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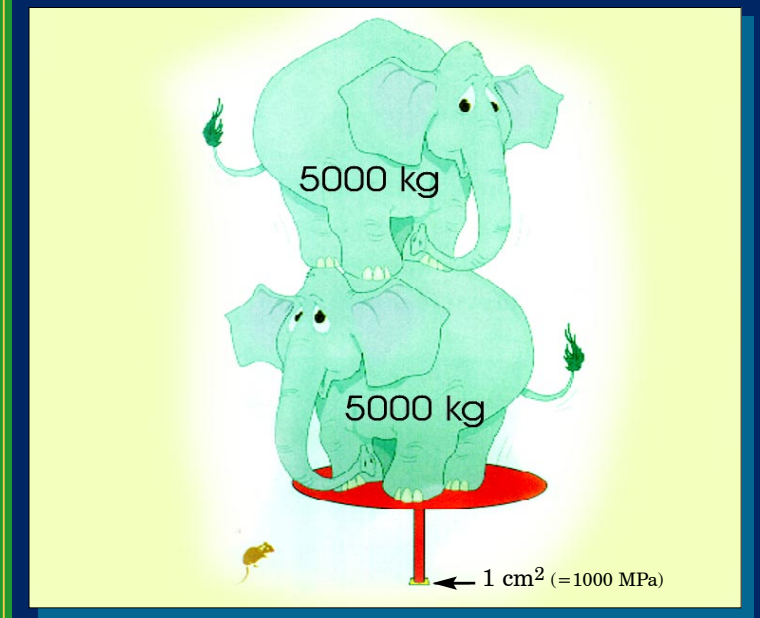
High Pressure Processing of Dairy Foods

Drs. Tom Beresford and Cáit Lane

High Pressure Processing accelerates proteolysis in Cheddar Cheese which should result in accelerated ripening. It is also effective in inactivating both food spoilage and pathogenic micro-organisms, especially when combined with bacteriocins (natural inhibitors). However the cost of this technology is still a major deterrent to commercial exploitation.



Teagasc
The Science of Farming & Food

This report is based on two complementary projects:
** High pressure technology - design and process application (ARMIS No. 4339)*
** High pressure treatment of liquid foods and derived products (ARMIS No. 4403)*

(High Pressure Processing of Dairy Foods)

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Summary and Conclusions

The term High Pressure Processing (HPP) is used to describe the technology whereby products are exposed to very high pressures in the region of 50 - 800 MPa (500 - 8000 Atmospheres). The potential application of HPP in the food industry has gained popularity in recent years, due to developments in the construction of HPP equipment which makes the technology more affordable. Applying HPP to food products results in modifications to interactions between individual components, rates of enzymatic reactions and inactivation of micro-organisms.

The first commercial HPP products appeared on the market in 1991 in Japan, where HPP is now being used commercially for products such as jams, sauces, fruit juices, rice cakes and desserts. The pioneering research into the application of HPP to milk dates back to the end of the 19th century. Application of HPP to milk has been shown to modify its gel forming characteristics as well as reducing its microbial load. HPP offers the potential to induce similar effects to those generated by heat on milk protein.

Recent reports have also indicated that HPP could accelerate the ripening of cheese. Much of the Irish cheese industry is based on the production of Cheddar cheese, the ripening time for which can vary from 4 - 12 months or more, depending on grade. A substantial portion of the cost associated with Cheddar manufacture is therefore attributed to storage under controlled conditions during ripening. Thus, any technology which may accelerate the ripening of Cheddar cheese while maintaining a balanced flavour and texture is of major economic significance.

While food safety is a dominant concern, consumers are increasingly demanding foods that maintain their natural appearance and flavour, while free of chemical preservatives. HPP offers the food industry the possibility of achieving these twin goals as this technology can lead to reduced microbial loads without detrimentally effecting the nutritional or sensory qualities of the product.

The development of food ingredients with novel functional properties offers the dairy industry an opportunity to revitalise existing markets and develop new ones. HPP can lead to modifications in the structure of milk components, in particular protein, which may provide interesting possibilities for the development of high value nutritional and functional ingredients.

Hence these projects set out to investigate the potential of HPP in the dairy industry and to identify products and processes to which it could be applied.

Main Conclusions and Achievements

** Application of HPP to Cheddar cheese resulted in increased levels of proteolysis, a key measure of maturity, indicating that HPP has the potential to accelerate the ripening of Cheddar. However, the level of acceleration observed was not sufficient to support the commercial exploitation of the technology.*

** Both food spoilage and pathogenic micro-organisms were inactivated by HPP. The level of inactivation was found to be dependent on a number of parameters including the treatment pressure, length of exposure, composition of the food system and the species of the target organism. Combining HPP with other technologies, such as bacteriocins, was shown to be particularly effective in reducing pressures required for microbial inactivation. This offers the potential to control pathogens in non-pasteurised dairy foods.*

** High pressure may be used as an alternative to heat for the preparation of cold-set milk protein gels. However, the obvious limiting factor would be cost since very high pressures (600 MPa) appear to be required for gelation. Alternative pressure treatments (i.e. lower pressures x longer times) or a combination of heat and pressures may prove to be an acceptable alternative for the development of acid-set gels with novel gelation properties.*

** Pressure treatments of 0 - 600 MPa for 0 - 45 min. had little effect on the emulsification or whipping/foaming properties of sodium caseinate. Pressure treatments of 200 or 400 MPa x 30 min. did not alter the susceptibility of sodium caseinate to hydrolysis by a commercial protease preparation and hence had little effect on the characteristics of the resultant hydrolysates.*

Research and Results

Pre-study investigations

Prior to undertaking these studies a consultant's report outlining the potential benefits of HPP to the Irish Dairy Industry was prepared. The report highlighted several important aspects of HPP including: its effect on food quality and on individual food components (e.g. proteins, lipids and starches), engineering aspects, and potential applications in the dairy industry, including cheese ripening, ice cream ageing, milk protein hydrolysates and milk proteins with modified functionality.

Because of the increasing use of this new technology in Japan in particular, a study tour was undertaken to that country as part of a feasibility study to investigate the impact of HPP on food systems. Recommendations outlined in the report directed the focus of the research undertaken in these studies.

Cheese Ripening

HPP conditions of 50 MPa at 25°C for 3 days was previously reported to have the potential to accelerate the ripening of Cheddar cheese. These conditions were applied to commercial Cheddar cheese. Proteolysis of casein is one of the most significant events to occur during ripening of Cheddar cheese. Products of proteolysis were monitored in HPP and control cheeses by a number of methods.

The data obtained from urea-polyacrylamide gel electrophoresis indicated a significant increase in the rate of formation of $\propto_{s1}$-1- casein on exposure to HPP which indicates that rennet activity can be enhanced by HPP treatment *(Fig 1)*. The results for water soluble nitrogen as a percentage of total nitrogen (%WSN/Tn) and free amino acids (FAA) in the water soluble nitrogen fraction (WSN) indicated that approximately a doubling in the levels of proteolysis was observed, when young commercial Cheddar was exposed to HPP. Similar results were obtained as the cheese age at pressurisation increased, however, the levels of enhanced proteolysis decreased relative to the control indicating that the application of HPP is most efficient if young Cheddar is treated *(Fig 2)*. Reverse phase HPLC was used to separate the peptides in the WSN on the basis of their hydrophobicity. The data obtained demonstrate that qualitatively very similar peptide distributions were obtained between HPP and control cheese, however quantitative differences were noted in the relative abundance of particular peptides present in the WSN. This indicated that while HPP was enhancing the rate at which proteolysis occurred, it did not lead to altered pathways of proteolysis. This finding was very significant, as alterations in the pathways of proteolysis would likely lead to atypical flavour and texture development, which would be undesirable to commercial Cheddar manufacturers. A further significance of these data, was the HPP treated Cheddar would likely be defined as "substantially equivalent" to traditional non-HPP treated Cheddar in terms of peptide distribution in the WSN.

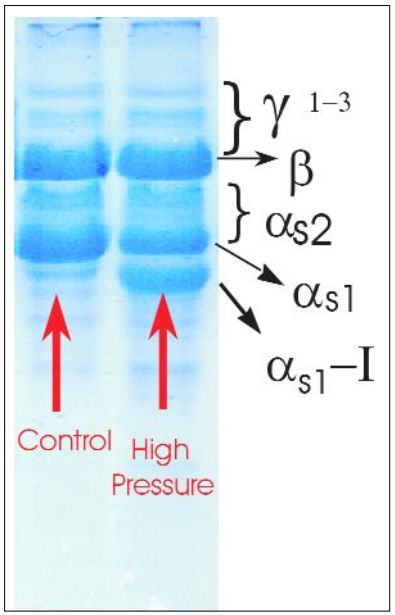

Fig 1: Urea-polyacrylamide gel electrophoretogram of control cheese and experimental Cheddar cheese following treatment at 50 MPa at 25°C for 3 days at day 2 of ripening.

Post HPP treatment cheese samples were stored under normal ripening conditions and assayed for products of proteolysis 30 days later. The overall conclusion from this part of the study was that proteolysis reverted to normal rate on removal of HPP, but that proteolysis in general remained more advanced in HPP treated cheese. Texture analysis was also performed on stored samples, the data indicated that HPP had no effect. Data from commercial grading at 3 months of ripening post HPP treatment was inconclusive, as the sample size available for analysis was too small.

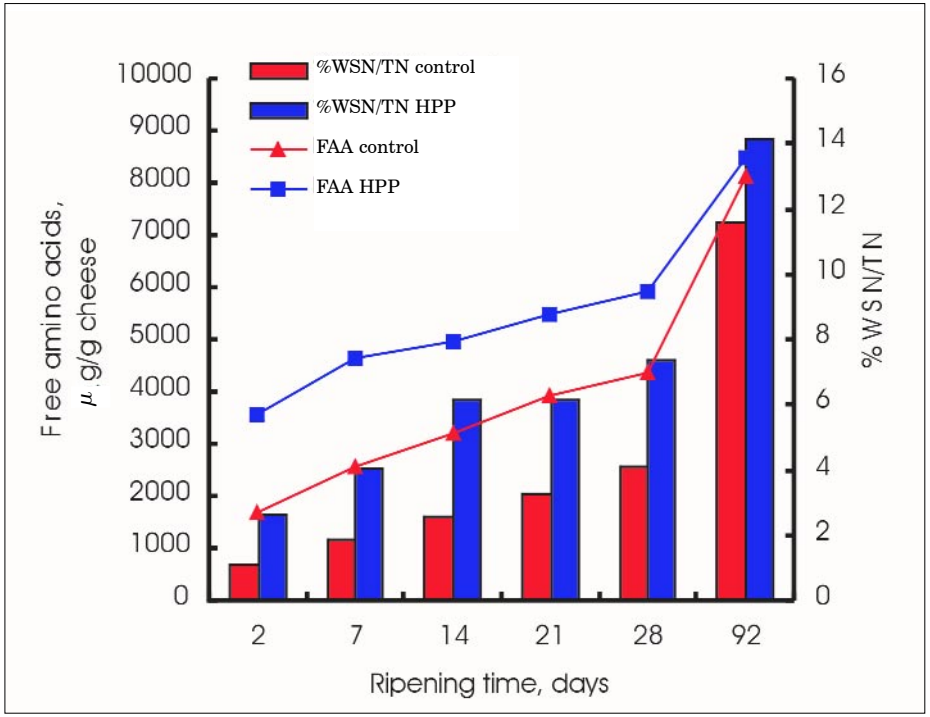

Fig 2: Effect of HPP at 50 MPa at 25°C for 3 days at different stages of ripening on % WSN/TN and free amino acids (FAA) in the WSN fraction of the cheese.

Commercial exploitation of this technology requires that the exposure time to HPP be considerably reduced. A series of experiments were set up to investigate the relationship between time of exposure to HPP and the degree of proteolysis observed in the cheese. The data obtained indicated that a linear relationship existed between increases in WSN and time of exposure to HPP. HPP had little effect on the production of FAA up to 48 hours of exposure and then significant levels were produced. The delay in the production of FAA probably is accounted for by the fact that they are produced by the enzymatic degradation of peptides in the WSN and thus could not be liberated in significant amounts until sufficient peptides were available.

It was also previously reported for some cheese varieties that HPP at very high pressures (400 MPa) for short time periods (5 minutes) followed by exposure to lower pressures (50 MPa) for up to 3 days significantly increased the rate of ripening. Similar experiments were undertaken with commercial Cheddar. The data obtained indicated that no significant difference ($p = 0.5$) in the levels of WSN was obtained between the samples treated at 400 MPa for 5 minutes followed by ambient pressure for 72 hours and samples treated at 400 MPa for 5 minutes followed by 50 MPa for 72 hours.

Autolysis of the starter in the cheese matrix plays a significant role in cheese maturation and cheeses made with autolytic cultures tend to ripen at accelerated rates. Autolysis of cultures in the cheese matrix was determined by assaying for the release of the intercellular enzyme lactate dehydrogenase. Cheddar cheese was manufactured with individual starter strains and the effect of HPP on the levels of lactic dehydrogenase in cheese juice was determined. The data obtained indicated that while HPP causes strain specific inactivation of lactococcal starters, autolysis was not induced by HPP *(Fig 3)*.

The overall conclusion from this research was that the HPP conditions investigated, while increasing the level of proteolysis, were not sufficient to support the commercial exploitation of this technology for achieving enhanced rates of cheese ripening.

Inactivation of micro-organisms

Most research to date has concentrated on the application of HPP in buffers or synthetic growth media, where micro-organisms can exhibit high sensitivity to inactivation by HPP, with very little attention focused on inactivation in food systems. To this end, the inactivation of micro-organisms in dairy food systems, with particular reference to cheese, was initiated. Initially a model cheese slurry system, incorporating cheese chips with salt and pH conditions typical for Cheddar was used to generate an inactivation matrix. Three

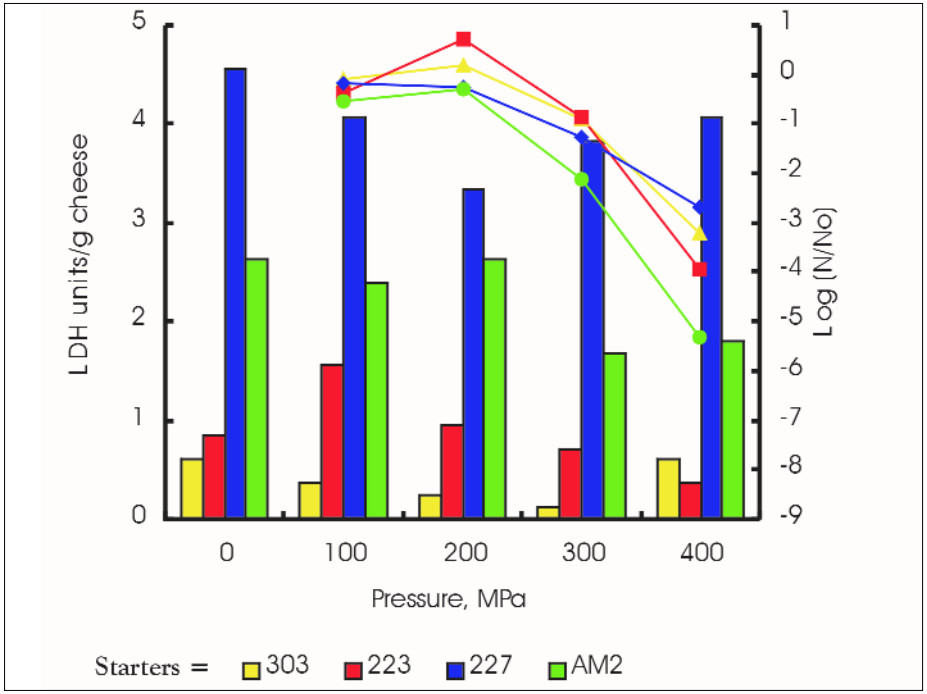

Fig 3: Effect of HPP at 100 - 400 MPa for 20 minutes at 20°C on inactivation (Line graph) and autolysis (Bar graph) of four starter cultures within the cheese matrix.

organisms, *Escherichia coli, Staphylococcus aureus* and *Penicillium roquefortii* representative of a Gram negative, Gram positive and mould species were inoculated into the slurry and pressure treated at 50, 100, 200, 300, 400, 500, 600, 700 and 800 MPa for 20 minutes at temperatures of 10°C, 20°C and 30°C. After pressure treatment the micro-organisms were detected and enumerated on selective media. The selective media were tested and found to be effective in enumerating individual species in the mixed culture of micro-organisms.

The results obtained indicated that the level of microbial inactivation obtained was a function of pressure and temperature applied. For example a treatment of 400 MPa at 20°C resulted in significant inhibition of all three micro-organisms - ca. 3 log reduction in the case of the bacterial species and 6 log in the case of the *Penicillium* mould. The Gram positive *S. aureus* species was more resistant to pressure than the Gram negative *E. coli* species with the latter showing increasing sensitivity to pressure on going from 10°C to 30°C. The mould species, though at lower pressures (<300 MPa) was more resistant than the bacteria, was much more sensitive at higher pressures.

These experiments were repeated with a number of other strains of the three species to determine the degree of variability among strains to HPP treatment. In the case of *E. coli* and the moulds similar trends and degree of inactivation by HPP were observed for strains within species. In the case of *S. aureus*, however, results were more variable suggesting a wider range of response among strains to HPP. Culture age was also shown to be an important determinant of microbial response to HPP with log phase cells more sensitive to pressure than stationary phase cells.

The kinetics of inactivation indicate a linear response to HPP at lower pressures (300 MPa) with deviations from this at higher pressures particularly in the case of *E. coli* and the mould species. In the case of the microbial species, it was shown that HPP induces a component of sub-lethal injury, as well as kill and in the results obtained for *E. coli* there is an over estimation of kill in the HPP data, whereas the data reflects true kill in the case of *S. aureus.*

Following establishment of optimum conditions for inactivation of micro-organisms in the model system, the conditions established were tested in buffer and actual Cheddar cheese. Relative sensitivity of the three microbial species to HPP in Cheddar cheese was as demonstrated previously for the model cheese slurry system i.e. *P. roquefortii* > *E. coli* > *S. aureus.* However there were substantial differences within species particularly in the case of *E. coli* where organisms in cheese were much more sensitive to HPP than in the cheese slurry system *(Fig 4)*. The same trend was evident in the case of *S. aureus* and *P. roquefortii* though the effect was less dramatic. The greater sensitivity of the micro-organisms in the cheese compared with the cheese slurry system may be explained by possible acid injury to the bacteria during the fermentation process. The result underlines the importance of taking product and processing conditions into account when assessing the impact of HPP on the viability of food micro-organisms.

The pressures required to achieve microbial kill are relatively high which presents difficulties in the application of the technology in industrial situations. It was demonstrated that combination of a bacteriocin with HPP significantly reduces the pressure required to

inactivate Gram positive microbial species such as *S. aureus* and *Listeria* spp. *(Fig 5)*. *This data demonstrates the potential of HPP to inactivate micro-organisms. However, it also demonstrates that the medium i.e. food v buffer, has a major impact on the levels of inactivation achieved. Combining HPP with other processes such as bacteriocins was very effective in reducing the number of viable organisms in a product and also allowed significant inactivation at lower pressures.*

Gelling properties of 'simulated yoghurt milk' (SYM)

Native WPI contained approximately 5% denatured protein *(Fig. 6)*. For a constant time (20 min.) and temperature (25°C), pressures of < 150 MPa caused only a small increase in the amount of denatured protein in WPI while increasing pressures above 150 MPa resulted in a steady increase in the level of denaturation. Pressures of > 400 MPa were required to cause greater than 50% denaturation of whey protein *(Fig. 6)*. Extensive denaturation (i.e. 91.4%) was evident after pressure treating WPI at 700 MPa which was comparable to, although slightly lower than, that caused by heating WPI at 78°C x 30 min. (i.e. 93.7%).

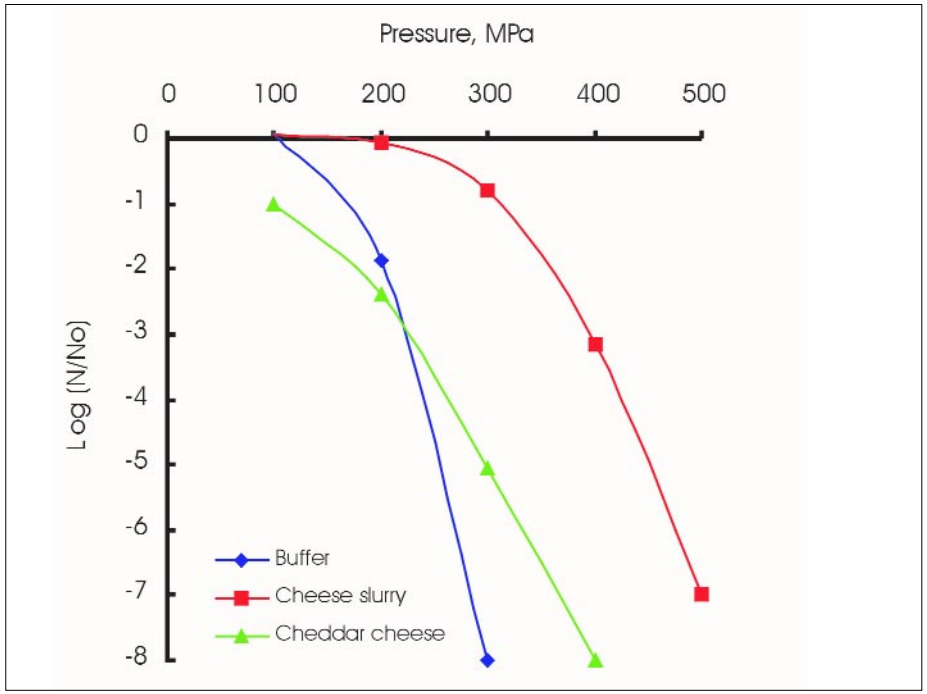

Fig. 4: HPP inactivation of E. coli *K12 in three different media (N_o is the total number of colony forming units in the control and N is the number of cells detected following pressurisation treatments).*

The use of high pressure as an alternative to heat treatment in relation to cold-set gelling of milk proteins was studied using a 'simulated yoghurt milk' (SYM) system, containing

phosphocasein and whey protein isolate in a ratio of 4:1. Gels were made by acidification of SYM with glucono-δ–lactose (GDL) at 40°C to pH 4.6. Gels were prepared from SYM containing pressure- or heat-treated WPI. Pressure-treating WPI at 250 or 400 MPa for 20 min. at 25°C prior to the preparation of SYM did not induce cold-set gelation of SYM, as seen by the lack of increase in G' values during the 2h acidification period *(Fig. 7)*. Pressures of 600 and 700 MPa supported the formation of cohesive gels when SYM acidified with GDL *(Fig. 7)*. These samples had similar gelation times and values for pH of gelation and yielded maximum G' values of 445 and 480 Pa, respectively *(Table 1)*.

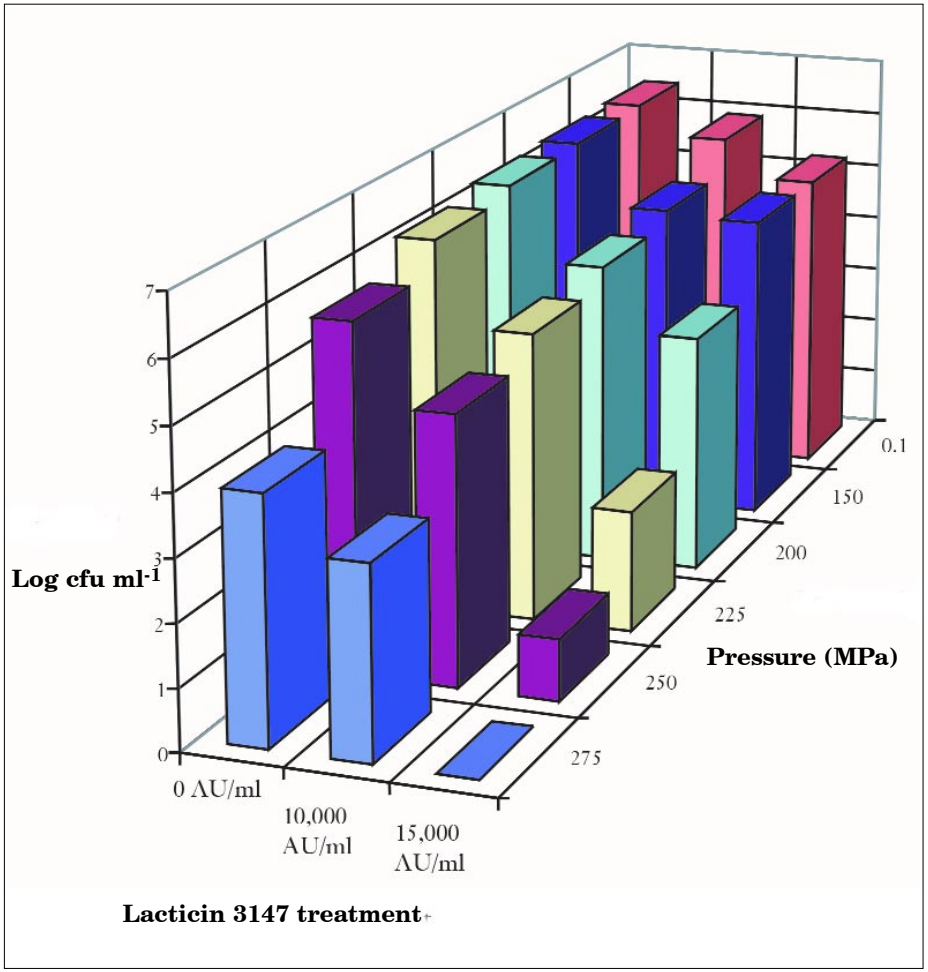

Fig. 5: Inactivation of Listeria innocua *DPC1770 through combinations of HPP and the bacteriocin lacticin 3147.*

Heating WPI at 78°C x 30 min. prior to preparation of SYM yielded shorter gelation times, higher values for pH of gelation and higher maximum G' values than any of the samples of SYM containing pressure-treated WPI *(Table 1)*. Although the profiles of G' as a function of time were similar for gels prepared from SYM containing pressure- (i.e. 600 and 700 MPa) or heat-treated WPI, G' values were considerably higher for the heat-treated sample at all times during the 120 min. gelation process *(Fig.7)*.

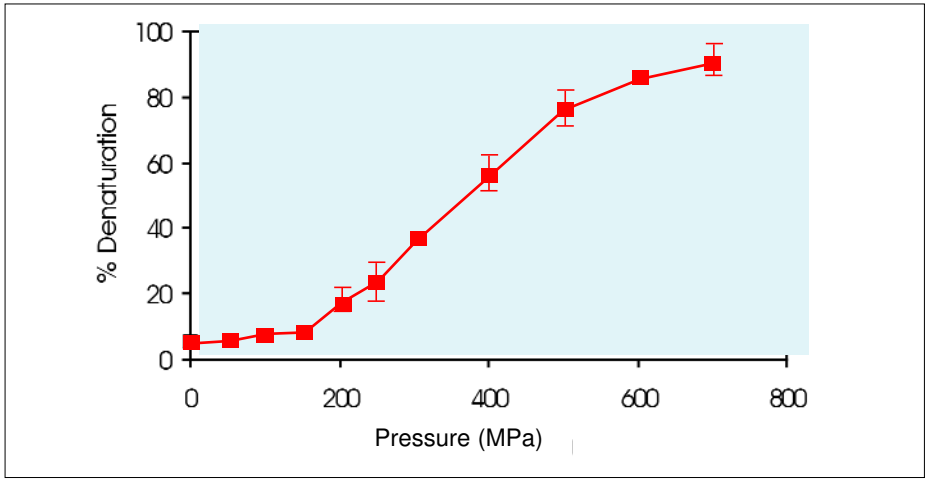

Fig. 6: Protein denaturation in whey protein isolate (WPI) as a function of HPP at 25°C for 20 min.

High pressure may be used as an alternative to heat for the preparation of cold-set milk protein gels. However, the obvious limiting factor would be cost since very high pressures (> 600 MPa) appear to be required for gelation. Alternative pressures treatments (i.e. lower pressures x longer times) or a combination of heat and pressures may prove to be an acceptable alternative for the development of acid-set gels with novel gelation properties.

Table 1. Gelation properties of SYM samples during acidification with GDL (2%,w/v) at 40°C for 2h.

Treatment	Gelation time* (min)	pH of gelation	Maximum G'** (Pa)
Control (i.e. no heat or pressure)	no gel	no gel	no gel
250 MPa x 20 min	no gel	no gel	no gel
400 MPa x 20 min	no gel	no gel	no gel
600 MPa x 20 min	15.5	5.55	445
700 MPa x 20 min	15.0	5.56	480
78°C x 30 min	6.5	5.83	598

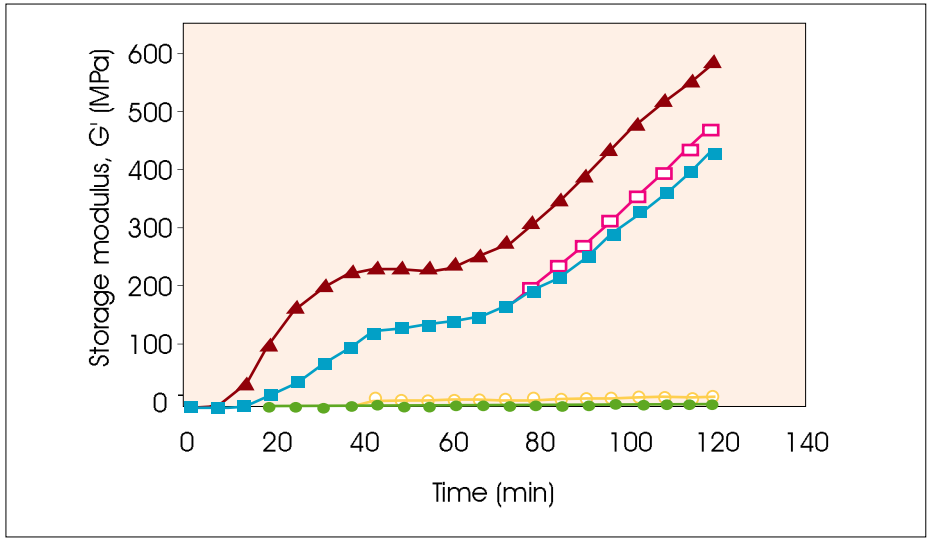

Fig. 7: Storage modulus [G'] as a function of time for gels made at 40°C with GDL from simulated yoghurt milk (SYM). Gels were made from SYM containing WPI that was subjected to 250 (●), 400 (○), 600 (■) or 700 MPa (□) for 20 min. at 25°C or WPI that was heated at 78°C for 30min. (▲).

* Gelatine time was the time at which G'>1.0 Pa
** Maximum G' was recorded 2h after addition of GDL

Functionality of sodium caseinate

The turbidity of sodium caseinate solutions at pH 7.0, 6.5 and, in particular, pH 6.0 decreased with increasing pressures up to 400 MPa, indicating pressure-induced changes in the structure of sodium caseinate particles. However, these changes appeared small and were not reflected by changes in functionality since pressure treatments (0 - 600 MPa for 0 - 45 min.) had little effect on the emulsification and whipping/foaming properties of this protein.

Pressure treatment of 200 or 400 MPa x 30 min. did not alter the susceptibility of sodium caseinate to hydrolysis by a commercial protease preparation since little difference was observed between the hydrolysates prepared from control or pressure-treated sodium caseinate with regard to % degree of hydrolysis (% DH), % digestibility or molecular mass distribution profiles. Although hydrolysis of sodium caseinate to approximately 1 or 2% DH reduced % foam drainage and increased foam expansion, most of the differences observed in functional properties between hydrolysates prepared from control and pressure-treated sodium caseinate did not appear to be the result of pressure *(Figs. 8a and b)*. However, increasing pressure in the range of 0 - 400 MPa seemed to cause a reduction in % foam expansion when foams were made with hydrolysates of sodium caseinate *(Fig. 8c)*. Subjecting sodium caseinate to pressures of < 400 MPa prior to hydrolysis by a commercial protease preparation had no positive effect on the characteristics of the resultant

hydrolysates.

Hence, HPP at 0 - 600 MPa for up to 45 min. had little effect on the emulsification or whipping/foaming properties of sodium caseinate. Furthermore, pressure treatments of 200 or 400 MPa x 30 min. appeared not to alter the susceptibility of sodium caseinate to hydrolysis by a commercial protease preparation and hence had little effect on the characteristics of the resultant hydrolysates.

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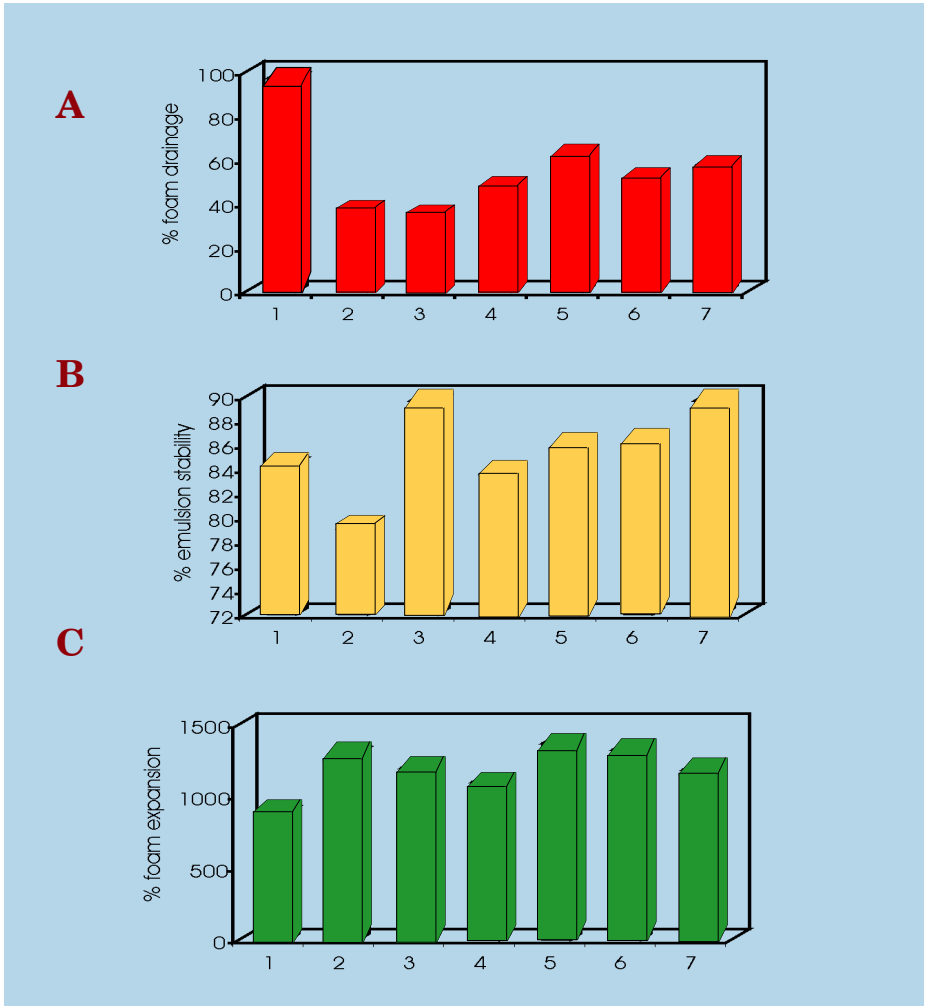
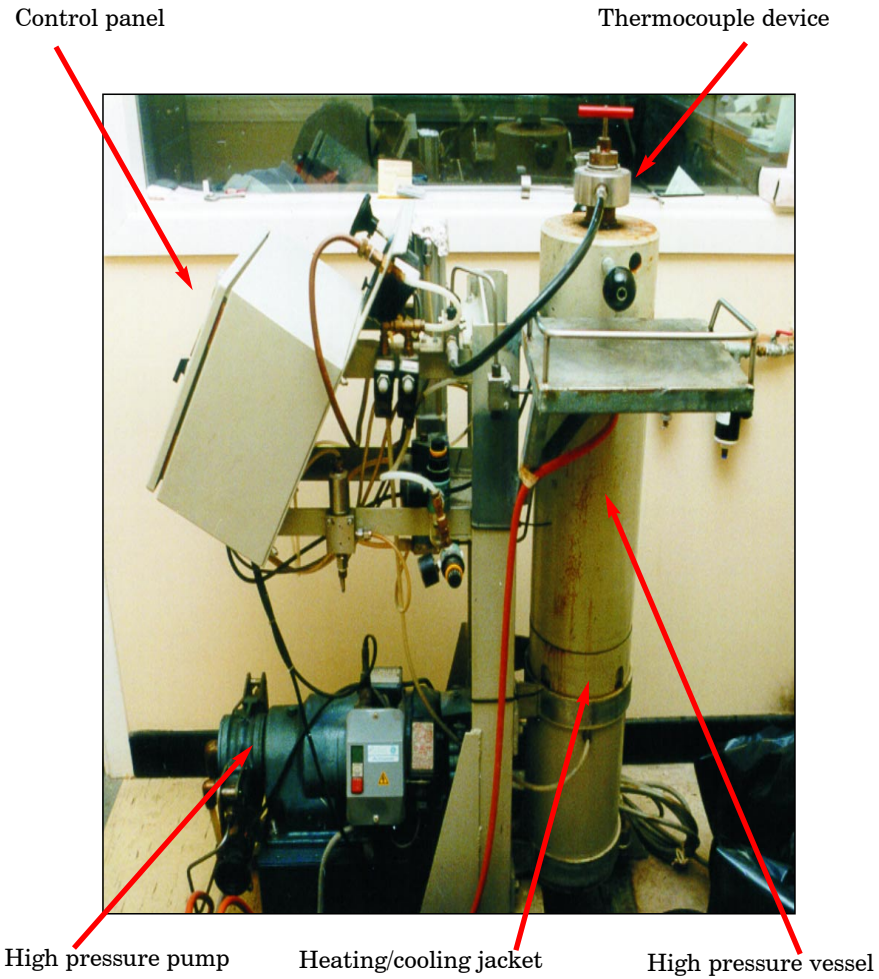
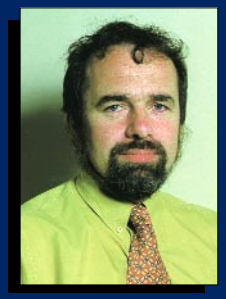
Fig. 8: Functional properties of sodium caseinate (1), sodium caseinate hydrolysed to approx. 1% (2) or 2% DH (5), sodium caseinate subjected to 200 (3 and 6) or 400 MPa (4 and 7) and subsequently hydrolysed to approx. 1% (3 and 4) or 2% DH (6 and 7).



High Pressure Rig Used in Experiment





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