Controlled stress rheometry compared with Formagraph measurements for characterization of the enzyme induced gelation of whey proteins at various pH

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ABSTRACT

The influence of pH (5.0-7.5) on the enzyme induced gelation of whey protein isolate was studied. Results obtained with the Formagraph (gel time, gel strength and rate of gelation) were compared with measurements using a controlled stress rheometer. Good agreement between the two methods was found, although the Formagraph, is not suitable for high gel strengths.

INTRODUCTION

Whey proteins are soluble over a wide pH range and exhibit good gelling properties, which is utilized by the meat and bakery industries. Two types of heat induced whey protein gels have been observed[1]: particulate, opaque gels formed at pH 4.5 to 6, and fine-stranded, transparent gels formed outside this pH range. Recently it has been shown, that partial enzymatic hydrolysis by a specific protease isolated from *Bacillus licheniformis* can induce gelation in whey protein[2].

Protein gelation, whether it is thermally or enzymatically induced, is often a time-consuming process. In order to describe the influence of various parameters (pH, addition of salts, concentration etc.) on gelation, it would be of interest to screen several samples at the same time.

The Formagraph, originally developed for use on cheese milk, is an instrument that uses small pendulums submerged in the samples to record an amplitude when the samples are exposed to a linear oscillation, and the results are given as a gelation curve, that shows an amplitude as soon as a gel is formed. Ten samples can be analyzed at the same time.

In the present study we have used the Formagraph to characterize the influence of pH on the enzymatically induced gelation of whey protein isolate, and compared the results with measurements obtained with the Bohlin CVO (a controlled stress rheometer).

MATERIALS AND METHODS

The whey protein isolate (WPI) used was a commercial preparation (BiPro, batch no. 157B-3-STP 27-341, Bio-Isolates PLC, Deeside, UK) with the specifications described by Ju et al[3] . Solutions of 9% WPI were prepared one or two days before use and kept at 5°C. Denatured WPI solutions were made by heating a solution adjusted to pH 7.0 at 80°C for 30 min. pH was adjusted with either 4 M HCl or 2 M NaOH. However, at pH levels below 6.2 the denatured WPI instantly formed a gel.

The enzyme used (BLP) was a serine protease from *Bacillus licheniformis*, specific for Glu-X and Asp-X bonds, and was obtained from Novo Nordisk A/S (Bagværd, Denmark). It had an activity of 20500 Anson U/kg according to the supplier. Enzyme solutions were prepared by dissolving 100 mg BLP in 0.5 ml distilled water. In all the

experiments the enzyme/substrate ratio was 1%.

The BLP-induced gelation of the WPI solutions was assessed with the Formagraph (Type 11700, Foss Electric, Hillerød, DK) in the following manner: Ten 10 ml samples were placed in the 10 individual wells in the cell block, which had been preheated to 50°C. The enzyme solution was added simultaneously to all the samples by a mixer fitted with ten spoons. 0.8 ml of vegetable oil was put on the surface of the samples to prevent evaporation. Gel time, rate of gel firming and gel strength was determined as described by Ju et al[4]. The mean and standard deviation from triplicate determinations were used.

The gelation of the whey protein solutions was also followed using a controlled stress rheometer (Bohlin CVO, Bohlin Ltd., Cirencester, UK), in the autostrain mode. The C 14 measurement system was used with a frequency of 0.5 Hz, and an autostrain set to 0.1. The gelation time was recorded as the time when the phase angle (δ) was equal to 45°, the rate of gel firming was taken as the maximum slope of the complex modulus (G*) vs time curve, and the gel strength was taken as G* at a time equal to twice the time of gelation.

RESULTS

Concerning the time of gelation at the various pH values (Figure 1), there is very good agreement between the results obtained with the Formagraph and with the Bohlin CVO.

The observed variations in gel time probably reflects the enzyme activity as well as the ionic charges of the proteins in solution. BLP has an optimum activity in the range of 6-10, and at pH-values higher than 6.8, where the whey proteins are kept in solution through electrostatic repulsion, it is the extent of hydrolysis that determines the gel time. As

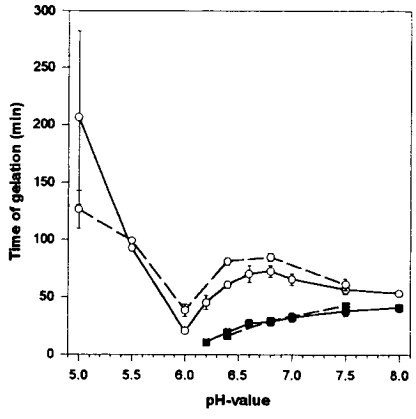

Figure 1. The time of gelation WPI (9%, 50°C) as measured by Formagraph (solid lines) and Bohlin CVO (dashed lines). White circles indicate native WPI and black squares pre-denatured WPI.

the pH is lowered, the net negative charge is decreased, and random aggregation of the protein from the native WPI solution takes place, resulting in white, opaque,

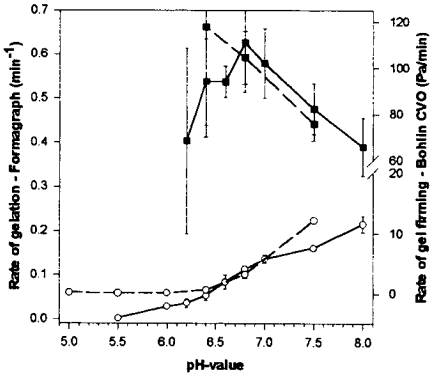

Figure 2. The rate of gel firming of WPI (9%, 50°C) as measured by Formagraph (solid lines) and Bohlin CVO (dashed lines). White circles indicate native WPI and black squares pre-denatured WPI

acid induced gels.

The pre-denatured WPI formed fine-stranded, translucent gels at the pH-values indicated, with gel times increasing with pH, perhaps reflecting that for these solutions, the charge of the protein is critical in determining the time of gelation.

The rate of gel firming (Figure 2) also shows a good agreement between Formagraph and Bohlin CVO measurements - especially in the pH range 6 to 7.

The rate of gel firming of the native WPI solutions was proportional to the pH, probably reflecting increased enzyme activity with increased pH. Denatured WPI gels faster than native WPI.

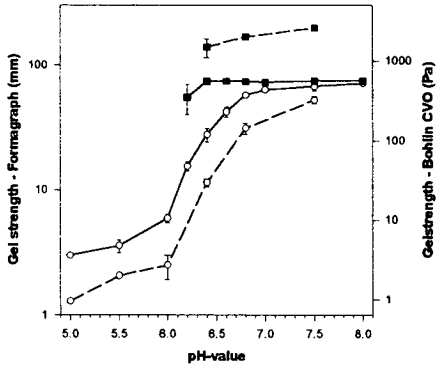

Figure 3. Gel strength of WPI (9%, 50°C) as measured by Formagraph (solid lines) and Bohlin CVO (dashed lines). White circles indicate native WPI and black squares pre-denatured WPI.

The gel strength (Figure 3) measured on the Formagraph shows good agreement with the Bohlin CVO, for the native WPI solutions in the pH range 5.0 to 6.8, where the gel strength did not exceed 70 mm (Formagraph). At higher gel strengths, however, the Formagraph is not able to differentiate between samples, whereas it appears from the Bohlin CVO measurements, that the gel strength of both native and denatured WPI solutions increased with pH, most likely due to the accompanying increase in enzyme activity.

CONCLUSION

Formagraph measurements showed good agreement with controlled stress measurements, until the upper limit of the instrument was reached.

Denatured WPI, hydrolyzed by BLP gelled faster and resulted in stronger gels than equivalent gels made from native WPI

The gelation properties varied with pH, due to a combined effect on protein net charge and enzyme activity.

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