GELATION OF BOVINE MYOFIBRILLAR PROTEIN INDUCED BY 1,5-GLUCONOLACTONE

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SUMMARY

Gels were formed at 4°C from myofibrillar proteins isolated from bovine *m. cutaneus trunci* by the addition of 1,5-gluconolactone (GdL). Compression was used to study the rheological characteristics of the gels.

Levels of 0, 1, 2, 4, 6, 8, 10 and 12% (w/w) GdL were added to myofibrillar proteins (20% solids) with 30% added water. Gels were formed at levels 2% GdL and greater. A concentration of 2% GdL (pH 4.5) resulted in formation of optimal gels in terms of gel rigidity and stickiness. Gel rigidity was strongly dependent on final pH. It is suggested that slow lowering of pH results in the formation of uniform myofibrillar gels below the isoelectric point. No differences were observed in gelation at temperatures in the range 0°C to 20°C.

The application of gelation by addition of a slow release food acid to a whole meat system could provide a method for restructuring meat at 4°C.

INTRODUCTION

Much research has been undertaken on the fundamental aspects of thermally induced binding of meat and its protein fractions. VADEHRA and BAKER (1970) stated that binding between meat pieces is a heat initiated reaction since no binding was observed in the raw state. However, FRETHEIM et al (1985) found that solutions of myosin (10 mg/ml) in 0.6 M KCl formed gels at 5°C if the pH was decreased slowly, by dialysis, to a value in the region of 2.5 to 5.5. Differential scanning calorimetry revealed that the myosin of pH-induced gels absorbed no thermal energy when heated, implicating acid-induced denaturation as the basis of gel formation. HERMANSSON et al (1986), in an investigation of the effect of pH and ionic strength on thermally induced myosin gels, also observed that myosin solutions dialysed against pH 4.0 buffers in 0.6 M KCl formed gels spontaneously at 4°C.

The aim of this study was to investigate gels of bovine myofibrillar proteins formed at 4°C as a result of the slow lowering of pH through the addition of 1,5-gluconolactone (GdL). Compression was used to study the rheological characteristics of the gels.

MATERIALS AND METHODS

Myofibrillar Proteins

Bovine *m. cutaneus trunci* was obtained within 1 h of slaughter. The muscle was trimmed of excess visible fat and connective tissue, cut into approximately 3 cm cubes and stored at 4°C overnight. Ice and water was added to the cubed meat (1:1:1, w/w/w) in a Jeffco Wet Disintegrator (model 291, Jeffress Bros Ltd, Brisbane, Australia). The mixture was stirred for 5 min. The meat slurry was poured into a motorised mixer with a further addition of iced water to slurry (2:3, w/w). The mixture was stirred for 10 min. Collagen was removed with a spoon while stirring. The meat slurry was strained through a 3 mm stainless steel mesh removing further collagen. Discs were removed from a Westfalia Separator AG (model LWA 205, Westfalia Oelde, Germany). The meat solution was added slowly to the separator at 12000 rpm until a maximum amount of myofibrillar protein was retained. Liquor was collected and run once through the separator. The myofibrillar protein was mixed in a Kenwood Chef Cakemixer (model A703C, Australia) to give a homogenous system. The protein was vacuum packaged and heat sealed using a chamber type vacuum packaging machine. They were stored as discs, less than 5 mm thick, at -20°C in PVDC nylon polyethylene polymer bags with low oxygen permeability and low moisture permeability. Myofibrillar protein was used within 5 months of preparation.

Gel Preparation

Water used for gel preparation was boiled distilled water that had been passed through a Milli-Q reagent water system (Millipore

Corporation, Bedford, Massachusetts). GdL and HCl used were analytical grade chemicals. All percentages are based on the weight of the myofibrillar protein sample used.

Myofibrillar protein (20% w/w solids) was thawed for 30 min at room temperature (approximately 20°C) and then ground in a mortar and pestle for less than 30 s. Water (10%, v/w) was added to the myofibrillar protein sample approximately 1 h 20 min after removal of the protein from the freezer. The mixture was stirred for 30 s. Solutions of GdL (0%, 1%, 2%, or 4%, w/w) in water (20%, v/w) were added to the protein mixture 5 min later. The mixture was stirred for 30 s. Samples were syringed into PVC tubing (20.0 mm x 17.5 mm i.d.) and vacuum packaged. The ends were levelled and covered with polyethylene all-purpose food film. The gel preparations were stored at 4°C, 2.5 h after the myofibrillar proteins were removed from the freezer. The time measurements at which samples were removed for testing were taken from the addition of GdL to the proteins. Gels prepared with concentrations of 6%, 8%, 10% and 12% (w/w) GdL had the GdL added as slurries.

Gels were also prepared using HCl instead of GdL at molar equivalents corresponding to 1%, 2% and 4% (w/w) GdL.

Dialysis

Gels were prepared as above at GdL concentration of 0%. Samples were suspended in a solution of 0.17 M HCl (molar equivalent to 4% w/w GdL in total solution) for 18 h, 2.5 h after removal of the protein from the freezer. The dialysis membrane was viscose cellulose, molecular weight cut-off approximately 12,000 to 14,000 dalton (Union Carbide 453105).

Youngs Modulus Measurements

The degree of gelation of the myofibrillar proteins was estimated from the force strain relationship for the uniaxial compression of cylinders of the material in an Instron Universal Testing Machine (model 4502, Instron Ltd, High Wycombe, England) equipped with a Phillips Computer (model Pro9CM082, Taiwan). A two bite program was used with a constant displacement of 7.5 mm, a crosshead speed of 39.6 mm/min and a 1 kN load cell. The surfaces in contact with the samples were lubricated with soya oil. Youngs Modulus and springiness (the height that the food recovers during the time that elapses between the end of the first bite and the start of the second bite, BOURNE, 1978) were calculated from averages of force displacement curves generated from four replicates. Displacement for Youngs Modulus calculations was less than 1 mm.

pH Measurements

Direct pH measurements were taken upon completion of compressive tests at 20°C using a standard pH meter (digital ionalyzer, model 701, Orion Research Inc, USA) equipped with a glass electrode. pH measurements were calculated from the average of 4 replicates.

Analysis of Data

All effects of the various treatments on Youngs Modulus and springiness were determined by analysis of variance for a factorial arrangement of treatments in a completely randomized design using a Stats-Packets Statistical Analysis Package.

RESULTS

Glicksman (1982) defined gelation as the association or crosslinking of randomly dispersed polymer chains in a solution to form a three dimensional network which immobilizes the liquid in the interstitial structures and which resists flow against pressure. The gels in this series of experiments were not shown to be true gels according to this definition of gelation and may be very viscous solutions. However, the term 'gel' is used to describe the resulting solids.

Youngs Modulus.

Youngs Modulus is the modulus of elasticity which does not directly relate to product texture. It is therefore not an entirely appropriate parameter for characterising gels and there is much subjectivity in estimating the slopes of force displacement graphs in its calculation. However, this method of measurement was chosen due to its ease of implementation, because it relates to the molecular structure of the material and because it is one of the major distinguishing parameters in gel systems (GLICKSMAN, 1982). To avoid the complexity of

compression tests when the strain exceeds 0.05-0.1 (CULIOLI and SHERMAN, 1976), strain for all Youngs Modulus measurements was 0.05. It must be noted that the Youngs Modulus often did not describe the type of gel; for example, a high Youngs Modulus could result from compression of a crumbly mass of material, exuding much liquid or from a cohesive strongly elastic gel. Therefore, Youngs Modulus should not be taken as a measure on its own, but in conjunction with springiness and qualitative aspects.

Gelation with HCl

When samples of myofibrillar proteins were dialysed against a solution of 0.17 M HCl for 18 h gels were formed. Direct addition of HCl resulted in the immediate gelation of myofibrillar proteins. It was extremely difficult to add the HCl solutions evenly due to the rapid gel formation and much of the HCl at the 4% level was not incorporated into the gel structure. Slow reduction of pH by dialysis resulted in a more homogenous gel of pH 1, but with a solid white core.

Gelation with GdL.

Four levels of GdL (0%, 1%, 2%, and 4%) were dissolved in water just prior to addition to the proteins. The Youngs modulus was monitored with time. At concentrations of 2% and 4% GdL, gels of appreciable Youngs Modulus were obtained. As seen in Figure 1, the majority of the modulus of elasticity was obtained within the first 10 h after addition of GdL. Figure 2 illustrates the springiness of the gels which unlike the graph of Youngs Modulus, shows that the gel formed using 4% GdL does not decline to values less than that of 2% GdL. Table 1 shows the subjective characteristics and final pH measurements of the gels.

The concentration of GdL was increased to levels of 6%, 8%, 10% and 12%. Gels formed almost immediately upon addition of the GdL to the proteins. At these increasing concentrations, the stickiness of the gels caused difficulties in manipulation. pH ranged from 3.0 to 3.5.

Samples stored at 0°C, 10°C, 20°C, and 30°C, 2.5 h after the addition of 2% GdL showed little difference in the Youngs Modulus values up to 20°C.

DISCUSSION

FRETHEIM et al (1985) studied the effect of lowering the pH of solutions of myosin and found that solutions of myosin (10 mg/ml) in 0.6 M KCl form gels at 5°C if the pH is decreased slowly, by dialysis, to a value in the region of 2.5 to 5.5. Differential scanning calorimetry revealed that the myosin of pH induced gels absorbed no thermal energy when heated, implicating acid-induced denaturation as the basis of gel formation. HERMANSSON et al (1986), in an investigation of the effect of pH on thermally induced myosin gels, also observed that myosin solution (7-9 g/l) dialysed against pH 4 buffers in 0.6 M KCl, formed gels spontaneously at 4°C.

1,5-Gluconolactone hydrolyses gradually in water to form gluconic acid and is used in foods such as sausages, frankfurters, yoghurt and various dessert mixes. It was proposed that addition of GdL to myofibrillar proteins could provide slow pH reduction similar to that achieved by dialysis. Within several hours of GdL addition to the myofibrillar protein at levels of 2% and 4% GdL, gels of appreciable strength had formed. At the same time the myofibrillar protein slurry had changed from a pink, crumbly mass to a red, glassy gel. At 4% GdL the further changes in qualitative characteristics to a pink, crumbly mass indicate a deterioration of the gel formed. Optimal strength and texture gels were formed at 2% GdL concentration (pH 4.5). It is suggested the application of gelation by addition of a slow release food acid to a whole meat system could provide a method for restructuring meat at 4°C.

Increased concentration of GdL lowered the final pH of the gels as a result of hydrolysis of GdL. Gel rigidity showed a strong dependence on final pH. These results support the suggestion of FRETHEIM et al (1985) that pH reduction provides stronger denaturing conditions leading to stronger gels as compared to increasing the temperature of heat induced gelation.

The differences in gels formed as a result of presumed conformational changes with GdL indicates a complex set of reactions involving both GdL and the myofibrillar proteins. The effects of sodium chloride and pyrophosphate are currently being investigated and preliminary results suggest that gelation is enhanced by the addition of sodium chloride. In addition, investigations on the mechanism of GdL induced gelation have commenced.

CONCLUSION

GdL was shown to induce gelation of bovine myofibrillar proteins at levels of 2% and greater. Optimal gels in terms of stickiness and gel rigidity were formed at 2% GdL concentration (pH 4.5). The effects of sodium chloride and pyrophosphate are currently being investigated. The application of gelation by addition of a slow release food acid to a whole meat system could provide a method for the restructuring of meat at 4°C.

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Figure 1: Time development of Youngs Modulus (Y.M.).

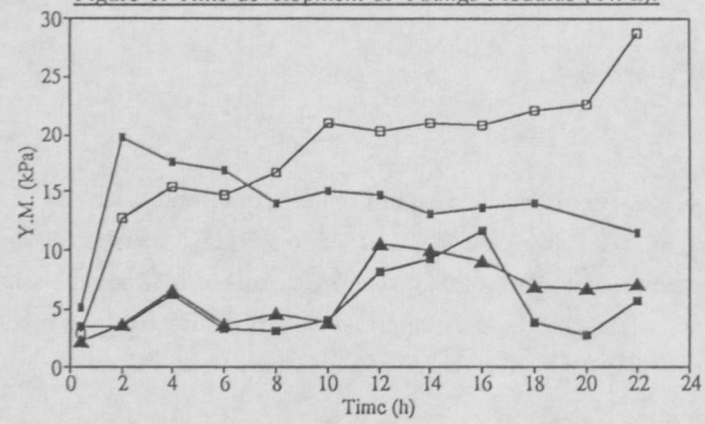

Figure 2: Time development of gel springiness.

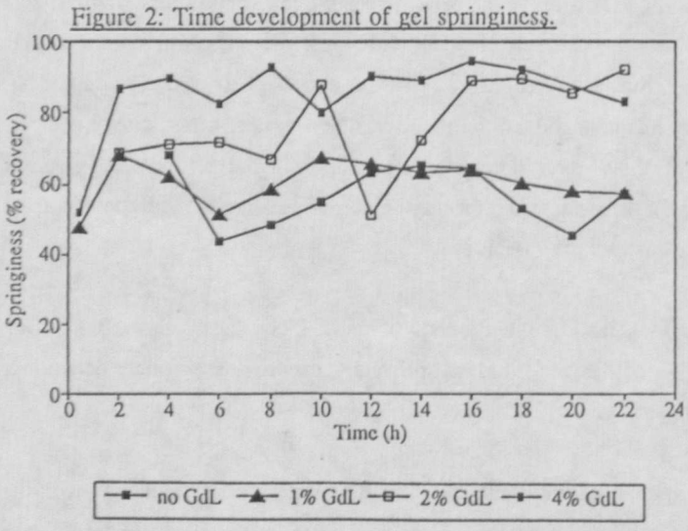

Table 1: Qualitative characteristics and final pH measurements of myofibril gels.

Appearance	GdL conc. (%)
pink/white, shape is not stable, pH 5.8	0
pink/white, crumbly, shape is not stable, pH 4.8	1
red, glassy, becomes sticky, initially shape is not stable, pH 4.5	2
initially pink and crumbly, becomes red, glassy and sticky, stable shape, pH 3.9	4