

DAIRY PRODUCTS RESEARCH CENTRE

MOORE PARK

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## **The use of Cold Setting Whey Proteins to enhance the Gelation Properties of Foods**

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*In cold-set applications, whey proteins need to withstand heating without gel formation. In this study a pre-heated whey protein ingredient improved the consistency of surimi and a cold-set dessert, and their potential for use in improving the consistency of acidified dairy products, such as yoghurt, and to stabilise calcium fortified milk-based beverages, was demonstrated.*



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## **High-Gelling Whey proteins as Functional Food Ingredients**

(The use of Cold Setting Whey Proteins to enhance the Gelation Properties of Foods)

ARMIS No. 3990

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## Summary and Conclusions

Whey proteins are widely used as ingredients in formulated foods because of their ability to gel on heating. During gelation, proteins unfold and re-aggregate in such a way that large quantities of water are bound by the denatured proteins. This process is salt and pH dependent. At neutral pH and under conditions of low salt, the heat treatment of whey proteins at temperatures over 70°C results in protein unfolding and denaturation without aggregation and gel formation. These pre-heated whey protein dispersions can subsequently be gelled at low temperatures through the addition of salts to give cold-setting whey protein products. The products have certain advantages in food systems where low temperature processing is the norm such as minced fish paste (surimi), meat products and dessert systems.

Hence, the main objective of this project was to produce dried, denatured, whey protein-based powders, which on reconstitution in food formulations show an increased ability to bind water in the presence of added salts, especially in the ambient temperature range. To achieve this, a number of secondary objectives were set to observe the behaviour of the whey protein system. These included the effects of salt on increases in viscosity during the heating process, the requirement for pH adjustment during processing and the ability of the pre-treated whey protein to interact with fat.

The main conclusions were as follows:

- \* It was shown that, compared to a commercial 75% whey protein concentrate, a pre-heated whey protein ingredient (cold-setting whey protein) improved the consistency of surimi and a cold-set dessert system.
- \* For cold-setting applications, the whey proteins need to withstand heating without gel formation. For example, as the protein concentration was increased, the salt concentration had to be decreased and pH increased to prevent the initiation of gelling during processing. When the salt concentration was increased, a lower heat treatment was needed to prevent viscosity increase. However, lower heat treatment resulted in a lower degree of protein unfolding and weaker cold-set gels. This example implies that only certain whey sources are suitable starting materials for cold-set applications.
- \* Model oil-in-water emulsions were studied using whey proteins pre-treated at different homogenisation and heating conditions to evaluate the potential of cold-setting whey proteins in yoghurt, mayonnaise and sauces. It was found that with these pre-treatments, emulsion viscosity increases were observed at very low whey protein concentration (< 1%), when salt was added after emulsion formation, indicating that cold-set whey proteins are much more effective gelling agents than normal whey protein ingredients. For this reason, they have potential in acidified dairy products such as yoghurt.
- \* Pre-heated whey protein dispersions are also capable of binding and stabilising calcium phosphate. This property can be exploited in the stabilisation of calcium-fortified milk-based beverages.

- \* *The commercial production of cold-setting whey protein ingredients will depend on the ability to retain whey protein solubility during processing. A number of mechanisms exist to achieve this but, in all cases, very exact control of the process is required.*
- \* *Because low salt levels prevent the aggregation and gelling of denatured whey proteins, whey protein isolate is an ideal starting material for the production of these ingredients, but due to the high cost, de-mineralised whey was chosen instead as the starting material. Careful consideration has also to be given to the processing equipment and the economics involved.*
- \* *The development of whey protein ingredients especially for cold-set end uses is a product specific exercise. General guidelines were developed in the current work, but further work with industry partners will be necessary before commercial success is achieved.*

## Research and Results

The main objective of this project was to produce dried, denatured but unaggregated whey protein powders which, on reconstitution in the presence of added salts in food formulations, show an increased ability to increase viscosity or gel, especially at ambient temperatures. Heating causes the globular protein molecules to unfold and form linear aggregates, but electrostatic repulsion opposes protein-protein interactions and hence gel formation, when the salt content is low. The addition of salts or their presence in the end-food product will initiate gelation of whey proteins resulting in cold-setting gels, provided the whey proteins have been denatured beforehand.

*Table 1. Composition of dessert formulation.*      Model Food Systems

Ingredient	(%)
Sugar	5.0
Starch	4.5
Whole Milk Powder	10.0
10% Whey Protein Solution	20.0
Water	60.5

This study was limited to foods where water immobilisation is the main functional requirement of the cold-setting whey protein. Gels were made from surimi by incorporation of 2% NaCl and heating to 80°C, followed by cooling to 25°C. The development of gel strength during the heating up stage is outlined in *Fig. 1*. The introduction of standard WPC-75 into the surimi prior to heating reduced the firmness (G\*) of the resulting gel.

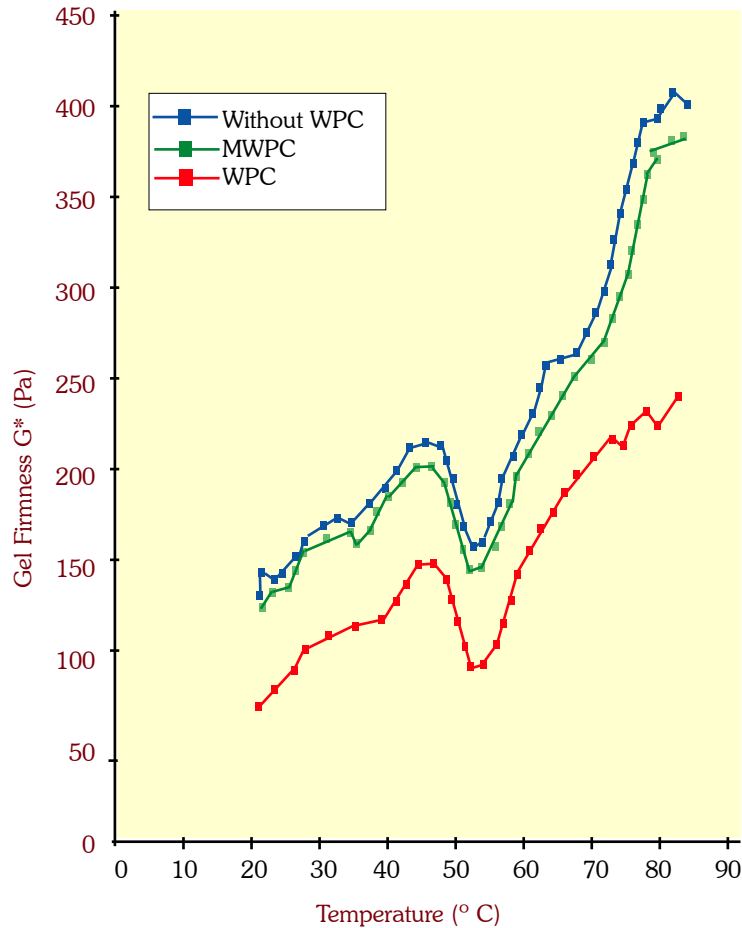


Figure 1. Effect of whey protein type on the gel strength of Surimi during heating from 25 - 80°C.

When part of the fish protein was replaced by pre-heated WPC (MWPC), there was little difference between the firmness of the control gels and pre-heated WPC gels.

A typical cold-set dessert formulation is outlined in *Table 1*. The recipe depends on the cold-dispersible starch to generate consistency and this model system was used to evaluate the whey protein ingredients. A comparison between the consistencies of the basic formulation without whey protein, with added standard WPC-75 and pre-heated WPC is outlined in *Table 2*.

The results indicated that the pre-heated WPC markedly enhanced the consistency ( $k^*$ ) of the gel, increasing the consistency from 98  $\text{Pas}^{n^*-1}$  for the control system to 200  $\text{Pas}^{n^*-1}$  with the pre-heated whey protein. The standard WPC-75 had a negative effect on the final consistency as with surimi.

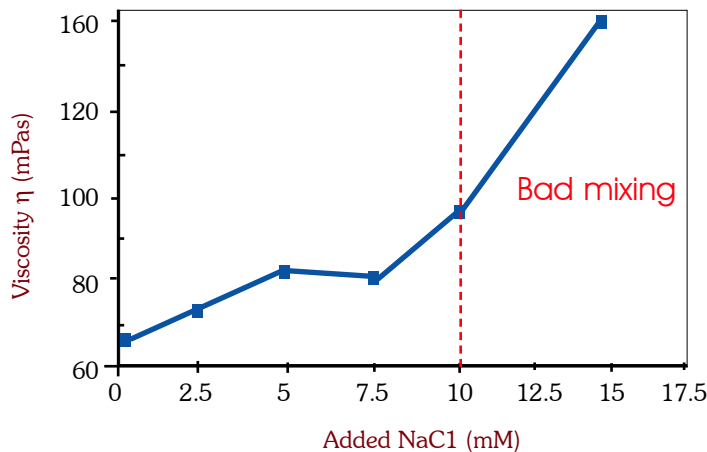
Thus, the inclusion of cold-setting whey protein was shown to have a positive effect in a fish gel and a cold-formulated dessert system compared to standard WPC.

*Table 2. Consistency of desserts with added whey protein (2%).*

Added Ingredient	Consistency $k^*$ (Pas $n^{*-1}$ )	$n^*$
No added WPC	98	0.125
Commercial WPC	64	0.097
Modified WPC	200	0.123

#### The effect of salt level

Cold-setting gels were formed by dissolving NaCl to a final concentration of 200 mM in preheated whey protein dispersions (10% protein), and the viscoelastic properties of the gels were measured at 40°C over 120 min. At a constant heat treatment, interactions between the unfolded proteins increased with increasing sodium content resulting in higher viscosities ( $\eta$ ) (Fig. 2). If the viscosity of the pre-heated whey protein dispersion exceeded 60 mPas, due to too high (> 7.5 mM) salt concentrations, it was not possible to obtain uniform mixing of the protein dispersion after cooling, when salt was added to induce cold-setting. Similar cold-set gels were obtained as long as the viscosity of the pre-treated whey protein dispersion after the heat treatment was maintained below 60 mPas by limiting the preheat treatment or salt added to the dispersion (Fig. 3).



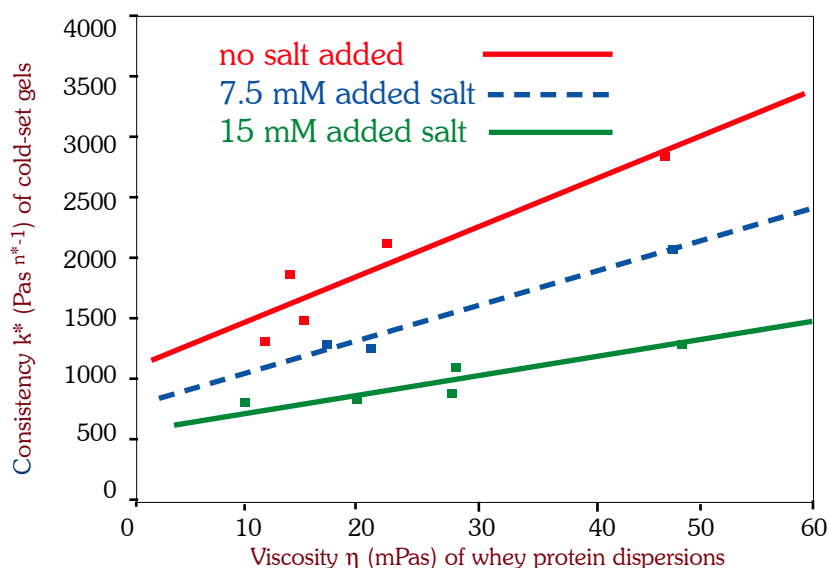
*Fig. 2. Effect of added NaCl on the viscosity of whey protein isolate (10% protein, pH 7.0, 40°C) after pre-heating at 90°C for 30 min.*

When the sodium level in the dispersion was greater than 7.5 mM, a milder heat treatment was required to prevent the viscosity exceeding 60 mPas. Milder heat treatment resulted in a lower degree of protein unfolding and consequently weaker cold-set gels.

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### Oil-in-water emulsions

Solutions of 1.25 or 10% whey protein isolate were heat-treated at 90°C x 30 min and/or homogenised using a Rannie Labo 60 valve homogeniser at 45 MPa. Emulsions containing 20% sunflower oil and 1% whey protein were formed using an homogenisation pressure of



*Figure 3. Effect of the salt-dependent viscosity of whey protein dispersions (10% protein, pH 7.0, pre-heated at 90°C for 30 min) on the consistency of cold-set gels formed at 40°C for 120 min by addition of 200 mM NaCl after heating.*

45 MPa. Fat globule size distributions and fat globule aggregation in the presence of CaCl<sub>2</sub>, NaCl or at reduced pH were measured. The rheological characteristics of emulsions made with native whey proteins and pre-heated whey proteins after 2-10 homogenisation passes at 45 MPa is outlined in *Table 3*. The viscosities ( $\eta$ ) of the emulsions stabilised with native whey proteins were stable in the presence of NaCl (30 mM) and increased to approximately 90 mPas with CaCl<sub>2</sub> (10mM). The viscosity of pre-heated whey protein emulsions increased in the presence of NaCl (30 mM) but gels were formed in the presence of 10 mM calcium over 60 min at 20°C. The consistency ( $k^*$ ) of these gels increased with increasing passes of the emulsion through the homogeniser from 78 Pas $n^{*-1}$  at 2 passes to 600 Pas $n^{*-1}$  at 10 passes. It should be noted that all measurements were terminated after 60 min, and therefore do not represent the final gel strength achievable. Pre-heated whey proteins had a lower emulsification efficiency than native whey proteins. The homogenisation of the whey protein suspensions prior to emulsification increased the emulsification efficiency but resulted in emulsions which formed gels on the addition of CaCl<sub>2</sub> (10 mM). *These pre-treatments by either homogenisation or heating can therefore be used to extend the use-range of whey protein ingredients in food emulsions.*

**Table 3.** Effect of salt addition (NaCl, 30 mM; CaCl<sub>2</sub>, 10 mM) on the rheological characteristics of emulsions stabilised by native whey proteins or by whey proteins which had been pre-heated (90°C, 30 min, 10% protein), as influenced by the number of homogenisation passes at 45 MPa.

Passes (No. of)	Native Whey Proteins		Heated Whey Proteins	
	NaCl, 30 mM Viscosity (mPas)	CaCl <sub>2</sub> , 10 mM Viscosity (mPas)	NaCl, 30 mM Viscosity (mPas)	CaCl <sub>2</sub> , 10 mM Consistency (Pas <sup>n*-1</sup> )
2	2.7	13.1	4.2	78
5	3.4	90.4	36.8	399
8	3.2	90.3	43.5	450
10	3.2	94.2	46.3	604

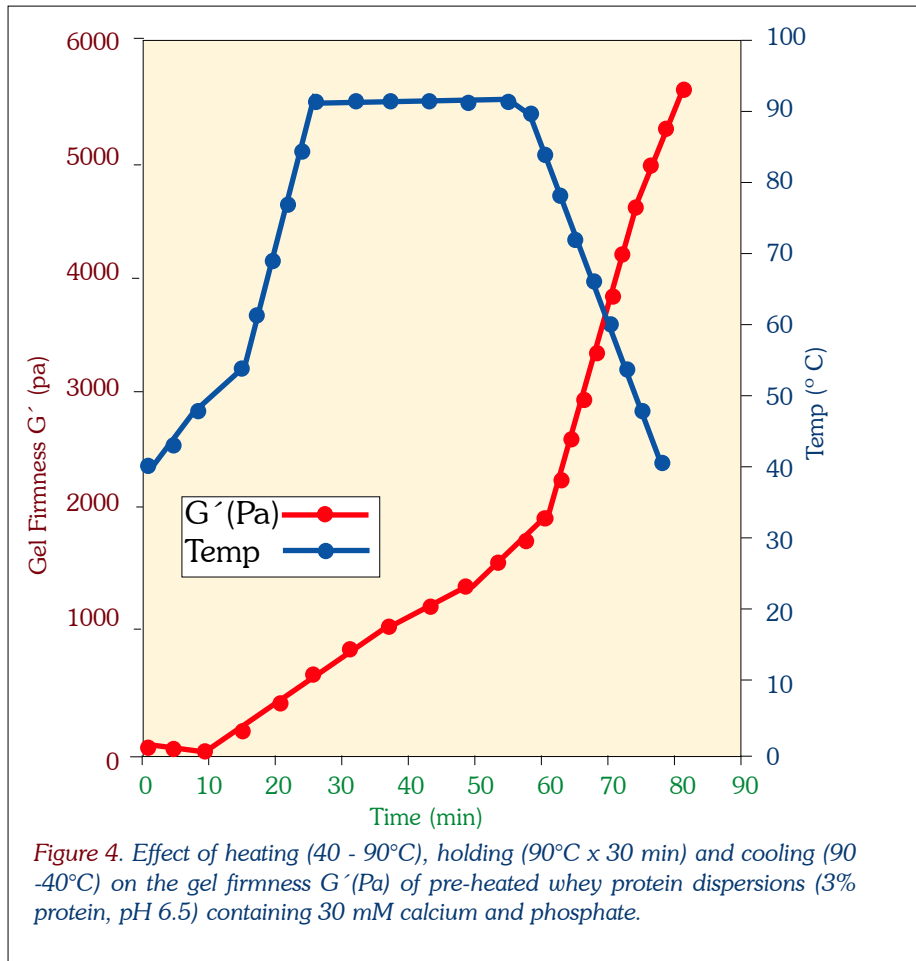
### Calcium phosphate effects on whey protein gelation

The objective of this part of the study was to examine the effect of calcium and phosphate on the gelation of pre-denatured whey protein dispersions. Pre-denaturation of a whey protein isolate solution (3% protein, w/v) was carried out using heat (70 - 90°C for 0 - 30 min). The whey protein dispersions were cooled to 20°C and titrated with calcium, phosphate, and citrate over a range of concentrations (10 - 30 mM) to selected final pH values (6.2 - 7.0). Low viscosity dispersions varying in opacity were obtained at a concentration of 3% protein under the conditions outlined. The effect of subsequent heating to 90°C led to gelation of the system (Fig. 4). The strength of the gel obtained was dependent on the pH of the initial whey protein-stabilised calcium phosphate dispersion. Maximum gel strength occurred at an initial pH of 6.5 and gels were not formed at pH 6.7 - 7.0. The effect of change in pH by addition of GDL at 25°C on the gelation characteristics was then quantified as outlined in Fig. 5. Pre-heated whey protein dispersions (3% protein) containing 10 mM calcium and phosphate formed gels at pH 6 and were significantly stronger than pre-heated control gels without added calcium and phosphate. *Thus, whey proteins dispersions which form gels at a concentration of 3% protein have potential in acidified dairy products while whey protein dispersions which bind calcium and phosphate and still maintain good heat stability (in the range pH 6.7-7.0) have potential applications in calcium-fortified milks.*

### Production of cold-gelling ingredients

Electrodialysed, ultrafiltered whey was used as a raw material because it allowed a high degree of pre-heating without major viscosity increases, albeit, at pH 7.5.





After drying this dispersion, the reconstituted powder had a higher viscosity than the same dispersion prior to drying. This indicated that the spray drying process had an aggregating effect on the pre-denatured whey proteins. However, for viscosity reasons, a protein level of 10% in the concentrate is a significant economic issue in drying denatured whey protein. The incorporation of additional extra carrier material, such as lactose for the drying process may also be a requirement for the maintenance of functionality.

*Demineralised ultrafiltered whey with greater than 20% solids and a protein level of <50% of the solids prior to pre-heating the concentrate is the best option at present for the preparation of a cold-setting whey protein ingredient.*

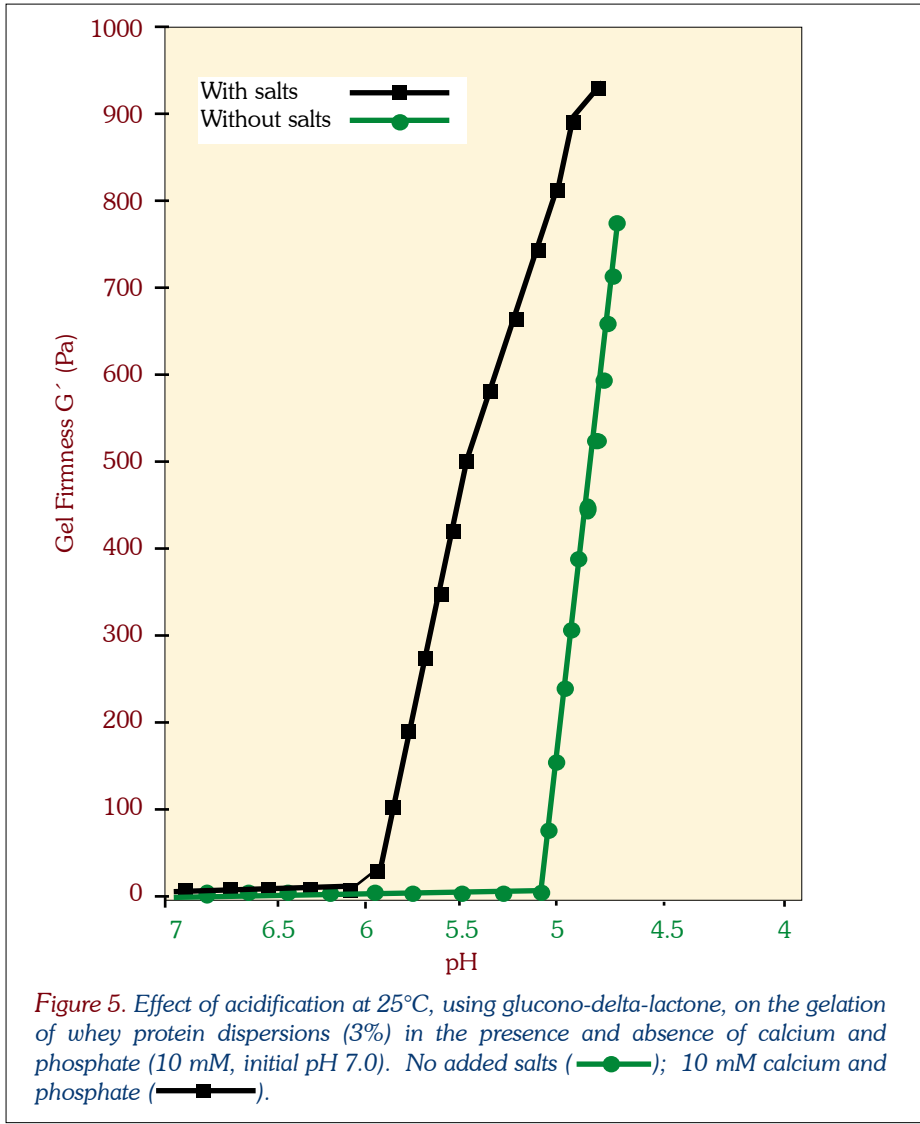


Figure 5. Effect of acidification at 25°C, using glucono-delta-lactone, on the gelation of whey protein dispersions (3%) in the presence and absence of calcium and phosphate (10 mM, initial pH 7.0). No added salts (—●—); 10 mM calcium and phosphate (—■—).

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## Publications

McClements, D.J. and Keogh, M.K. (1995). Physical properties of cold-setting gels formed from heat-denatured whey protein isolate. *Journal of Food Science* 69, 7-14.

Keogh, M.K. The effect of composition on whey protein gelation. *Bohlin Rheology User Group Meeting* at Bristol Univ., UK. 12/13 September 1995.

Twomey, M. and Keogh, M.K. The gelation characteristics of whey protein. *Food Sci. and Tech. Res. Conf.* University College, Cork, 12/13 Sept 1995

Keogh, M. K. (1996). Report on *Food Ingredients Europe 1995*, Frankfurt, Germany. 7-9 November 1995.

Keogh, M. K. (1996). Report on Milk Protein Structure and Function *Conf. Wadahl*, Norway, 11-13 April, 1996.

O'Kennedy, B.T. and Twomey, M. (1996). Applications of whey proteins in foods. In: *Proceedings of 3rd Food Ingredient Symposium*, pp. 57-75.

O'Kennedy, B.T. (1997). Cold-setting whey proteins. *Workshop on Advances in Dairy Protein Applications*, Moorepark, Fermoy, December 10th.

Twomey, M., Keogh, M.K., Mehra, R. and O'Kennedy, B.T. (1997). Gel characteristics of  $\beta$ -lactoglobulin, whey protein concentrate and whey protein isolate. *J. Texture Studies* 28, 387-403.

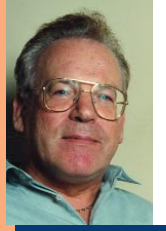
Halbert, C., Hallihan, A., Twomey, M. and O'Kennedy, B.T. (1997). Calcium phosphate effects on whey protein gelation. 27th Annual Food Science and Technology Research Conf. 18th September. *Irish Journal of Agricultural and Food Research* 36, 268 - 269.

Bramaud, C. and O'Kennedy, B.T. (1997). Effect of whey protein pre-treatment on the formation and stability of oil-in-water emulsions. 27th Annual Food Science and Technology Research Conf. 18th September. *Irish Journal of Agricultural and Food Research* 36, 270.

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