

## DAIRY PRODUCTS RESEARCH CENTRE

### MOOREPARK

END OF PROJECT REPORT 2000 DPRC No. 40

## **$\beta$ -Lactoglobulin: A Whey Protein Fraction with Enhanced Functionality**

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*The aims of this research were to source and/or produce sufficient quantities of  $\beta$ -lactoglobulin ( $\beta$ -lg)-enriched ingredients obtained through whey protein fractionation using different technologies, and evaluate their functionality in model and food systems.*

*The studies showed that downstream processing of  $\beta$ -lg can be manipulated to influence the composition and functional properties of  $\beta$ -lg-enriched fractions.  $\beta$ -Lg-enriched fraction has clear advantages over conventional whey protein products (WPC, WPI), in that it can be tailor-made to have specific functional properties desired in particular food products.*



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## Functionality and Applications Development of $\beta$ -Lactoglobulin Produced by Different Technologies

( $\beta$ -Lactoglobulin: A Whey Protein Fraction with Enhanced Functionality)

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## Summary and Conclusions

*Infant formula manufacturers are progressively moving towards the development of the next generation of infant milk formula based on the inclusion of  $\alpha$ -lactalbumin-enriched ingredients in order to further 'humanise' baby milk, as well as to reduce the allergenicity associated with the presence of  $\beta$ -lactoglobulin ( $\beta$ -lg). Since  $\alpha$ -lactalbumin represents one of the two major whey protein fractions in bovine milk, the viability of new fractionation processes currently under development will depend inter alia on the functional value that will attach to the remaining fraction, namely  $\beta$ -lg. Since this protein fraction influences whey protein functionality for the most part, it is to be expected that its availability in an enriched form should lead to further enhancement of its key functional properties, and stimulate further market opportunities. It is therefore imperative that attention is given to the processes and functionality of  $\beta$ -lg produced by different processing approaches.*

*Hence, the overall objective of the project was:*

- To source and/or produce sufficient quantities of  $\beta$ -lg-enriched ingredients obtained through whey protein fractionation using different technologies, and to evaluate their functionality in model and food systems.*
- To investigate the influence of thermal treatments and ionic environment on the molecular structure of purified  $\beta$ -lg in order to understand their effect on protein functionality (gelation).*
- To improve the water-holding capacity of  $\beta$ -lg-enriched fraction so that it could compete more favourably with carbohydrate hydrocolloids in food applications.*

### Main Conclusions and Achievements

- \* Downstream processing of  $\beta$ -lg was manipulated to influence the composition, and hence the functional properties of  $\beta$ -lg-enriched fractions.*
- \*  $\beta$ -Lg-enriched fractions had enhanced functional properties compared to WPC 75 and WPI.*
- \*  $\beta$ -Lg-enriched fraction has clear advantages over conventional whey protein products (WPC, WPI), in that it can be tailor-made to have specific*

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*functional properties desired in particular food products.*

*\* Water-binding properties of  $\beta$ -lg-enriched fraction could be improved by multi-stage heating.*

## Research and Results

### **$\beta$ -Lactoglobulin ( $\beta$ -lg) - enriched fractions produced from whey protein subsequent to selective precipitation and removal of $\alpha$ -lactalbumin fraction**

A laboratory method for the preparation of  $\beta$ -lg-enriched protein fraction from whey protein concentrate (WPC), following selective isoelectric precipitation and removal of  $\alpha$ -lactalbumin, was scaled up to pilot plant level. Kilogram quantities of four different  $\beta$ -lg-enriched powders (Betagalac A, Betagalac G, Betagalac C and Betagalac B) containing varying mineral levels were produced by differential downstream processing of the soluble  $\beta$ -lg rich fraction. Powders were subjected to compositional analysis and functional tests in both model systems (nitrogen solubility, heat stability and heat induced gelation) and food products (meringues, frankfurters, and clear, fruit flavoured, acidic drinks).

### Properties of $\beta$ -lg-enriched fractions

#### *Composition*

The protein powders had total protein content of between 82 - 88%, fat content of less than 1%,  $\beta$ -lg enrichment of between 70 - 85% of the true protein, and protein denaturation of between 0 - 5%. The variable mineral levels and balance between the monovalent and divalent ions in the powders were the result of the different processing conditions applied to the  $\beta$ -lg-enriched stream (calcium 0.11 to 6.84, potassium 0.11 to 2.32, sodium 0.34 to 6.47, phosphorus 1.57 to 1.95, all expressed as mg/g protein (*Table 1*)). This is compared with 75% protein, 8% fat, 50 - 55% of  $\beta$ -lg and 29% protein denaturation in commercial 75% protein WPC.

Table 1: Mineral composition of  $\beta$ -lg-enriched powders.

$\beta$ -Lg-enriched powders	Sodium (mg/g protein)	Potassium (mg/g protein)	Calcium (mg/g protein)	Phosphorus (mg/g protein)
Betalac A	6.47	2.32	6.48	1.95
Betalac G	10.09	0.72	2.88	1.68
Betalac C	1.02	0.34	0.68	1.59
Betalac B	0.34	0.11	0.11	1.57

### Nitrogen Solubility

Nitrogen solubility of 1% protein dispersions of  $\beta$ -lg-enriched powders in distilled water was measured over the pH range 2 to 8.

Except for Betalac A, all other  $\beta$ -lg-enriched powders showed > 97% nitrogen solubility at all pH values (Fig. 1). In addition, Betalac B showed complete clarity up to 3% protein level.

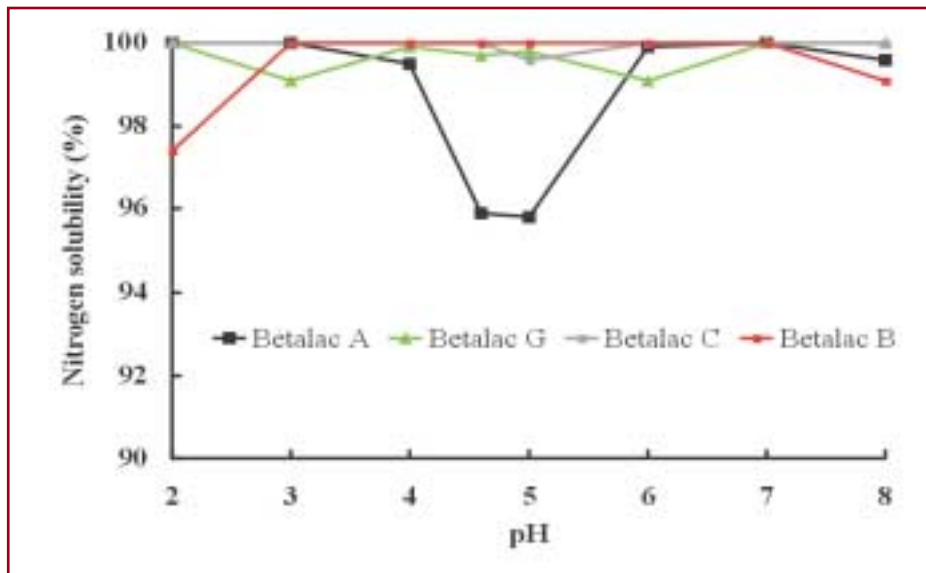


Fig. 1. Nitrogen solubility of  $\beta$ -lg-enriched powders.

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### Heat Stability

Heat stability of 3% protein solutions of the powders in distilled water was determined at 140°C at acidic and near neutral pH values.

Heat stability of  $\beta$ -lg-enriched ingredients was influenced by the mineral balance and pH. While there were only slight differences in heat stability of the different powders at acidic pH values, there were dramatic differences in heat stability at near neutral pH values. At pH 6.8, heat stability ranged from 0 to 30 min, and at pH 3.8 from 23 to 25 min (Fig. 2). Heat stability increased as the mineral content decreased (at low ionic strength 30 min HCT at pH 6.8). In comparison, heat stability of standard WPC 75 was 1 and 0.66 min at pH 6.8 and 3.8 respectively.

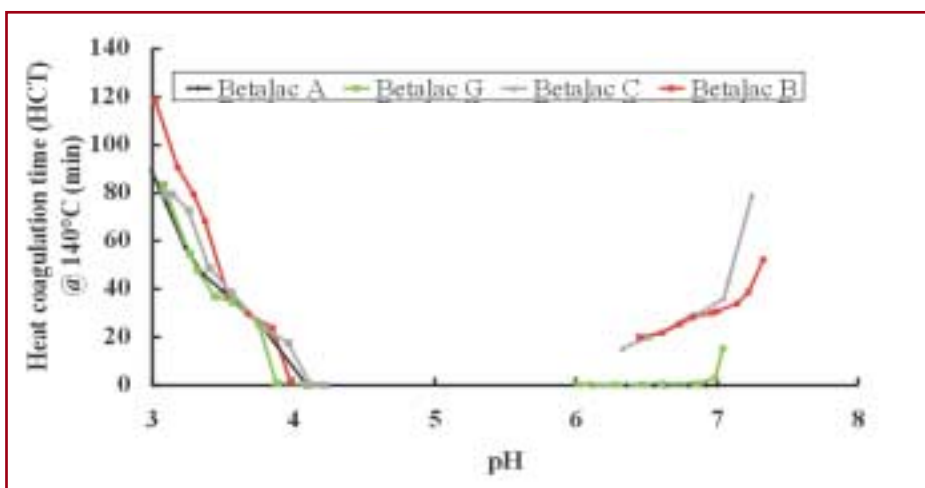
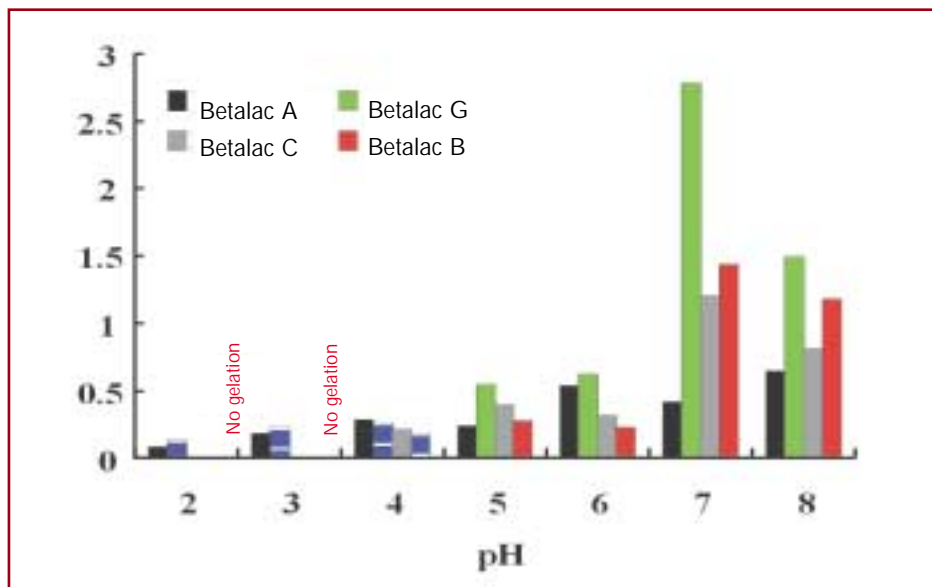


Fig. 2 Heat stability of  $\beta$ -lg-enriched powders.

### Heat-induced Gelation

Heat-induced gelation was characterised by measuring the strength of gels made from 10% protein solutions of the powders in distilled water over the pH range 2 to 8. Strength (Fig. 3) and appearance of gels was influenced by pH and mineral balance.  $\beta$ -Lg-enriched powders produced different types of heat-induced gels: from white to translucent, and strong to soft depending upon mineral composition and pH. This can form the basis for production of  $\beta$ -lg-enriched powders tailor-made for specific gelation



*Fig. 3 Gel strength of 10% protein dispersions of  $\beta$ -lg powders measured at 70% gel compression as a function of pH. Thatched bars indicate break strength of gels not reaching requirements in food applications.*

### **Foam Formation**

$\beta$ -Lg-enriched powders showed foam expansion values ranging from 720 to 1052% and foam drainage values ranging from 30 to 63% at pH 7.0. A commercial whey protein isolate (WPI) did not form a stable foam under the test conditions, while a commercial  $\beta$ -lg-enriched powder showed foam expansion value of 983% and 22% foam drainage.

In comparison, Actiwhite (an egg white powder used commercially to manufacture meringues) had a foam expansion of 995% and foam drainage of only 1% at pH 7.0.

Foaming properties of two high foaming  $\beta$ -lg-enriched powders were investigated in the pH range from 4.0 to 7.0. *In both cases, foam expansion increased with decreasing pH, and foam drainage was lowest at pH value of 5.0. Addition of sugar (sucrose) to dispersions of  $\beta$ -lg-enriched powders prior to foaming increased foam stability, but decreased foam expansion at pH 7.0.*

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*Heating dispersions of a  $\beta$ -lg-enriched powder at 68°C and pH 7 for 20 min led to foam expansion and drainage values similar to Actiwhite.*

## Food applications of $\beta$ -lg-enriched powders

### Meringues

$\beta$ -Lg-enriched powders were tested in meringues for their ability to stabilise foam under heat set conditions (egg-white replacer).



*Betalac A and G produced good foams but were unable to maintain shape and texture of meringues during cooking. Betalac C (and to a lesser extent Betalac B) showed foaming and heat set properties similar to egg-white. Thus, Betalac C type of  $\beta$ -lg-enriched powders could successfully replace egg-white in such food products.*

### Frankfurters



Frankfurters containing 30% fat (normal control), 12% fat (reduced fat control), and 12% fat plus  $\beta$ -lg-enriched powders (experimental) were prepared using beef/pork meat. They were measured for cook loss, sensory properties, and texture profile analysis.

*Frankfurters containing 12% fat and Betalac A resulted in sensory and textural properties*



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*comparable to frankfurters containing 30% fat. This indicated the potential for  $\beta$ -lg-enriched fraction in partially replacing and reducing fat in frankfurters, and binding water in processed meat and fish products.*

### **Clear, fruit flavoured, acidic beverages**

Betalac B was specifically produced for protein fortification of beverages. Its taste and clarity was evaluated in a clear orange and passion fruit flavoured acidic drink by a soft drinks manufacturer, and in blackberry, lemon and lime, and raspberry flavoured drinks in our laboratory.



*In all cases, its taste was acceptable between 1 and 2% protein level while its clarity was acceptable up to 3% protein level, the maximum concentration tested.*

### **An alternative method for preparation of $\beta$ -lg-enriched fraction (Amundson process)**

The Amundson method for fractionation of  $\beta$ -lg from whey was also investigated.

The method involves selective precipitation of  $\beta$ -lg by pH adjustment of low ionic (demineralised) whey protein concentrate to 4.65.

*Overall, the Amundson process, as reported in the literature by Amundson and evaluated by us, did not result in selective precipitation of  $\beta$ -lg using both low ionic (demineralised) and normal whey protein concentrate. The influence of thermal treatment at different pH values on precipitation of  $\beta$ -lg at pH 4.65 was also studied. The treatments employed did not effectively improve selective precipitation of  $\beta$ -lg from WPC.*

## **Summary of Main Conclusions from These Studies**

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- a)  $\beta$ -Lg-enriched powders (herein named Betalac) produced different types of heat-induced gels: white to translucent, and strong to soft depending on mineral composition and pH.
  - b) Betalac powders (except one) showed complete nitrogen solubility at all pH values.
  - c) Betalac B exhibited complete clarity at all pH values.
  - d) Betalac C and B showed high heat stability at acidic and near neutral pH values.
  - e) Betalac A enabled fat content to be reduced by > 50% in frankfurters.
  - f) Betalac C performed well as an egg white replacer in meringues.
  - g) Betalac B was acceptable for protein fortification of clear flavoured drinks
    - at 1% protein level for taste and colour
    - up to 3% protein level for clarity.

### Enhancement of the water-holding capacity (viscosity) of $\beta$ -Lg-enriched fractions

An investigation was undertaken to enhance the water-binding properties (viscosity) of  $\beta$ -Lg-enriched fractions at low temperature and low protein concentration by multi-stage heating. The principle of this approach was to preheat the protein solution at a pH where gelation or precipitation is avoided, and then adjust the pH to a range where further heating induces formation of viscous dispersions of the protein. Experiments were performed to determine the influence of protein concentration, salt, and pH at primary and secondary heating stages on the viscosity/gelation of the fractions.

#### Influence of pH at the primary heating stage

Protein dispersions (4%) of  $\beta$ -Lg-enriched powders containing different levels of indigenous minerals were adjusted to pH values between 3.5 and 8.0, heated (primary heating stage) at 80°C for 1 h, and then cooled. Appearance and colour of the heated samples were noted, and where possible, viscosity and turbidity were measured. In the case of powder with

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the highest level of minerals, samples between pH 4.1 - 6.5 formed gels, whereas samples outside this pH range remained fluid, having a white/grey colour. For other low mineral containing powders, samples between pH 4.1 and 5.7 formed gels, while others remained fluid.

*It was concluded that the pH at primary heating stage should be > 6.5 to avoid gel formation at the full range of mineral contents studied.*

### **Influence of salt and pH at secondary heating stage**

Protein dispersions (4%) of a  $\beta$ -lg-enriched powder that had high gelling properties (Betalac G) were heated (primary heating stage) at pH 8.0 for 1 h in the presence or absence of 0.1 M salt, cooled, and then adjusted to pH values in the range 5.2 to 7.5. Viscosity of the dispersions were measured, and are reported here for a shear rate of 50 s<sup>-1</sup>. Samples without salt showed little difference in viscosity before and after pH adjustment between 5.6 and 7.5. Viscosities were between 1 and 2 mPas. However, the viscosity increased to 9.7 and 46 mPas when pH was adjusted to 5.2 and 5.4 respectively. *On the other hand, samples with salt showed increase in viscosity (range 19 - 132 mPas) depending upon the adjusted pH (7.5 - 5.6).*

*Further heating (secondary heating stage) at 80°C for 1 h had no effect on viscosity of samples without salt and in the pH range 6.0 - 7.5; those at pH < 5.8 formed white gels. Samples containing salt formed white viscous dispersions in the pH range 7.0 - 7.5, while those at lower pH values formed white gels or precipitates. Results indicated that the water-binding properties of the  $\beta$ -lg-enriched fraction could be improved by multi-stage heating.*

*The duration of primary heating at pH 8.0 and 80°C (30 - 60 min) influenced the viscosity and turbidity of samples after secondary heating at pH 7.5 - 6.5 for 1 h. The longer heating times favoured development of viscous dispersions.*

### **Summary of Main Conclusions from These Studies:**

*a) The duration of primary heating at pH 8.0 and 80°C (30 - 60 min) influenced the viscosity and turbidity of samples after secondary heating at pH 7.5 - 6.5 for 1 h.*



Dr. Raj Mehra

### Publications

Mehra, R., O'Kennedy, B.T., Rawle, D. and Kelly, P. (1999). Properties and food applications of  $\beta$ -lactoglobulin-rich whey protein concentrates. Presented at the *29th Annual Food Science and Technology Research Conference* held at UCC, Cork on 15-17th September 1999 and abstracted in its Proceedings.

Mehra, R., O'Kennedy, B.T., Rawle, D. and Kelly, P. (2000). Properties and applications of  $\beta$ -lactoglobulin-rich whey protein concentrates. *Irish Journal of Agriculture and Food Research* **39**(1), 160.

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