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Effects of Protein Unfolding and Soluble Aggregates Formation on the Gel Strength of Whey Proteins

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Abstract

Heat-induced gelation is an important functional property of whey proteins. Preheating of calcium reduced whey was reported to increase gel strength. 5% whey-protein solutions were preheated at pH 7 and at various temperatures(60~80°C) for 15 minutes. The amount of soluble aggregates and denaturation enthalpy of preheated whey proteins were measured. Preheating temperature was negatively correlated with denaturation enthalpy(R^2=0.857, P=0.008) and positively with the amount of soluble aggregates(R^2=0.921, P=0.002). Denaturation enthalpy was negatively correlated with gel strength(R^2=0.93, P=0.002). Soluble aggregates and gel strength were positively correlated(R^2=0.972, P=0.0003). The formation of three dimensional gel network requires controlled protein denaturation and aggregation. Since preheating leads to the partial denaturation of proteins and the formation of soluble aggregates, preheated whey proteins have a higher gel strength than non-preheated one.

Key words: whey proteins, soluble aggregates, denaturation enthalpy

INTRODUCTION

Whey is a by-product of cheese manufacture which is drained from the curd(1). Whey proteins have good nutritional quality and many functional advantages, such as solubility, high water retention, foaming, and gelation (2,3). One of the most important functional properties of whey proteins is the formation of heat-induced gel(4). Generally, gelation is regarded as a two-stage process. The first step involves the unfolding of proteins and the second involves aggregation. Aggregated proteins can form a gel network stabilized by hydrophobic interactions, hydrogen bonds, electrostatic interactions, and disulfide linkages(5). Whey proteins can be used for egg-white replacement, surimi, or reformulated meat products(1). If we could increase the gel strength of whey proteins, the amount of whey proteins which is required to get a certain gel strength would be decreased. One possible method is to preheat whey protein solutions at proper pH and calcium content before spray drying(6-8).

Preheating of concentrated whey changes the properties of resulting whey proteins. Hwang(7) reported that preheated whey protein concentrates(WPC) contained more soluble aggregates than control whey. Many researchers (9-12) have reported that size exclusion HPLC analysis can be used to measure soluble protein aggregates. The HPLC procedure was relatively simple to perform, and appeared to give a good indication of the amount of functional proteins present in WPC. It is assumed that preheated whey proteins have lower denaturation enthalpy because they are already partially denatured. Differential scanning calorimetry(DSC) has been used to determine protein denaturation. In DSC, heat uptake, which is related to protein unfolding(denaturation), is measured from the peak area(13). WPC processed with intensive heat treatment showed smaller peak areas, indicating more severe protein denaturation(9,14,15).

In this study, the effect of soluble aggregates and denaturation enthalpy on gel strength was investigated. Hwang (7) showed that the amount of soluble aggregates was not significant when WPC solution was preheated below pH 7. The range of denaturation temperatures of whey proteins is known as 60~80°C. Therefore, pH 7 and temperature range of 60~80°C were selected as preheating conditions.

MATERIALS AND METHODS

Preheating of whey proteins

Whey protein isolates(protein 91.2%, calcium 0.15%,

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Le Sueur Isolates, Le Sueur, MN, USA) solutions(5%) were preheated at pH 7 and at temperatures of 60, 64, 68, 72, 76, and 80°C for 15 minutes. Preheated WPI solutions were freeze-dried

Gel strength

A 20ml of 10% protein solution with 10mM $CaCl_2$ was placed in glass tubes(850mm × 19mm, i.d.) and heated at 90°C for 20 minutes. After heating, the tubes were placed in an iced water bath for 1 hour. The resultant gels were cut into 2cm length. Gel strength was measured using a Texture Analyzer TA-XT2(Texture Technologies Corp., Scarsdale, NY, USA). Displacement speed was 1mm/sec and a cylindrical probe(2.5cm diameter) was used. Gel strength was expressed as the force in Newtons when the surface yield point was reached.

Soluble aggregates

Soluble aggregates were determined by the size exclusion HPLC procedure of Morr(16). A 10ml portion of 0.5% solution was injected on a TSK-Gel G 3000 SWXL column(30cm × 7.8mm, i.d., TosoHaas, Montgomeryville, PA, USA), and eluted with 0.1M, pH 6 sodium phosphate buffer containing 0.1M sodium nitrate at a flow rate of 0.6ml/min. Protein was monitored at 280nm using a Waters HPLC system(Millipore Corporation, Milford, MA, USA) consisting of 501 pumps, 700 Satellite WISP, and M-490 Programmable Multiwavelength Detector.

Denaturation enthalpy

A DuPont 910 Differential Scanning Calorimeter(DuPont Instruments, Hoffman Estates, IL, USA) equipped with a DuPont 9900 Thermal Analyzer was used. A 15mg sample of 10% protein solution was placed in hermetically sealed, stainless steel pan. Denatured whey protein was used as a reference. Samples were scanned at heating rates of 10°C/min over the temperature range from 25 to 120°C.

RESULTS AND DISCUSSION

Effect of preheating temperature on denaturation enthalpy, soluble aggregates, and gel strength

The relationships between preheating temperature and denaturation enthalpy as well as soluble aggregates are presented in Fig. 1. Preheating temperature was negatively correlated with denaturation enthalpy(R^2=0.857, P=0.008). Denaturation enthalpy rapidly decreased with heating above 72°C. Since β-lactoglobulin(β-lg) is denatured at 76~82°C, and amounts to 50% of whey proteins, this significant change is probably due to the denaturation of β-lg. As preheating temperature increased, the amount of soluble aggregates also increased(R^2=0.921, P=0.002). Soluble aggregates were formed when whey protein solutions were preheated above 64°C. This result was consistent with that of Parris et al.(17), who reported the amount of soluble aggregates increased almost linearly above 65°C. Whey proteins are reversibly denatured at 60°C, therefore preheating at 60°C can be regarded as

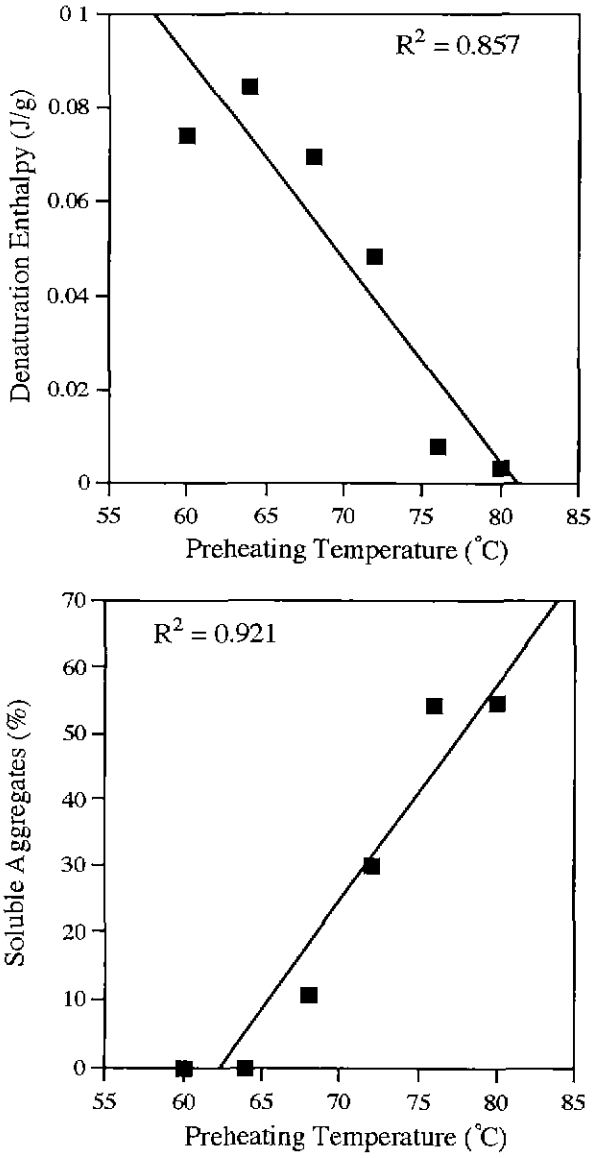

Fig. 1. The relationship between preheating temperature and denaturation enthalpy and soluble aggregates of whey proteins.

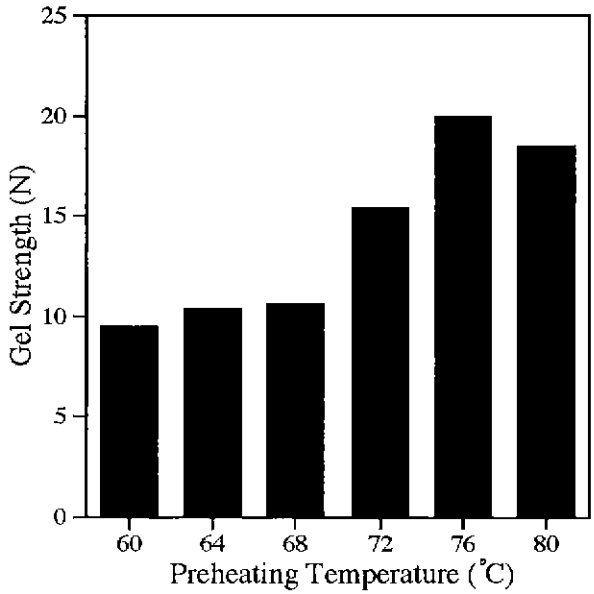

Fig. 2. The effect of preheating temperature on gel strength of whey proteins.

control, that is, the non-preheated sample. Preheated samples had higher gel strength than the control(Fig. 2). For example, preheating at 72°C resulted in 1.6 times higher gel strength than the control. Gel strength increased significantly between 68 and 72°C. It is likely that soluble aggregates played an important role in increasing gel strength because the formation of soluble aggregates started above 64°C. Hwang(7) reported that WPC which had a large amount of soluble aggregates formed stronger gels. It was concluded that whey protein solutions should be preheated above 70°C at pH 7 to increase gel strength. Whey protein solutions, which were preheated at higher temperatures, had more soluble aggregates and consequently formed stronger gels. However, preheating above certain temperatures may cause the formation of insoluble aggregates and leads to low solubility or high viscosity depending on preheating conditions. Many researches have shown that whey proteins are unfolded to a limited extent at 70°C, and irreversibly denatured at temperature above 70°C(1,18-22).

Effect of denaturation enthalpy and soluble aggregates on gel strength

Denaturation enthalpy was negatively correlated with gel strength(Fig. 3). The R^2 value was 0.93 and the p-value was 0.002. Whey proteins form heat-induced gels through controlled protein denaturation and aggregation. During heating, the extent of denaturation is important factor on protein gel system. If heating is too severe, the proteins

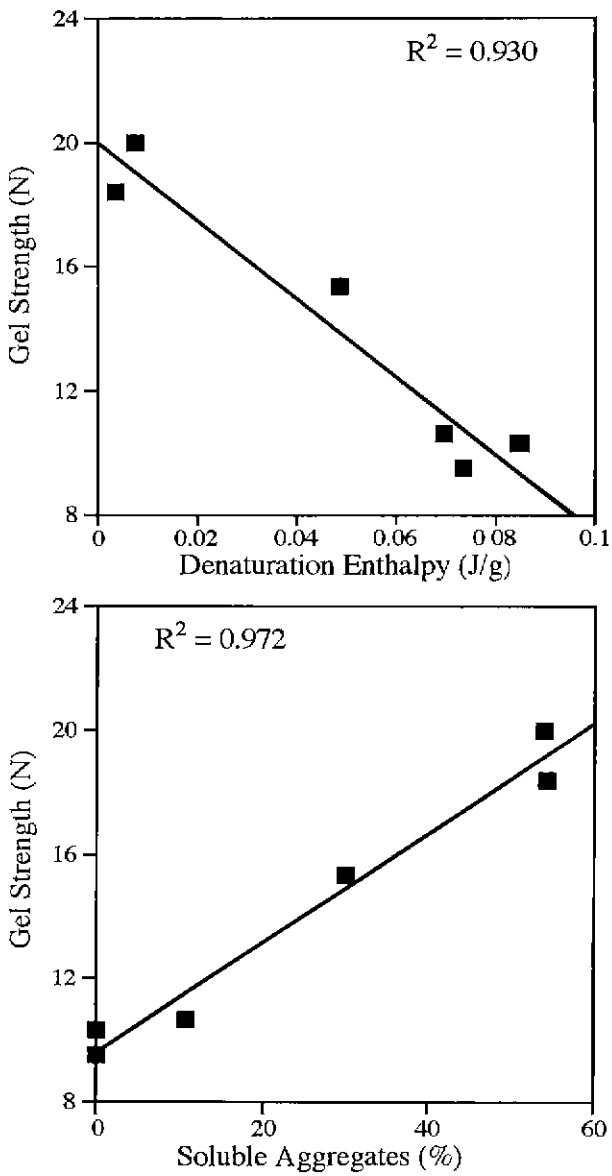

Fig. 3. The relationship between gel strength and denaturation enthalpy and soluble aggregates of whey proteins.

are extensively denatured, and precipitate prior to the formation of a gel network. Preheating at proper temperature causes partial denaturation of proteins. The formation of a heat induced gel requires a balance between the rate of unfolding and the rate of aggregation. Ideally, there would be complete unfolding and then aggregates formation. Proteins heated at temperatures of 80 to 100°C unfold and aggregate at the same time. Excessive aggregation prior to adequate unfolding results in a less structured and weaker gel matrix. Preheating at 70°C probably results in considerable unfolding as indicated by a decreased enthalpy of denaturation with only minimal aggregation. Minimal aggregation is suggested by the high solubilities of these samples. Therefore preheated

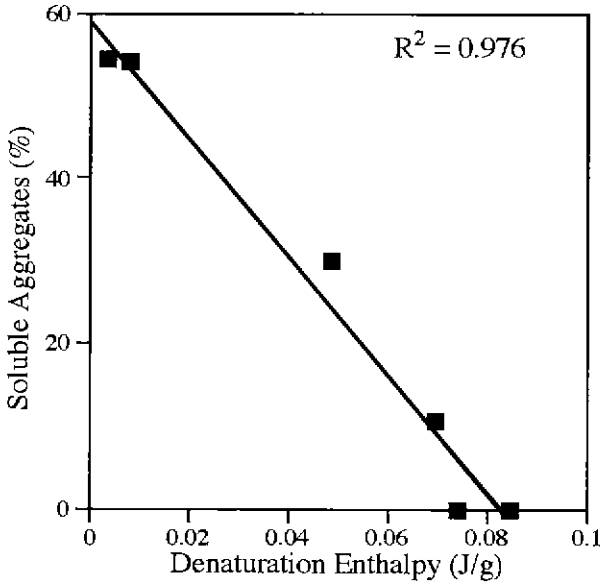

Fig. 4. The relationship between denaturation enthalpy and soluble aggregates of whey proteins.

samples, with low denaturation enthalpies, are able to form stronger gels than samples that have not been preheated. The sample which was preheated at 80°C had a lower gel strength than that of 76°C. This implies that denaturation above a certain level impairs gel strength. Soluble aggregates and gel strength were positively correlated (Fig. 3, R^2=0.972, P=0.0003). Soluble protein aggregates are regarded as intermediates required for the formation of gel networks(7,23,24). These results are consistent with those suggestions. Soluble aggregates and denaturation enthalpy were negatively correlated with the R^2 value of 0.976 and the p-value of 0.0002(Fig. 4). This suggests that soluble protein aggregates form after proteins are denatured.

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