH 7.5) at 50°C. The final concentration of casein solution was adjusted to 2.5%(w/v) and stored at 5°C until use. All chemicals used in the experiment were of reagent grade. Distilled water was used throughout the experiment unless otherwise specified.

Chemical modification

Degree of modification was determined by the method of Adler-Nessen(13). Free amino groups of casein

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was determined by trinitrobezene sulfonic acid(TNBS) before and after modification. Modified and unmodified casein was dissolved in 0.1M borate buffer(pH 9.5) followed by the addition of 1.1M TNBS with rapid mixing. The degree of modification(DM) was determined by comparing the absorbance at 420nm.

Heat coagulation

Kramer and Kwee method(14) was used to determine heat coagulation. Protein solution(2%, pH 6.8) was stirred for 15min. While stirring 1.0ml was taken out for protein determination by the biuret method. An aliquot (10ml) was placed into a screw cap test tube, heated in a water bath at 100°C for 20min, cooled to room temperature, and then centrifuged at 1935×g for 20min. Protein content of the supernatant was determined by the biuret method. Heat coagulation was reported as the percentage of protein coagulated by heating to total protein of the suspension before heating.

Calcium precipitability

The method of Choi et al.(15) was used to determine calcium precipitability. Protein sample was dissolved in an appropriate buffer to the final concentration of 0.1%(w/v), centrifuged at 2000×g for 20min and then 1ml aliquots were taken for protein determination by the biuret method. The remaining supernatant was divided into two 10ml aliquots followed by the addition of 5% $CaCl_2$ solution(0.2ml). The mixture were shaken at 350rpm for 10min, and then centrifuged at 2000×g for 20min. After centrifugation, 1ml of supernatant was taken for protein determination. Precipitability was expressed as percentage of protein precipitated by $CaCl_2$ to protein of the supernatant before $CaCl_2$ addition.

Foaming capacity and stability

The method of Wang and Kinsella(16) was followed to determine foaming capacity and stability. Protein solution(0.2g in 20ml of buffer of different pHs) was placed in 50ml graduated cylinder with stopper inserted. Cylinders were agitated horizontally at 25°C for 1min. A filter paper disk, having a diameter equal to that of the cylinder, was gently pushed onto the top of the foam layer. The height of the foam was recorded as an index of foaming capacity of the protein. Foam stability was expressed as percentage of foam height after standing at 25°C for 30min to foaming capacity value.

Emulsion activity and stability

To determine the emulsifying properties the method of Pearce and Kinsella(17) was used. Soybean oil(10ml) and 2% protein solution(30ml) were shaken together and homogenized in a Waring blender. Temperature was maintained at 25°C. Aliquot(1ml) of the emulsion were diluted serially with 0.1% sodium dodecyl sulfate solution to give absorbances of 0.01~0.6 at 500nm. Emulsion activity was designated as the absorbance unit mutiplied by the dilution values. After standing the emulsion at 100°C for 10min the same procedure was followed. Emulsion stability was expressed as percentage of the absorbance after heating to that before heating.

RESULTS AND DISCUSSION

Heat coagulation

Heat coagulation of modified casein was shown in Fig. 1. Acetylated casein exhibited relatively less changes in heat coagulation than other modified casein. At pH 7.0 virtually no effect of acetylation was observed on

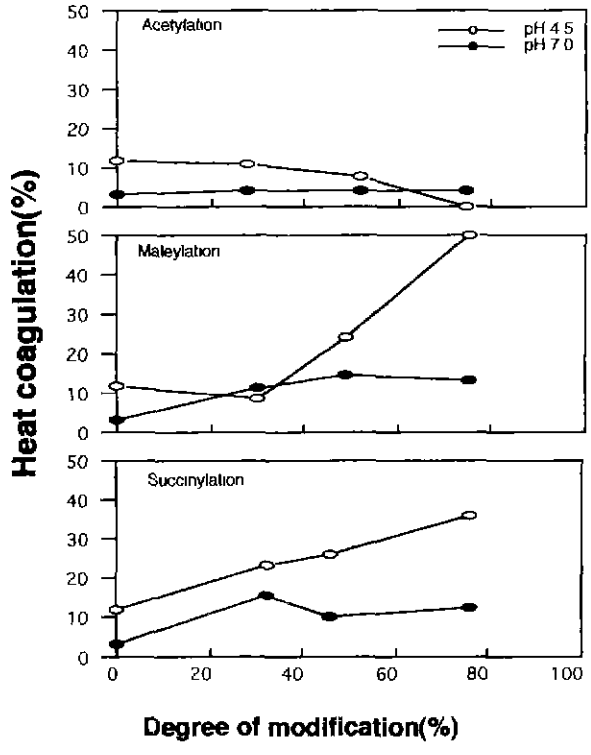

Fig. 1. Heat coagulation of chemically modified casein at pH 4.5 and pH 7.0.

heat coaguation property of casein; in contrast, at pH 4.5 74.1% acetylated casein did not show any coagulation at all. Maleylation increased heat coagulation; at pH 4.5, in particular, dramatic increase in heat coagulation was observed with DM of 48.5% or over. However, the increase in coagulation was not significant at DM of 30.4%. Heat coagulation property of succinylated protein was proportional to the DM at pH 4.5, which indicates that this property can be easily controlled. At pH 7.0 the tendency is almost the same as maleylated casein except for the case of DM of 32.4%, over which heat coagulation slightly decreased.

Heat-induced gelation is a requirement in such products as sausage and cheese analogues in which thermal gelation is necessary for structure and emulsion stability, and in egg white based cakes heat setting of foam batter without collapse is critically important(18). Based upon the result of heat coagulation, maleylated casein should increase the rate of gellation and firmness of gel when used in gel-type food. For use in can-type soup additives, on the contrary, low heat coagulation is required at pH 4.5; therefore, 74.1% acetylated casein is considered to be the most promising candidate.

Calcium precipitability

Chemical modification decreased the sensitivity of casein to calcium ions in most cases(Fig. 2). Calcium precipitability of maleylated or acetylated casein exhibited the inversely proportional relationship to DM at both pH values; in contrast, succinylated casein did not show significant changes in calcium precipitability except for DM of 76.0%.

Calcium precipitability of chemically modified casein was attributed to exposure and/or formation of hydrophobic groups, increase in negative ions, and conformation of protein structure. Acetylation should increase the number of hydrophobic groups; however, it is not likely to increase the calcium precipitability because the hydrophobicity of acetyl group is not any greater than that of other functional groups. In contrast, succinylation and maleylation will increase the number of negative ions as twice as acetylation, which facilitate the unfolding of proteins. Sensitivity to calcium ions, however, was greater in maleylated casein than that in succinylated counterpart. This difference might be caused by the presence of double bond in maleyl group, which increases electronegativity.

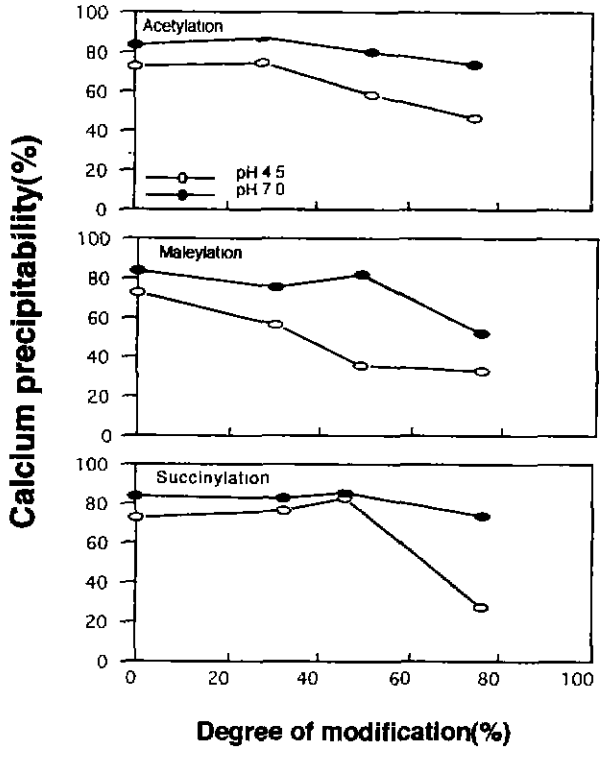

Fig. 2. Calcium precipitability of chemically modified ca-sein at pH 4.5 and pH 7.0.

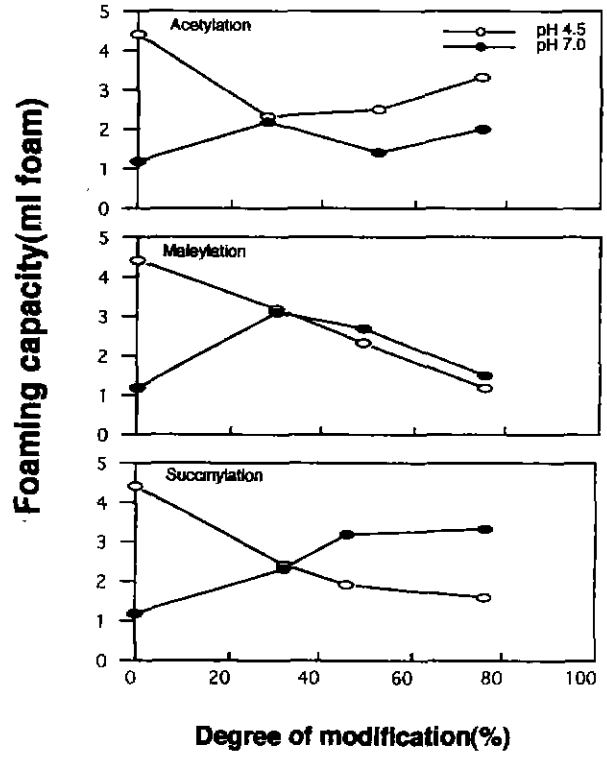

Fig. 3. Foaming capacity of chemically modified casein at pH 4.5 and pH 7.0.

For real food system(pH 4.5) fortified with calcium, 74.1% acetylated casein might be the best selection because it exhibited 0% heat coagulation and relatively low calcium precipitation as compared to other modified casein.

Foaming properties

Kinsella(12) summarized present thinking on foam formation of protein solutions. When an aqueous suspension of protein ingredient is agitated by whipping or aeraton processess, it will encapsulate air into droplets or bubbles that are surrounded by a liquid film.

Chemical modification of casein resulted in decreased foaming capacity(FC) at pH 4.5 compared to the control (Fig. 3). FC at pH 4.5 showed inversely proportional relationship with the degree of modification except for acetylated casein. FC increased after reaching the minimum at acetylation of 27.7%.

On the contrary, at pH 7.0 chemical modification significantly increased FC in most cases; succinylation, in particular, showed remarkable increase in FC. In contrast, FC of maleylated casein decreased after reaching the maximum at DM of 30.4%. Acetylation did not show large magnitude of changes in FC at pH 7.0.

FC is very important property in manufacturing bakery products and ice cream; however the foam should be stable to complete the manufacturing process. In Fig. 4, chemically modified casein showed different mode of stability depending upon pH. At pH 7.0 foaming stability(FS) decreased with increasing DM, whereas it was increased proportionally with DM. Since pH of most foods lies somewhere in pH 4.5, this result indicates that chemically modified casein can applied to the real food system for foaming property.

With respect to FC and FS, 74.1% acetylated casein showed the best foaming property because the increased hydrophobicity and low degree of ionization in carboxyl groups of casein reduce the interfacial tension between air and solution. At pH 7.0, on the contrary, 32.4% succinylated casein improved the foaming property; in this case, exposure of internal hydrophobic groups by increased negative ions is most likely to contribute to the enhanced foaming property. Compared to succinylated casein different behavior of maleylated casein can be explained by the electornegativity of double bonds in maleyl group.

Emulsifying properties

Emulsifying properties have long been utilized as

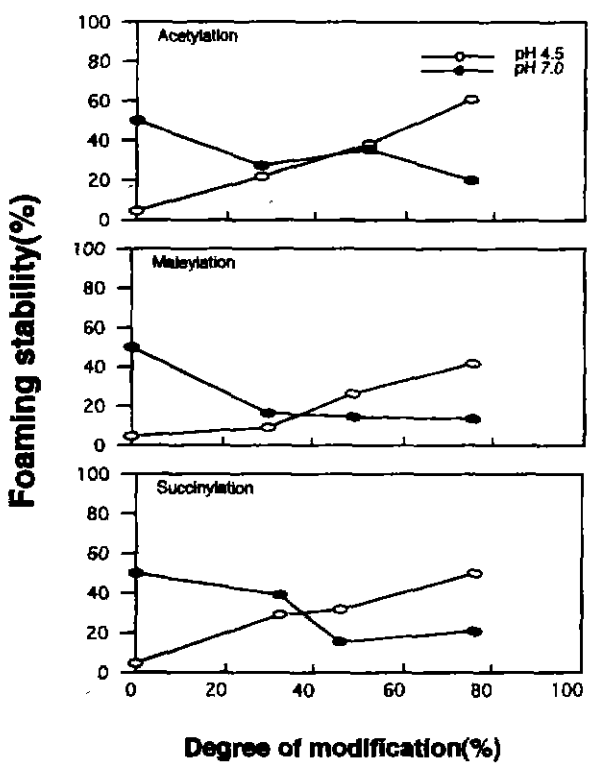

Fig. 4. Foaming stability of chemically modified casein at pH 4.5 and pH 7.0.

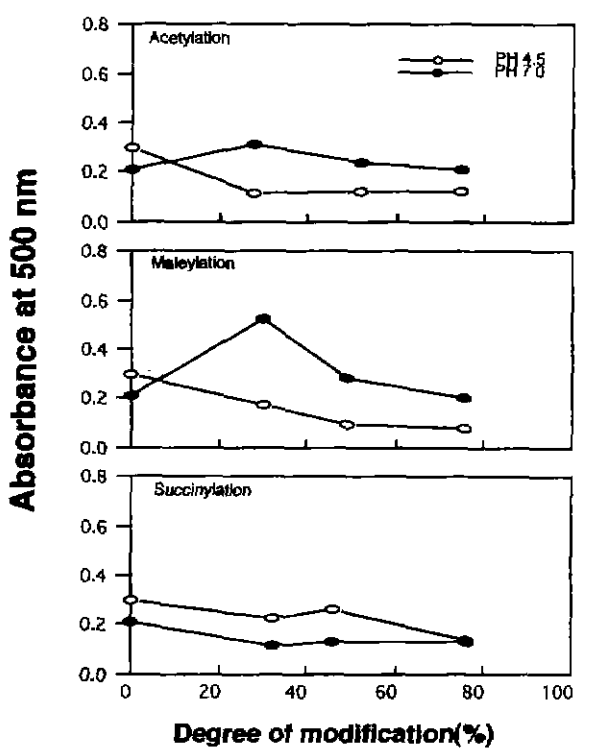

Fig. 5. Emulsion activity of chemically modified casein at pH 4.5 and pH 7.0.

processing aids in comminuted meats, coffee whiteners, milk-type beverages, etc. and expressed as emulsion activity and emulsion stability(19,20).

Regardless of types of chemical modifier, emulsion activity(EA) at pH 4.5 was decreased with increase in the degree of modification; in contrast at pH 7.0 EA showed singificantly higher as increasing DM than that at pH 4.5(Fig. 5). In particular, maleylated casein with DM of 30.4% exhibited the highest EA.

Emulsion stability(ES) tendency was opposite from the emulsion activity(EA). At all DM values acetylated or maleylated casein showed higher ES at pH 4.5 than that at pH 7.0; on the contrary, succinylated casein exhibited the strong ES at pH 7.0(Fig. 6). ES of maleylated casein was remarkably improved with increasing DM up to 48.5%.

With respect to EA and ES, maleylated casein showed considerable enhancement in emulsion properties. At pH 7.0 EA of 30.4% maleylated casein was increased 2.5 times as compared to the control; at pH 4.5 maleylation did not improve EA but ES was 100% for 30.4~48.5% maleylated casein.

In conclusion the functional properties of casein can be designed by employing proper chemical modifier and the extent of modification to fit the specific food system. However, further research is needed to confirm that this result can be extended to the real food products which should undergo more severe treatment during the processing.

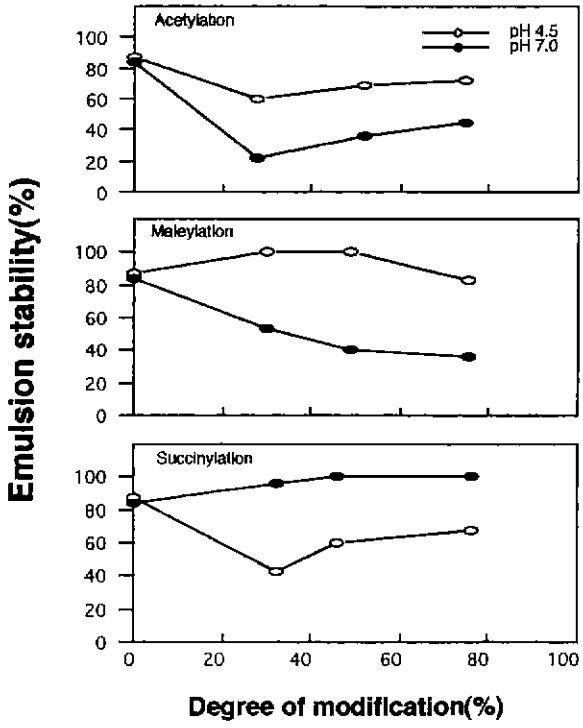

Fig. 6. Emulsion stability of chemically modified casein at pH 4.5 and pH 7.0.

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