CSIRO Food Research Quarterly

VOLUME 51
NO. 3 & 4 1991

CSIRO

Food Research Quarterly

Volume 51

Numbers 3 & 4, 1991

P.O. Box 52
North Ryde, NSW 2113

TELEPHONE: (02) 887 8333
TELEX: AA23407
TELEFAX: (02) 887 3107

Located at: Gate 1
51 Delhi Road, NORTH RYDE

Editor: Keith Richardson, Food Technology Liaison Officer
Division of Food Processing, CSIRO

Editorial
Committee: K.C. Richardson (Chairman)
J.H.B. Christian
D. Graham
I. Mathieson
J. Prentice
S. Zadow

Design &
typesetting: Eames Communications Pty Ltd

Printing: All Graphics Printing Pty Ltd

ISSN 0310-9070

Contents

From the Editor

Publication of the CSIRO Food Research Quarterly will cease with this combined issue of volume 51, numbers 3 and 4, 1991.

The journal was published first in March 1941 as 'CSIRO Food Preservation Quarterly' and has served as the Division's main extension publication since that time. In his editorial for the first issue, the late W.A. Empey wrote, '... it has been felt that the efficiency of extension services could be increased by the diffusion of knowledge through a special regular publication containing semi-technical articles which would be circulated directly to those persons responsible for the control of processes in the field of food preservation. In addition, there will be accounts of the nature and scope of the work in progress in the laboratories of the Division of Food Preservation, together with explanations of results of investigations already published.'

The Quarterly stayed close to these original aims throughout its half century existence and helped establish the reputation of the Division as a centre of high quality food research both in Australia and overseas. Many of our travelling scientists were delighted to find the Quarterly in libraries of food research laboratories throughout the world.

The Division's outstanding scientists during this time were never inhibited by terms such as 'strategic' and 'tactical' research. They moved across their scientific fields with relative ease and accepted as their responsibility the need to inform a wide readership of the advantages to be gained for manufacturer and consumer from the application of scientific methods to the processing and storage of foods.

It is now felt that with CSIRO collaborating with industry in many different ways, other vehicles for communication and extension are more effective than a quarterly journal. I would like to assure all our readers that the Division will be pursuing its extension activities with undiminished energy and enthusiasm.

My final task as editor of the Quarterly is to thank our numerous contributors to the journal over the years and to thank equally our readers whose support we have greatly appreciated.

K.C. Richardson
Chairman, Editorial Committee

New Dairy Science & Technology Leads to Novel Milk Protein Products

R.J. Pearce, J.A. Dunkerley, S.C. Marshall,
G.O. Regester & R.M. Shanley.

CSIRO Division of Food Processing, Highett, Vic. 3190.

A paper presented to the NSW Division of the Dairy Industry Association of Australia Annual Conference at Sydney on 22nd May, 1990.

INTRODUCTION

The objective of this paper is to demonstrate the importance of maintaining a continuing effort in pursuit of fundamental scientific information about milk and dairy systems for the furtherance of dairy technology. With just a few examples currently under investigation at the CSIRO Dairy Research Laboratory which relate to the development of novel milk protein products, it will be shown how knowledge of the structure and properties of the individual milk proteins together with developments in technological facilities provide the route to new process technology and high added-value products. The examples chosen span a range of stages of development from early technological exploration to near commercialisation.

Thermal fractionation of whey proteins

Whey contains a complex mixture of proteins, as shown in Figure 1. On the basis of the known properties of the major whey proteins, the ability to concentrate the mixture of proteins from whey using membrane technology to manufacture whey protein concentrates (WPC) was hailed as the panacea of the dairy-based, food ingredient industry. Unfortunately, for reasons still poorly understood, WPC even at 75% protein content is in general not a very functional product.

Basic scientific studies conducted at the CSIRO Dairy Research Laboratory led to the discovery that the solubility of α-lactalbumin could be changed under appropriate conditions of pH and temperature. It was shown (see Figure 2) that at about pH 4.2 in the temperature range 55-65°C, α-lactalbumin shows a solubility minimum, demonstrated as a turbidity maximum, whereas the solubility of β-lactoglobulin under these conditions is

Figure 1. Typical distribution of major proteins in cheese whey

Protein type	% of total protein
β-lactoglobulin	45
casein-derived peptides	20
α-lactalbumin	18
serum albumin	5
immunoglobulins	5
lipoproteins	5
enzymes	2

scarcely changed. Application of these conditions to whey and examination of soluble and insoluble products by electrophoresis demonstrated that not only was α-lactalbumin rendered insoluble but other lesser proteins also (Pearce, 1983). After development of a process to pilot scale, two whey protein products were obtained termed α- and β-fraction which contained proteins as shown in Figure 3.

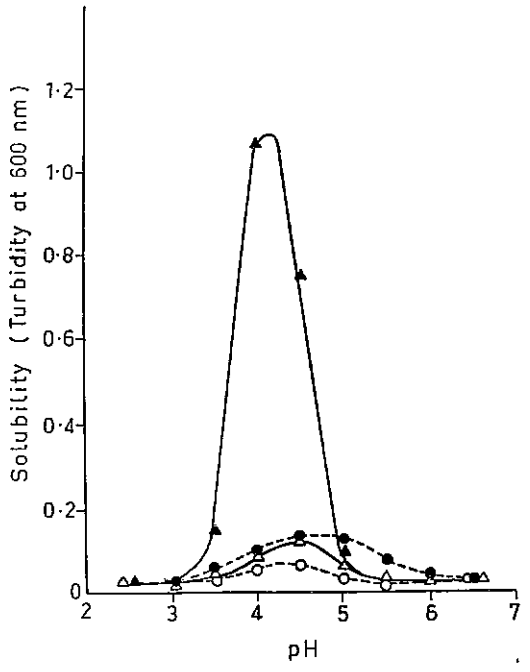
A major advantage of this new process from a commercial development standpoint is the simplicity of facilities that are required. The process has been outlined in schematic form here shown in Figure 4 (Pearce, 1987). Ultrafiltration facilities as used for WPC manufacture may be utilised, requiring only additional pH control, heating and centrifugal separation equipment.

The value of the thermal fractionation process is realised in the functional properties of the products relative to WPC. Some of these have been summarised in Figure 5. In Figure 3 it may be seen that residual lipid, as lipoprotein, in whey is fractionated in the α-fraction leaving the β-fraction essentially fat-free. The consequence of this can be seen in the relative turbidities of solutions of WPC, α- and β-fractions.

In acidic beverages, β-fraction may be utilised since, even after UHT treatment at say 90°C for 30 seconds, the proteins remain soluble. The major protein of β-fraction is β-lactoglobulin and this protein remains soluble below pH 3.7 even though denatured by this heat treatment (Jelen and Buchheim, 1984).

As a foaming agent whey

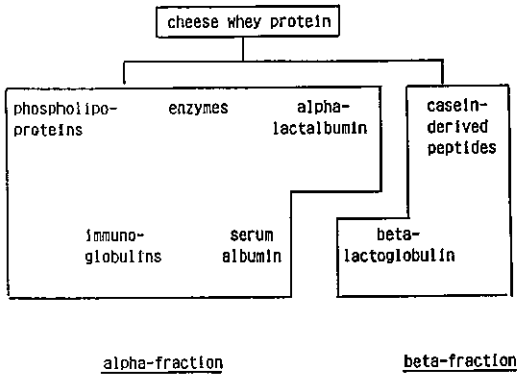
Figure 2. Solubility of alpha-lactalbumin & beta-lactoglobulin at 65°C.



Alpha-lactalbumin Δ 1 mg/mL, ▲ 2 mg/mL
Beta-lactoglobulin O 2 mg/mL, ● 4 mg/mL

From Pearce (1983)

Figure 3. Fractionation of whey proteins into alpha- and beta-fractions.



protein products have in general been effective.

However, to function as an egg white alternative, the foam must demonstrate heat set properties. WPC and α-fraction do not have this ability but β-fraction will form meringue-type products as well as or better than egg white, as shown in Figure 5.

Thermal gelation with high water holding capacity is a prerequisite in many composite meat and fish products to facilitate the 'juiciness' of the cooked product. WPC and α-fraction do not demonstrate this ability, however β-fraction may yield heat-induced gels of greater breaking strength than egg white and greater elasticity, together with opaqueness or near-clarity as required, as shown in Figure 6.

In summary, in this first example it was the discovery of new physico-chemical properties of one of the major whey proteins, α-lactalbumin, that led to the development of a new process yielding a new dairy product of superior functional performance for food application.

Isolation of casein-derived peptide

In Figures 1 and 3 above, a component, perhaps not strictly a whey protein, has been designated as casein-derived peptide (CDP) and may represent as much as 25% of the whey protein. Until quite recently the abundance of this material in whey was not recognised because of its unique compositional characteristics, established only in the past decade.

The structure of the casein

Figure 4. Schematic representation of the thermal fractionation process for whey proteins (Pearce, 1987).

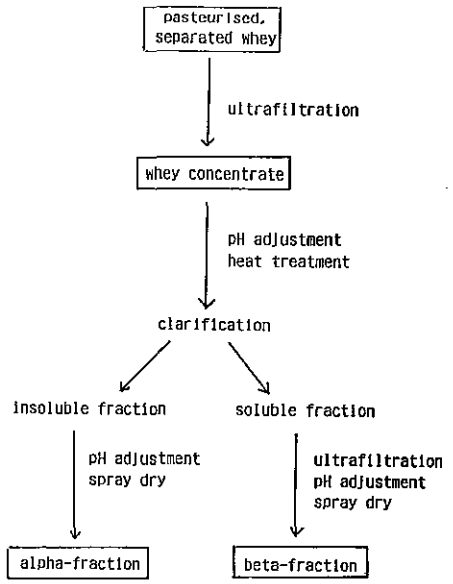


Figure 5. Summary of some of the functional properties of whey protein fractions compared to whey protein concentrate (75% protein).

Functional property	WPC-75	alpha-fraction	beta-fraction
Appearance in suspension	turbid	milky	clear/ opalescent
Solubility after pasteurisation at low pH	turbid with sediment formation	sediment formation	clear, soluble
foam capacity and stability	variable, non-heat setting	high capacity, and stability non-heat setting	high capacity, high stability, high sugar-foam stability, heat-setting
thermal gelation and water holding capacity (WHC)	thick coagulum low WHC	fine coagulum low WHC	uniform gel, clear to white, high WHC

Figure 6. Heat-set gel prepared from beta-fraction (enriched beta-lactoglobulin) derived from Cheddar cheese whey.

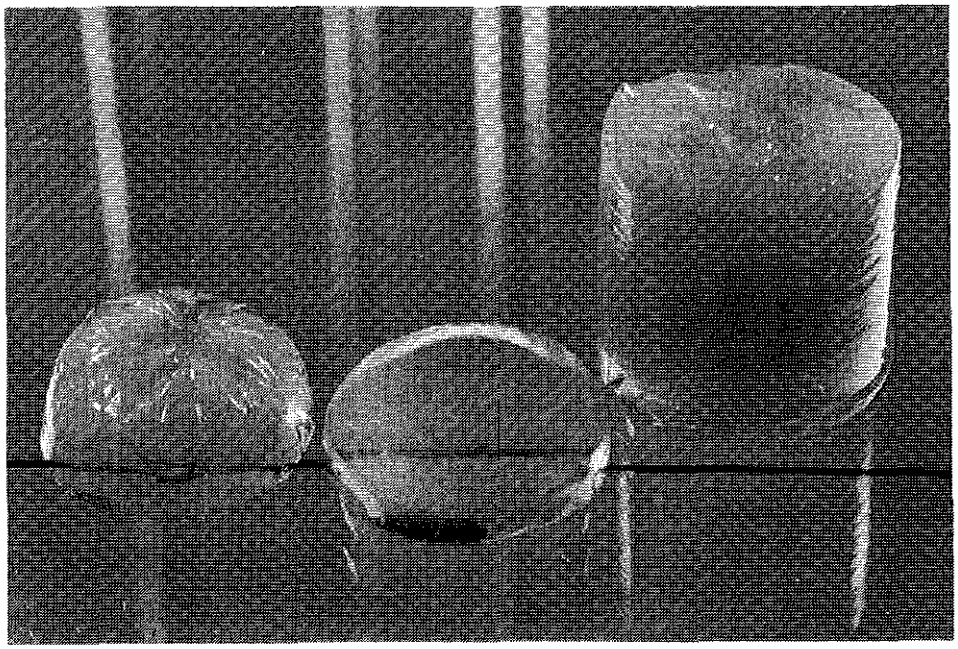


Figure 7. Amino acid sequence of bovine kappa-casein showing the point of cleavage by rennin and the generation of the casein-derived peptide (macropeptide).

Para κ-casein Part

[1]PyrGlu – Gln – Gln – Asn – Glu – Glu – Glu – Pro – Ile – Arg – Cys – Glu – Lys – Asp – Glu
Val – Tyr – Gln – Ile – Pro – Ile – Tyr – Lys – Ala – Ile – Lys – Asp – Ser – Phe – Phe – Arg
Leu – Ser – Arg – Tyr – Pro – Ser – Tyr – Gly – Leu – Asn – Tyr – Tyr – Gln – Gln – Lys – Pro
Lys – Ala – Tyr – Tyr – (Pro – Tyr – Pro) – Leu – Phe – Gln – Asn – Asn – Ile – Leu – Ala – Val
Pro – Ala – Ala – Val – Arg – Ser – Pro – Ala – Gln – Ile – Leu – Gln – Trp – Gln – Val – Leu
Met – Thr – Thr – Pro – Gln – Ala – Gln – Cys – Ser – Lys – Ala – Pro – Val – Thr – Asn – Ser
Ala – Arg – His – Pro – His – Pro – His – Leu – Ser – Phe

Rennin

Probable bonding site for N-acetylgalactosamine + galactose + terminal N-acetylneuraminic acid.

Macropeptide Part

Asp – Gln – Asn – Lys – Lys – Pro – Pro – Ile – Ala – Met
Lys – Thr – Glu – Ile – Pro – Thr – Ile – Asn – Thr – Ile – Ala – Ser – Gly – Glu – Pro – Thr
Glu – Leu – Thr – Ala – Val – Thr – Ser – Glu – Val – Ala – Glu – Ile[2] or Thr – Thr – Pro – Thr – Ser
Ala[2] or Asp – Ser – Pro – Glu – Val – Ile – Glu – Ser – Pro – Pro – Glu – Ile – Asn – Thr – Val – Gln
HO-Val – Ala – Thr – Ser – Thr – Val

[1]Pyrrolidonecarboxylic acid.
[2]Genetic variant κA-casein contains Thr and Asp in residues 136 and 148, κB-casein contains Ile and Ala.

protein in milk has been a matter of debate for more than thirty years and remains far from fully elucidated. Current concepts of structure have been reviewed (Swaisgood, 1982). Casein is commonly represented as having a micellar nature, each micelle being constructed of subunits and each subunit being a composite of four primary gene products, namely α-s_1, s_2, β- and κ-casein. It has been established that κ-casein is susceptible to cleavage by the enzyme chymosin or rennet and releases a large peptide, the casein-derived peptide, from the micelle which subsequently coagulates. Recent studies mostly by French scientists established the amino acid sequence of κ-casein and the point of enzymatic cleavage as shown in Figure 7 (Swaisgood, 1982). With this sequence data, firstly a mechanism for the proteolytic process was able to be deduced and, secondly, it demonstrated conclusively a feature, presumed by those working with CDP, namely that CDP is devoid of aromatic amino acids since it shows negligible absorbance in the appropriate part of the UV spectrum most used for protein detection. Thirdly, it also explained the high acidity and solubility of this material due to the abundance of the amino acids glutamic and aspartic acid.

With this fundamental data it required only the vision to recognise that CDP might represent a valuable source of protein for patients suffering from phenylketonuria (PKU) or forms of hepatic disease and the biochemical experience to utilise the physico-chemical properties of the material in an isolation process.

The development of such a process necessitated monitoring of the material to be isolated; however, as indicated above CDP was not greatly considered earlier because of detection difficulty. For CDP isolation it was possible to adapt an FPLC-based method established for studying whey protein separation in the thermal fractionation process described above. Figures 8a and 8b show how effectively CDP was isolated from cheese whey using an ion exchange adsorption process as described by Skudder (1986).

The amino acid composition of product so obtained was very similar to that predicted by the sequence data shown in Figure 7 and indicated a high degree of purity.

The advantage of using a proteinaceous product like CDP lies in its ability to be incorporated as a bland ingredient into a range of food product types, whereas current maintenance diets for PKU patients rely on unpleasant tasting, synthetic amino acid mixtures administered as a beverage. Since PKU patients at most risk are infants, appropriate food applications have been established such as infant milk formulae, teething rusks, biscuits and fruit jellies.

Basic dairy science resulted in the purification and detailed characterisation of the κ-casein protein molecule. Amino acid sequence data then provided the directions for both the isolation procedure and the application for this potentially very high value new dairy product.

Preparation of α-lactalbumin & whey lipoproteins

Biochemical studies have shown that α-lactalbumin is the principal protein of human milk, that it represents half of the enzyme system responsible for lactose synthesis in cow, human and other mammals, and that the structure and composition of the protein are well conserved between species (Shukla, 1973). Thus, with the trend towards infant formulae more closely resembling human milk, α-lactalbumin of bovine origin manufactured in a purified form should provide an opportunity for a closely 'humanised' product to be prepared.

From the thermal fractionation process described above, a product designated α-fraction was derived containing proteins as shown in Figure 3. The major protein of this fraction is α-lactalbumin. However, directly from this process, α-lactalbumin is derived as part of an insoluble product not suitable for infant feeding.

The solution to the problem was derived from a detailed understanding of the physical chemistry of α-lactalbumin. Differential scanning calorimetry, a technique used to measure changes in the energy levels of a system occurring as a change of state during heating or cooling, was used to measure the temperature at which proteins denature. Once heat denatured and cooled most proteins are no longer capable of showing again the energy transition on reheating; however, α-lactalbumin has been shown to be an exception (Ruegg *et al.*, 1977). α-Lactalbumin was denatured by heat-

ing but on cooling appeared to renature and could again be denatured on reheating. The value of this property of α-lactalbumin was utilised in the isolation of α-lactalbumin from α-fraction. It was considered that in the thermal fractionation process the α-lactalbumin was reversibly denatured on heating and subsequently coagulated at its isoelectric point. When the protein was cooled and the pH restored to neutral the protein renatured and became soluble again.

Utilising the newly emerged membrane technology of microfiltration, it was possible to separate the resolubilised α-lactalbumin from the much larger complex aggregates of lipoprotein in a procedure schematically outlined in Figure 9. From infant food manufacturers a specification was established that an enriched α-lactalbumin product for use in a highly humanised formulation should contain not less than 80% of the protein as α-lactalbumin. The procedure depicted above has enabled such a level of enrichment to be achieved as shown in the analytical chromatograms (Figs 10a and 10b).

Thus again detailed knowledge of the structure and physico-chemical properties of the protein to be isolated enabled a process protocol to be devised and another novel dairy product, enriched α-lactalbumin, to be obtained for use in a high value food product.

Clearly, from Figures 3 and 9 the lipoproteins are obtained as a significant byproduct for which applications are being investigated and if successful will provide yet another new dairy-derived food ingredient.

Figure 8. Comparison of the protein compositions of (a) cheese whey & (b) casein-derived peptide preparation by HPLC analysis.

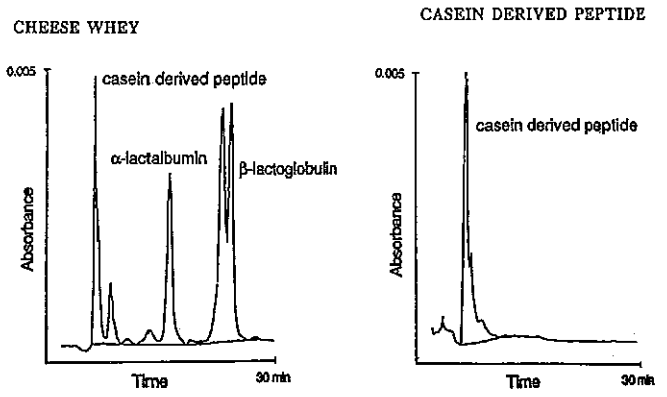
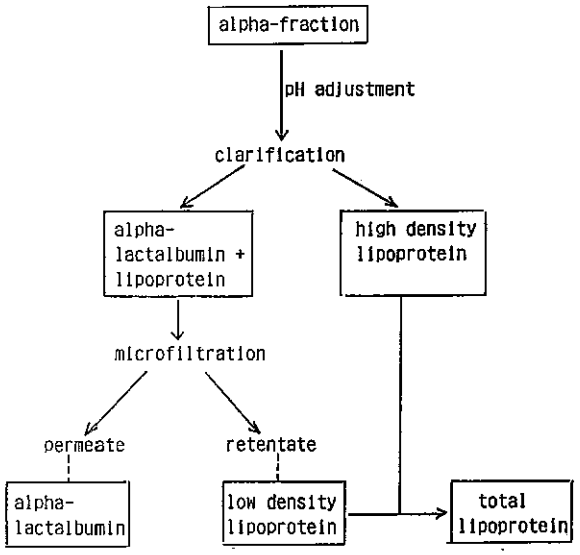


Figure 9. Schematic outline of the procedure for the subfractionation of alpha-fraction into highly enriched alpha-lactalbumin and lipoprotein-rich products.



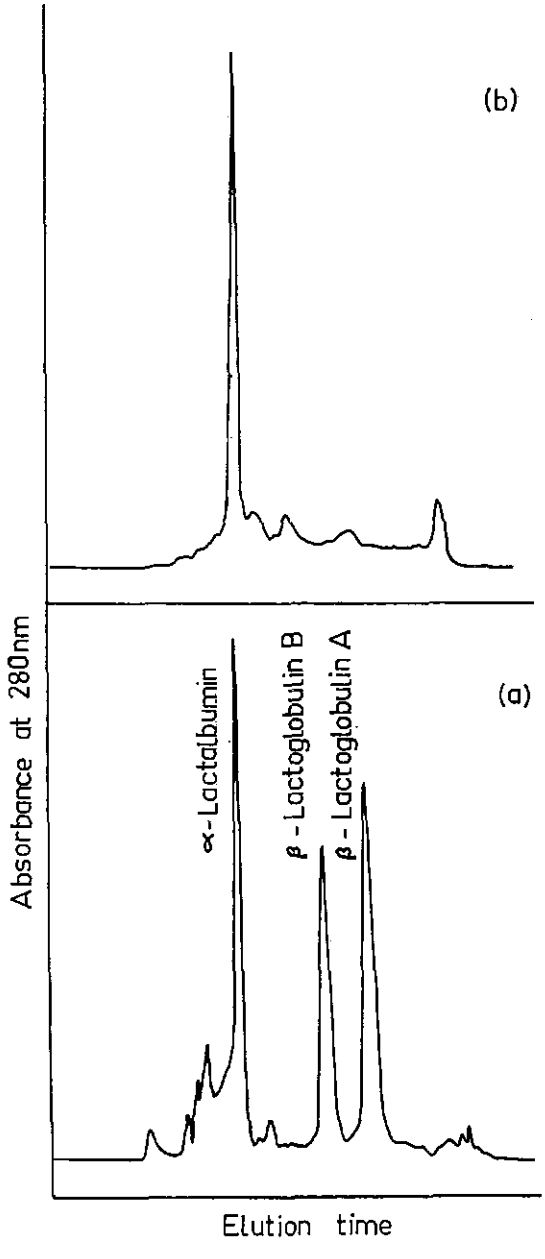
CONCLUSION

There are a number of further examples currently under development at the Dairy Research Laboratory that could be described, but space does not permit full coverage here. While these three examples relate largely to whey-derived products, studies aimed at new casein-derived products are also in progress but not yet so far advanced. Nevertheless, a common theme connects the processes devised for each new product; namely, that each has arisen as a direct result of new discoveries in the science of dairy proteins assisted by developments in processing facilities. If further process and product development is to proceed in the dairy industry, this will only be achieved if an appropriate ongoing commitment is made to strategic dairy science.

ACKNOWLEDGEMENT

We acknowledge G.W. Smithers for the FPLC analyses.

Figure 10. Comparison of the protein compositions of (a) cheese whey and (b) enriched alpha-lactalbumin product by FPLC analysis.



REFERENCES

Jelen, P. and Buchheim, W. (1984). Stability of whey protein upon heating in acidic conditions. *Milchwissenchaft.* **39,** 215-8.

Pearce, R.J. (1983). Thermal separation of β-lactoglobulin and α-lactalbumin in bovine cheddar cheese whey. *Australian Journal of Dairy Technology.* **38,** 144-8.

Pearce, R.J. (1987). Fractionation of whey proteins. *Bulletin of the International Dairy Federation.* **212,** 150-3.

Ruegg, M., Moor, U. and Blanc, B. (1977). A calorimetric study of the thermal denaturation of whey proteins in simulated milk ultrafiltrate. *Journal of Dairy Research.* **44,** 509-20.

Shukla, T.P. (1973). Alpha-Lactalbumin: the B protein subunit of lactose-synthetase. *CRC Critical Reviews in Food Technology.* **3,** 241-312.

Skudder, P.J. (1985). Evaluation of a porous silica-based iron-exchange medium for the production of protein fractions from rennet and acid whey. *Journal of Dairy Research.* **52,** 167-81.

Swaisgood, H.F. (1982). Chemistry of milk proteins. In 'Developments in Dairy Chemistry' Vol. 1 (Ed. P. F. Fox). (London: Elsevier Applied Science) pp. 1-59.

Trends in the Production & Utilisation of Dairy Protein Products: Production

D.M. Mulvihill
Food Chemistry Department, University College, Cork, Ireland

Production of Dairy Protein Products

Bovine milk contains 13% solids which include fat, lactose, protein, and organic and inorganic salts. Normal milk contains 3.5 g total protein per 100 ml which falls into two main categories based on solubility at pH 4.6 and 20°C. Under these conditions 80% of the total nitrogen precipitates and this fraction is referred to as casein, while 20% remains soluble in the serum or whey; 15% being whey proteins with the remainder being non-protein components. The characteristics of the caseins and whey proteins differ very significantly and some of these differences are exploited in industrial methods of casein and whey protein isolation.

However, they can also be isolated together in various high protein products referred to as co-precipitates. The casein and whey proteins are heterogeneous protein systems (Fox, 1989) and methods with industrial scale-up possibilities are now being developed to effect fractionation to individual proteins.

Production of Caseins

Casein has been produced commercially for at least 70 years. Initially, casein was used for industrial purposes, eg, glues, paper glazing and synthetic fibres. It was not until the 1960's that isolated casein became an important food protein, due mainly to pioneering work in Australia and New Zealand. Today, casein, produced by acid or rennet coagulation, is one of the principal functional food proteins with an annual world production of 250,000 tonnes. It has some special properties and cannot be replaced by other proteins in certain food applications. Methods for casein and caseinate manu-facture have been reviewed by Muller (1971, 1982); Mulvihill (1989) and Fox & Mulvihill (1990).

The first step in the isolation of the casein fraction from milk is the removal of fat by centrifugation to yield a skim milk from which the casein is isolated after destabilising it and rendering it insoluble. The use of skim milk ensures that the fat content of the casein is low enough to minimise flavour defects arising from deterioration of lipids in the dried product. Following destabilisation, the insoluble casein is separated from the soluble whey proteins, lactose and salts, washed to remove residual soluble solids and dried (Fig. 1).

Destabilisation/ Precipitation

a.) In the manufacture of mineral acid casein, precipitation is accomplished by spraying dilute (1-2 M) mineral acid, usually HCl, under pressure into milk (preheated to 25-30°C) flowing in the opposite direction to give a precipitation pH of 4.6. Steam is then injected to heat the acidified milk to the required precipitation temperature (50°C) and a holding or acidulation tube is used to ensure complete coagulation and agglomeration of the curd prior to separation of the curd and whey (Fig. 2).

b.) The pH of skim milk can also be reduced to the isoelectric point of casein by mixing skim milk at < 10°C with a cation ion exchange resin in

Fig. 1. Casein Manufacture

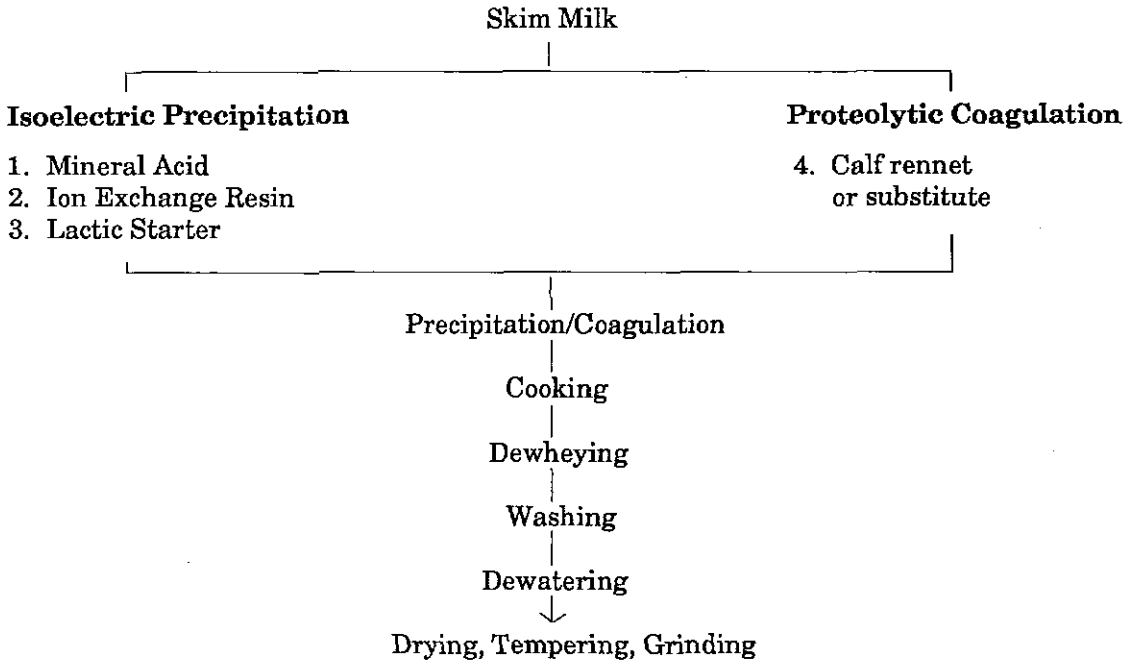
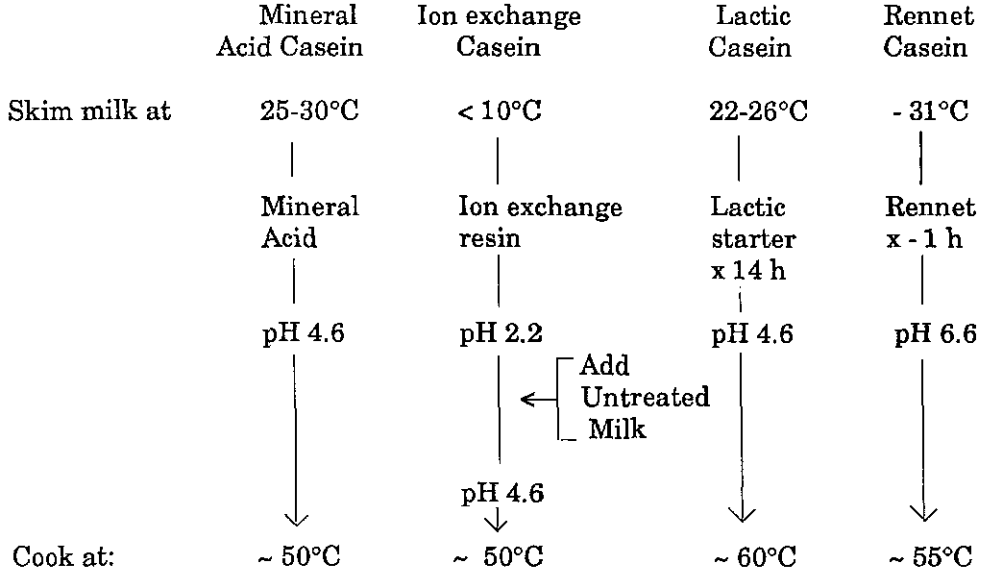


Fig. 2 Casein Precipitation Conditions



the hydrogen form in a reaction column; this replaces cations in the milk by H^+ to give milk of pH 2.2. The deionised, acidified milk is then mixed with untreated milk to give the final desired precipitation pH of 4.6. The mixture is then heated to the coagulation temperature by direct steam injection (Fig. 2).

c.) Precipitation in the manufacture of lactic casein is accomplished by inoculating pasteurised skim milk with a mixed or multiple defined-strain starter and incubating at 22-26°C. During an incubation period of 14-16 hours, the starter ferments some of the lactose to lactic acid slowly and a casein gel network or coagulum with good water holding capacity is formed as the pH of the milk falls slowly under quiescent conditions to the isoelectric pH of the casein. Following coagulation, the coagulum is pumped from the coagulation vats and cooked by direct steam injection. To permit the curd particles to agglomerate and to initiate syneresis, a period of contact with whey, termed acidulation, is allowed either in a holding pipe or vat prior to curd and whey separation (Fig. 2).

d.) Any of a number of proteinases can coagulate milk at its natural pH (6.7) in a two stage process. The first stage involves the specific hydrolysis of κ-casein to yield para-κ-casein and (glyco)macropeptides, while the second stage involves coagulation of the rennet-altered casein micelles by Ca^{2+} at temperatures above 20°C. When a coagulum of this nature is formed from skim milk, it can be further processed to yield rennet casein in a manner similar to that used for the manufacture of lactic casein following quiescent acid coagulation. Rennet casein has a high ash content, especially colloidal calcium phosphate (CCP), calcium and phosphate. The proteinases traditionally used, referred to as rennets, are crude preparations of gastric proteinases prepared from calf vells. However, the supply of rennet has been inadequate for many years and rennet substitutes are now widely used. The primary and secondary phases of rennet coagulation, including the mechanism of gel assembly and the factors influencing gel strength, are now well understood. In the traditional method for rennet casein manufacture, skim milk at pH 6.7, is 'set' with rennet (1:4500) in large jacketed vats at 31°C, in a manner similar to that practiced in cheesemaking. When coagulation has progressed to the desired stage, the gel is pumped from the coagulation vat to a cooking pipe where steam is injected to raise the temperature to 50-55°C for 45 seconds before separation of curds and whey (Fig. 2).

Dewheying

Following destabilisation of the casein, the curd is separated from the whey prior to washing. The efficiency of the 'dewheying' step, which is of the utmost importance in determining the volume of whey recovered for further processing, the efficiency of the washing operation and the quality of the final casein produced, depends on the pH and temperature of precipitation and the equipment used to achieve separation.

Washing

During washing, residual whey constituents (lactose, whey proteins, salts) and free acid are removed from the dewheyed curd to a limited extent by washing of the surface of the curd particles and to a much larger extent by diffusion from within the curd particles. The rate of diffusion depends on the size and permeability of the curd particles, the difference in the concentration of the constituents between the interior of the particles and the surrounding wash water and on the amount, temperature and movement of the wash water.

Washing systems used include multi-stage, counter-current systems and counter-current tower washing systems in which the curd falls through an ascending column of water.

In casein washing it is normal to use a gradient of wash water temperatures during the washing operation. A typical temperature profile for washing acid curd in a four stage washing system is 55, 65, 75 and 35°C for the first to the fourth stage, respectively.

Dewatering

When washing is complete, casein curd is mechanically dewatered to produce a curd of minimum moisture content to minimise the quantity of water to be evaporated and thus minimise the energy required during the subsequent thermal drying operation. The properties of the casein curd following washing should be such as to allow for maximum dewatering under the conditions of

operation of the dewatering machine while at the same time maintaining the curd in a suitable condition for subsequent drying. Mechanical dewatering devices include roller and screw presses and decanting centrifuges.

Drying, Tempering & Grinding

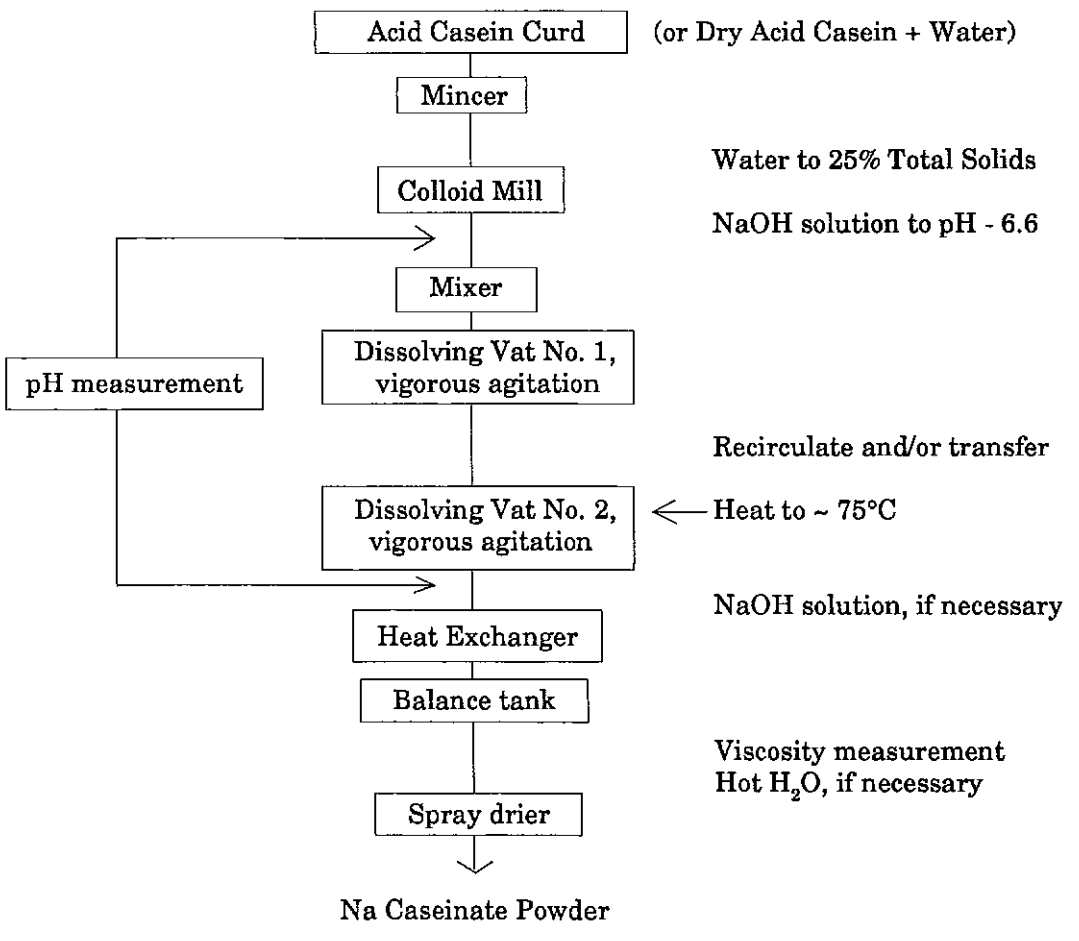
To produce a stable, long-life casein that meets the internationally recognised compositional standards for edible grade product, the casein curd is dried to <12% moisture in any one of a variety of drier types.

Dried casein is relatively hot as it emerges from the drier and the moisture content of individual particles varies. Therefore, it is necessary to temper and blend the dried curd to achieve a cooled final product of uniform moisture content. This is usually achieved by pneumatic circulation of the curd between a number of holding bins.

Following drying, tempering and blending, the casein is ground in roller or pin-disc mills to produce the small-sized particles required by users of casein. Milled material is separated on screens to products of desired particle-size range and oversized material is recycled for further milling.

A drying technique referred to as 'attrition drying', based on the principle of grinding and drying in a single operation, is now widely used in casein plants since it allows the production of a casein product that closely resembles spray-dried casein. The drier consists of a fast revolving, multichambered rotor and a stator with a

Fig. 3. Sodium Caseinate Manufacture



serrated surface. Turbulence, vortices and cavitation effects in the drier result in highly efficient grinding which pulverises the curd to very small particles with a large total surface area. These particles are simultaneously dried in a hot air stream that passes through the drier concurrently with the curd. The dried casein is very fine with an overall average particle size of 100μm. The particles have good wettability and dispersibility in water because they are irregularly shaped and many contain cavities due to the rapid evaporative process.

Production of Caseinates

Acid caseins are insoluble in water but will dissolve in alkali under suitable conditions to yield water soluble caseinates that may be spray- or roller-dried.

Sodium caseinate

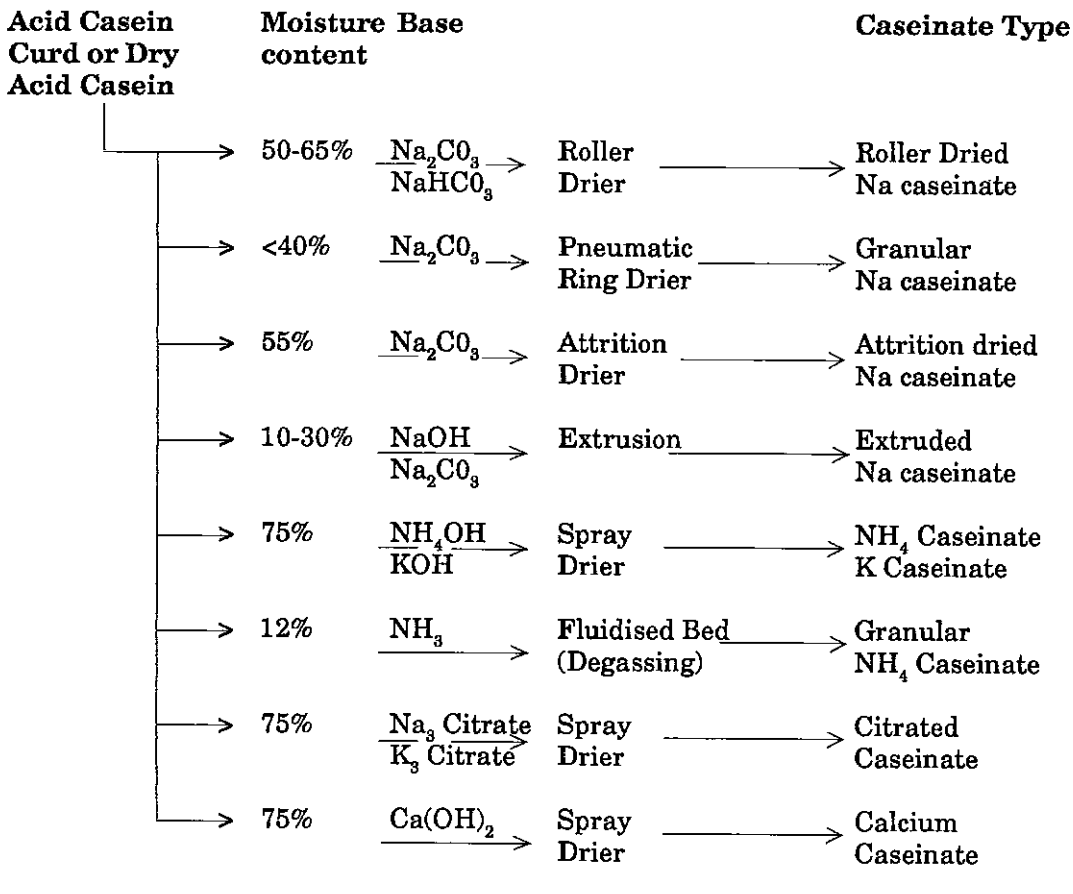
Sodium caseinate, usually prepared by solubilising acid casein with NaOH, is the water-soluble casein most commonly used in foods. The steps involved in its manufacture are outlined in Fig. 3.

a) Casein curd from a dewatering device (45% solids) is minced to disintegrate the curd and then mixed with water at 40°C to give a solids content of 25% before passing it through a colloid mill.

b) NaOH (2.5 M) is pumped into the casein slurry, emerging from the mill at <45°C and with the consistency of 'toothpaste', to give a final caseinate pH of 6.6-6.8. The NaOH and slurry must be efficiently mixed with a mixer capable of coping with the high viscosity.

c) The mixture is transferred to a vat where solubilisation occurs as the mixture is vigor-

Fig. 4 Methods of Manufacture of Different Caseinate Types



ously agitated and heated. The slurry is recirculated and/or transferred by pump to a second vat where solubilisation is completed as the solution temperature is raised to 75°C. An in-line pH meter is used to indicate whether the correct volume of NaOH solution has been added to the curd and to regulate the addition.

d) The caseinate solution is pumped to a balance tank through a heat exchanger in which the temperature is increased to 95°C. A second in-line pH meter is used to control further addition of NaOH, if necessary, to give a caseinate of the desired pH.

e) The solution is pumped from the balance tank to the spray drier via an in-line viscometer that regulates hot water addition to control viscosity and ensure efficient atomisation of the solution in the drier. During caseinate production care must be taken to minimise: i) the time for which the caseinate solution is held at high temperatures, since brown colouration may occur due to reactions between the protein and residual lactose and ii) the time for which the casein is exposed to high pH during dissolving, as this may lead to the production of lysinoalanine and the development of off-flavours.

Other caseinates

Other methods used to produce different caseinate types are set out in Fig. 4:

a) Production of roller dried sodium caseinate by feeding a mixture of curd (50-65% moisture) and an alkaline sodium salt (Na_2C0_3 or $NaHC0_3$) onto the drying drum of a roller-drier.

b) Production of granular sodium caseinate by lowering the moisture content of acid casein curd to <40%, reacting the curd with Na_2C0_3, with agitation, for up to 60 minutes and drying the resultant caseinate in a pneumatic ring drier or a fluidised bed drier. The resulting caseinate has a higher bulk density and improved dispersibility compared to spray and roller-dried products.

c) Drying a mixture of acid casein curd (45% dry matter) and Na_2CO_3 in an attrition drier to produce a product that looks like spray-dried sodium caseinate, but which has a much higher bulk density.

d) Conversion of casein to caseinate in the presence of a limited amount of water using extrusion techniques.

e) Production of ammonium and potassium caseinates in a manner similar to that used for the production of sodium caseinate by substituting NH_4OH or KOH for NaOH.

f) Production of granular ammonium caseinate by exposing dry acid casein to ammonia (gas) and removing excess ammonia with a stream of air in a fluidised bed degassing system.

g) Production of citrated caseinate by a method similar to that used for the preparation of spray-dried sodium caseinate by using a mixture of trisodium citrate and tripotassium citrate in place of NaOH.

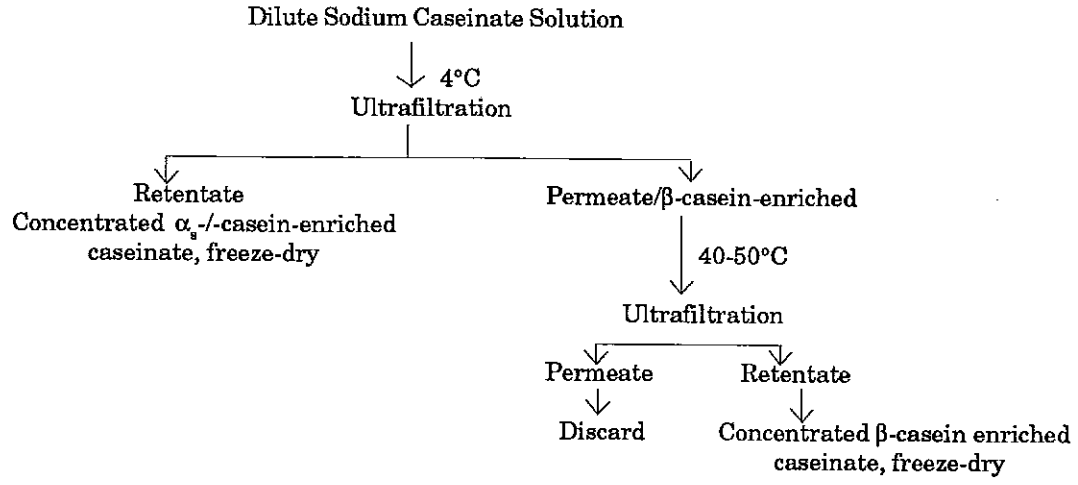
h) Production of calcium caseinate by i) passing 'soft' casein curd through a mixer to give evenly-sized particles; ii) mixing with water to 25% total solids; iii) passing the mixture through a colloid mill and adjusting the temperature to give a milled slurry at 35-40°C; iv) mixing the slurry with a metered volume of 10% aqueous Ca $(OH)_2$ slurry to give the desired final pH; v) agitating and recirculating in a low-temperature conversion tank until conversion is complete (>10 min.); vi) heating the dispersion in a tubular heat exchanger to 70°C and pumping directly to a spray drier.

Miscellaneous Methods of Casein & Co-precipitate Isolation

In addition to the 'traditional' methods described above, several new methods for the preparation of casein or co-precipitates have been reported, some of which may have commercial potential. One such method involves precipitation of milk proteins by addition of ethanol to reduce the dielectric constant of the mixture (Hewedi *et al.*, 1985). Addition of an equal volume of 60% (v/v) ethanol to pasteurised skim milk or to skim milk that had been heated at 90°C for 10 minutes, both adjusted to pH 6.3, caused precipitation of 82% and 90% of total nitrogen, respectively.

Another method used selective solubilisation of lactose from nonfat-dry-milk using ethanol. The insoluble residue, which may be regarded as a milk protein co-precipitate, had a lactose content of 2-4% using a one-step extraction process under optimum conditions (Hoff *et al.*, 1987).

Fig. 5 Method for Preparing α_s- and β-Casein-Enriched Caseins (Murphy & Fox, 1990)



Ultrafiltration of skim milk to a 4 or 6 volume concentration ratio (VCR = initial volume of milk/volume of retentate) to remove lactose, followed by storage of the retentate at -8°C for 1-4 weeks leads to cryo-destabilisation of casein and some whey proteins which are sedimentable by centrifugation at 5,000g for 10 minutes at 0-5°C (Lonergan, 1983).

Membrane filtration methods for the separation of casein micelles (phosphocaseinate) from all the other constituents of skim milk, including the whey proteins, are reported to be developed at laboratory level but have not as yet been scaled up to industrial level (Maubois, 1990).

Industrial Scale Fractionation of Caseins

A number of methods have been developed for fractionating casein into β-casein rich and α_s-/κ-casein rich fractions on a

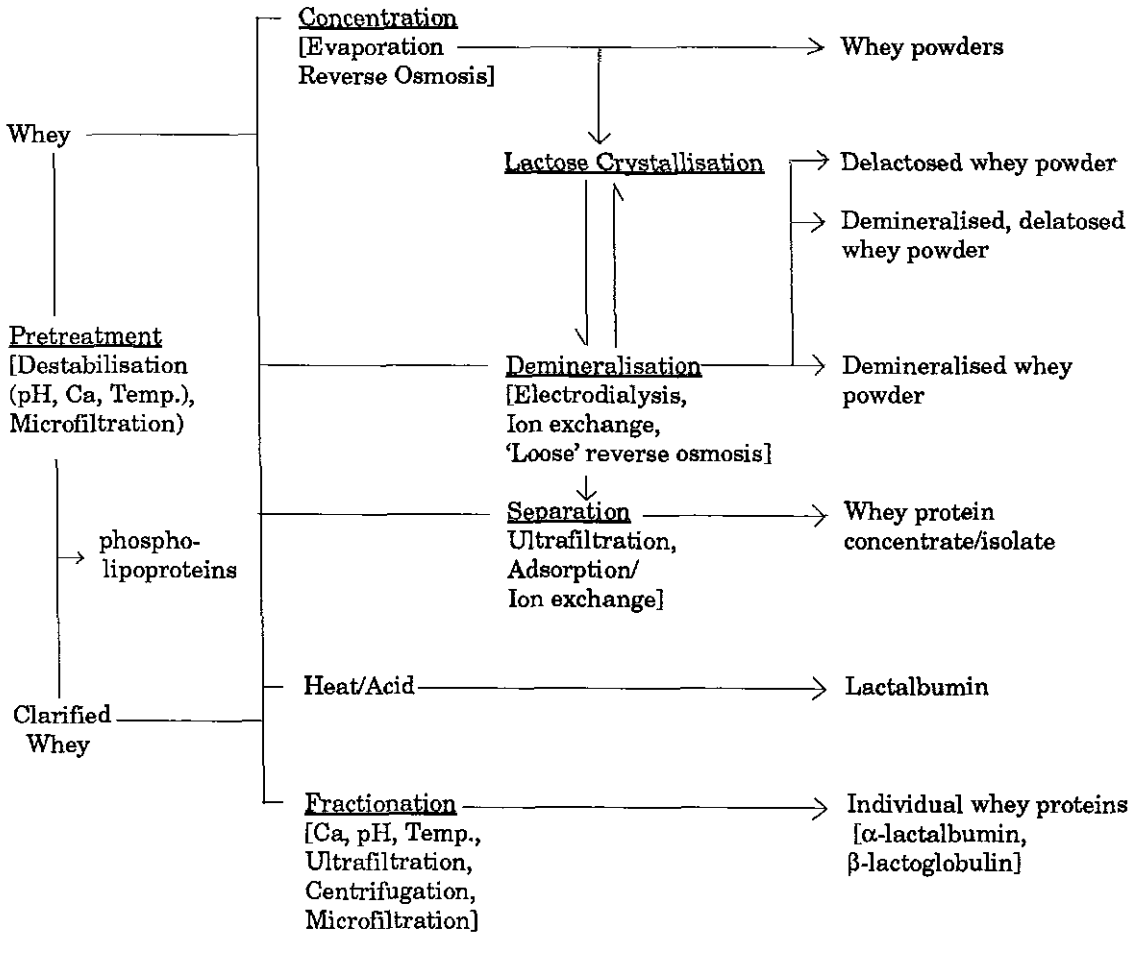
Table 1. Average composition and pH of sweet (rennet casein & cheddar cheese) and acid (lactic and mineral acid) wheys

Component	Composition (g/l)			
	Sweet wheys		Acid wheys	
	Rennet casein casein	Cheddar cheese casein	Lactic acid	Mineral acid
Total solids	66.0	67.0	64.0	63.0
Total protein (N x 6.38)	6.57	6.47	6.20	6.1
Non-protein nitrogen (NPN)	0.37	0.27	0.40	0.30
Lactose	52.3	52.4	44.3	46.9
Milk fat	0.2	0.2	0.3	0.3
Minerals (ash)	5.0	5.2	7.5	7.9
Calcium	0.5	0.4	1.6	1.4
Phosphate	1.0	0.5	2.0	2.0
Sodium	0.53	0.50	0.51	0.50
Lactate	-	2.0	6.4	-
pH	6.4	5.9	4.6	4.7

potentially industrial scale. These are based on the association characteristics of the caseins which are dependent on ionic strength and/or temperature. At low temperatures, β-casein exists in solution as monomers (Payens & Van Markwijk, 1963), a characteristic exploited by Allen *et al.* (1985) to prepare β-casein by rennet-ing calcium caseinate at 4°C. Under these conditions β-casein remains soluble while α_s- and para-κ-caseins coagulate. A method for the isolation of β-casein by microfiltration of calcium caseinate at 5°C was reported by Terre *et al.* (1986). Famelart *et al.* (1989) optimised the same technique to purify β-casein from whole casein at 4°C and pH 4.2-4.6. Murphy and Fox (1990) described a method for the fractionation of a dilute sodium caseinate solution by ultrafiltration into a β-casein rich permeate and an α_s-/κ-casein rich retentate (Fig. 5).

As many peptides derived from caseins have been shown to have biological function there is growing interest in methods of production and isolation of these peptides. However, commercial scale production and isolation methods have not as yet been reported.

Fig. 6 Industrial Isolation of Protein Products from Whey



Production of Whey Protein-enriched Products

Whey is the liquid remaining after removal of fat and casein from milk during the manufacture of cheese or acid and rennet casein. There are two principal types of whey, sweet whey (minimum pH 5.6) from the manufacture of cheese or rennet casein and acid whey (maximum pH 5.1) from the manufacture of acid casein. Average compositions of some whey types are shown in Table 1. Acid whey has a higher mineral/ash content and if the acidity has been formed by the action of starter bacteria, the lactose concentration is reduced. Whey proteins represent only 10% of the total solids of whey and on drying wheys the resulting powders have low protein contents. However, a number of processes (Fig. 6) have been developed and are now being exploited to recover the whey proteins in more concentrated forms. These processes have been reviewed by Marshall (1982), IDF (1987) and Morr (1989).

Whey and whey protein-enriched fractions are usually pasteurised using minimum temperature and holding times and maintained at low temperature to minimise microbial and physico-chemical deterioration of the proteins and other whey constituents that would adversely alter functional and organoleptic properties of the resulting protein-enriched products.

Prior to further processing, whey is commonly pretreated by methods involving pH and/or temperature adjustments, addition of calcium or calcium complexing agents and either quiescent standing, centrifugation or microfiltration to remove insoluble cheese curd or casein fines, milkfat and calcium lipophosphoprotein complexes. After preparation, whey protein-enriched solutions are also pasteurised, concentrated and spray dried.

Whey Powders/Modified Whey Powders

Whole whey powders containing less than 15% protein are produced by concentrating whey by evaporation or a combination of reverse osmosis and evaporation followed by spray drying. Demineralisation by 'loose' reverse osmosis, electrodialysis or ion-exchange and/or lactose crystallisation are used commercially to reduce the lactose and/or mineral concentration of whey and produce modified whey powders such as demineralised and demineralised-delactosed whey powders which contain 15-35% protein.

Lactose is traditionally crystallised from whey by concentration of the whey to a level of 58-62% total solids using a multiple falling film evaporator at a maximum product temperature of 70°C, controlled cooling of the concentrate and seeding to induce nucleation and crystal growth and separation of the crystals from the mother liquor by decanter centrifugation followed by washing (if desired) and crystal drying. The mother liquor is further concentrated and spray dried as a whey protein concentrate powder containing 30% protein.

Whey protein concentrate production by ultrafiltration diafiltration

Ultrafiltration (UF) is a pressure membrane filtration process that facilitates the selective separation of whey protein from lactose, salts and water under mild conditions of temperature and pH. It is a physico-chemical separation technique in which a pressurised solution flows over a porous membrane that allows the passage of only relatively small molecules. The retained solution (retentate) flows over the membrane, while under the influence of pressure, water flows through the membrane, together with the low molecular weight solutes. The protein is retained by the membrane and is concentrated relative to the other solutes in the retentate. Fat globules and suspended solids are also retained in the retentate.

The membranes used in UF are asymmetric microporous structures, the effective layers of which appear to contain pores with diameters ranging from 1-20nm. Commonly used membrane configurations include tubular, spiral wound, plate and frame and hollow-fibre, with each configuration offering advantages and disadvantages for particular applications. The membranes are manufactured from synthetic polymers (eg, polysulphone or polyamide). They are characterised by resistance to high temperature (up to 100°C), ability to withstand a wide pH range (1-13) and that they can be cleaned with the materials normally used in the dairy industry (eg, nitric acid and sodium hydroxide). Although

UF is currently the method of choice for commercial manufacture of WPC of varying protein concentrations, it has several major problems that limit its operational performance. These problems include: high capital and operating costs; membrane fouling with accompanying loss of permeate flux rate; incomplete removal of low molecular weight solutes without diafiltration (dilution of retentate with water and repeated UF); cleaning, sanitation and related microbial problems; the disposal of large volumes of permeate fraction.

Whey pretreatments, referred to earlier, are used to increase flux during ultrafiltration to prevent fouling of the membranes and to modify the properties of the whey protein concentrates.

Limits for whey concentration by UF on modern plants are 24% total solids with a protein/total solids ratio limit of 0.72:1. Diafiltration is employed to achieve higher protein/total solids ratio limits of 0.80:1 and total solid limits of 28%.

Whey protein isolate (WPI) production by ion exchange adsorption

Whey proteins are amphoteric molecules and may be considered as charged ions. At a pH lower than their isoelectric point (pH 4.6) whey proteins have a net positive charge and behave as cations which can be adsorbed on cation exchangers. At a pH above their isoelectric point the proteins have a net negative charge and behave as anions which can be adsorbed on anion exchangers. Media with suitable pore sizes and surface characteristics have been developed specifically for recovery of protein from dilute solutions depending upon the pH of the medium. Two major ion exchange fractionation processes have been commercialised for manufacturing WPI.

The 'Vistec' process uses a cellulose-based exchanger in a stirred tank reactor. The process involves a series of steps that are performed as a fractionation cycle: i) whey is adjusted to pH <4.6 with acid, pumped into the tank reactor and stirred to allow protein adsorption onto the ion exchanger; ii) lactose and other non-protein materials are eluted with water; iii) the pH is adjusted to >5.5 with alkali to release the proteins from the ion exchanger; iv) the proteins are eluted from the tank reactor, concentrated by ultrafiltration and evaporation and spray dried as WPI containing 95% protein. UF treatment of the protein eluate fraction is essential for purifying and concentrating the protein fraction.

The 'Spherosil' processes use either cationic Spherosil S or anionic Spherosil QMA ion exchangers and the fractionation is accomplished in fixed-bed column reactors. Acidified whey at pH <4.6 is applied to the Spherosil S column reactor to allow protein adsorption. After lactose and other solutes are eluted with water, the pH is raised by the addition of alkali and the proteins are eluted from the reactor. The protein eluate fraction is concentrated by UF and evaporation and spray dried to produce WPI. Sweet whey at pH >5.5 is applied to the Spherosil QMA column reactor to permit negatively charged protein molecules to adsorb onto the ion exchanger. After elution of non-protein materials the proteins are released by lowering the pH with acid. Released proteins are concentrated and spray dried as WPI as for the Spherosil S process.

These adsorption processes recover 85% of the protein under ideal operating conditions and the recovered concentrates are characterised by high protein and low lactose and lipid concentrations and have high functionality. However, several major problems are associated with these ion exchange processes. These problems include: i) production of large volumes of rinse, chemical solutions and deproteinised whey that must be processed and disposed of; ii) the need to concentrate and purify the dilute protein eluate fraction by UF, evaporation and drying; iii) the excessive time requirement for conducting each fractionation cycle and iv) microbial contamination of the reactor.

Lactalbumin production

Whey proteins are globular proteins and they are readily denatured on heating. On transformation from their globular structures to more random structures, sulphydryl and hydrophobic groups are exposed and protein-protein interactions occur. The extent of aggregation and precipitation of the denatured proteins depends on heating temperature and holding time, pH and level of calcium. Commercial precipitation conditions depend on whey type and final product characteristics required and whey may be preconcentrated and/or demineralised prior to

Fig. 7 Conventional Co-Precipitate Manufacture

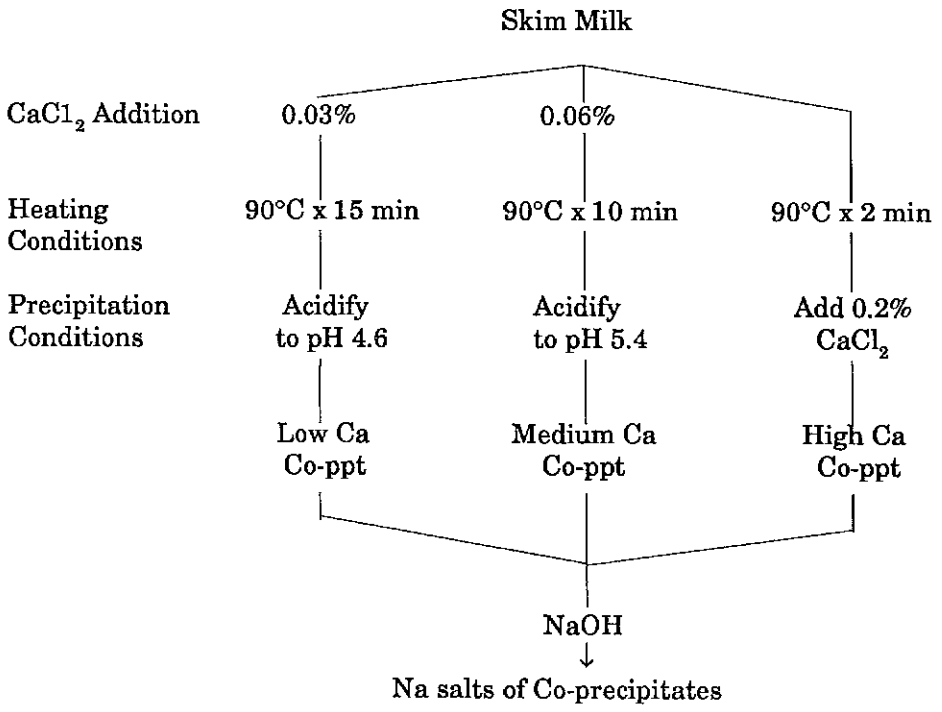
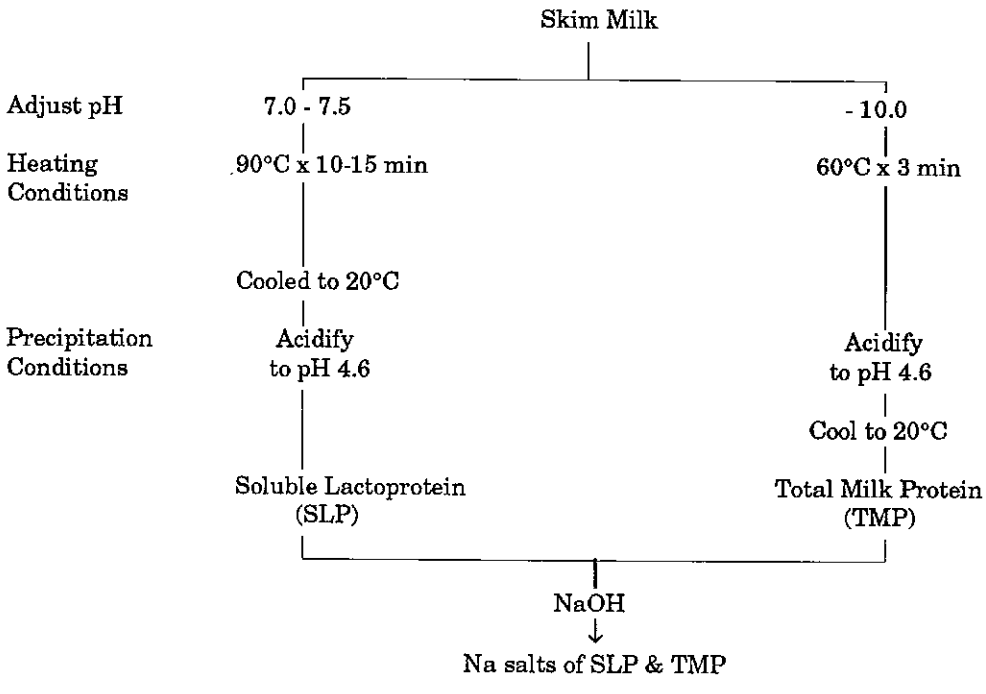


Fig. 8. Methods for Manufacture of Soluble Lactoprotein & Total Milk Protein



precipitation. The precipitated protein, which is referred to as lactalbumin, may be recovered by settling and decanting, vacuum filtration, self-desludging centrifuges or horizontal solid bowl decanters. The precipitate may be washed to reduce mineral and lactose contents and dried in spray, roller, ring or fluidised bed driers depending on the final solids content. Protein yields may be up to 80% of that in the whey and lactalbumin containing up to 90% protein on a dry basis may be recovered depending on precipitation pH and degree of washing.

Fractionation of whey proteins

A number of methods have been developed to either pilot scale or to the point of showing possible commercial scale potential for the fractionation of the major whey protein components, β-lactoglobulin and α-lactalbumin and production of whey protein concentrates enriched in these fractions. These methods depend on either mild heat treatments of whey concentrate or clarified whey under controlled pH and ionic conditions or demineralisation of whey concentrate under controlled pH conditions, to effect selective reversible precipitation of α-lactalbumin or β-lactoglobulin enriched fractions (Fig. 6). There is also considerable interest in the isolation of biologically active proteins such as lactoferrin and lactoperoxidase and biologically or functionally active peptides from whey.

Co-precipitate Production

Following precipitation of caseins from milk by acidification or renneting, the whey proteins remain soluble in the whey. However, these can be precipitated in combination with the casein by first heating milk, at its natural pH, to temperatures that denature the whey proteins and induce their complexation with casein, followed by precipitation of the milk protein complex by acidification to pH 4.6 or by a combination of added $CaCl_2$ and acidification (Fig. 7). Products produced in this manner are referred to as casein-whey protein co-precipitates. Yields of 92-95% of total milk protein are obtained compared to <80% for acid or rennet caseins. Co-precipitates produced by this method have poor solubility properties. However, processes for the manufacture of similar products with good solubility have been described (Fig. 8). These processes involve preadjusting milk to pH 7.0-7.5, heating at 90°C for 15 minutes or preadjusting milk to pH 10 and heating at 60°C for 3 minutes prior to isoelectric precipitation.

Milk Protein Concentrate Production

Skim milk is also being processed directly by ultrafiltration/diafiltration to yield milk protein concentrates that contain a range of protein contents up to 80% and in which the casein is in a similar, micellar form to that found in milk, while the whey proteins are also reported to be in their native form. The products have a relatively high ash content as protein bound minerals are retained.

References

Allen, I.M., McAuliffe, A.G. and Donnelly, W.T. (1985). Simplified approaches to casein fractionation. *Irish Journal of Food Science and Technology,* **9:** 85.

Famelart, M.H., Hardy, C. and Brule, G. (1989). Optimisation of the preparation of β-casein-enriched solution. *Le Lait,* **69:** 47-57.

Fox, P.F. and Mulvihill, D.M. (1990). Casein. In *Food Gels.* (Harris, P., Ed.) (Elsevier Applied Science Publishers, London) pp. 121-74.

Hewedi, M.M., Mulvihill, D.M. and Fox, P.P. (1985). Recovery of milk protein by ethanol precipitation. *Irish Journal of Food Science and Technology,* **9:** 11-23.

Hoff, J.E., Nielsen, S.S., Peng, I.C. and Chambers, J.V. (1987). Ethanol extraction of lactose from non-fat dry milk: production of protein raffinate. *Journal of Dairy Science,* **70:** 1785-96.

International Dairy Federation (1987). Trends in whey utilisation. *Bulletin of the International Dairy Federation,* **212.**

Lonergan, D.A. (1983). Isolation of casein by ultrafiltration and cryodestabilisation. *Journal of Food Science,* **48:** 1817-21.

Marshall, K.R. (1982). Industrial isolation of milk proteins: whey protein. In *Developments in Dairy Chemistry - 1.* (Fox, P.F., Ed.) (Elsevier Applied Science Publishers, London.) pp. 339-73.

Maubois, J.L. (1990). Applications of membrane techniques in the dairy industry - proposals for a new IDF group of experts. *International Dairy Federation*, **244:** pp 20-9.

Morr, C.V. (1989). Whey proteins: manufacture. In *Developments in Dairy Chemistry - 4.* (Fox, P.F., Ed.) (Elsevier Applied Science Publishers, London.) pp. 245-84.

Muller, L.L. (1971). Manufacture and uses of casein and co-precipitate. *Dairy Science Abstracts*, **33:** 659-74.

Muller, L.L. (1982). Manufacture of caseins, caseinates and co-precipitates. In *Developments in Dairy Chemistry - 1.* (Fox, P.F., Ed.) (Elsevier Applied Science Publishers, London.) pp. 315-37.

Mulvihill, D.M. (1989). Caseins and caseinates: manufacture. In *Developments in Dairy Chemistry - 4.* (Fox, P.F., Ed.) (Elsevier Applied Science Publishers, London.) pp. 97-130.

Murphy, J.M. and Fox, P.F. (1990). Fractionation of sodium caseinate by ultrafiltration. *Food Chemistry*, **39:** 27-38.

Payens, T.A.J. and van Markwijk, B.W. (1963). Some features of the association of β-casein. *Biochimica et Biophysica Acta*, **71:** 517-530.

Terre, E., Maubois, J.L., Brule, G. and Pierre, A. (1986). FR 2, 592 769.

News From The Division

Retirement of Stan Shenstone

Stan ('Bill') Shenstone is retiring after a long period of distinguished service in the Division of Food Preservation and its successors. In fact, it is doubtful whether any other officer has served so long - 48 years - and in so many of the Division's Sections.

He joined the Division in 1943 as a laboratory assistant in the Fruit Storage Section at Homebush, under Dr 'Sandy' Trout, transferring in 1945 to the Vegetable Dehydration Group under Dr Thelma Reynolds.

For some years, the Microbiology Section had been studying the bacterial spoilage of shell eggs. In 1948 it was decided to terminate these studies and to look into other aspects of egg quality. Stan (or 'Bill', as he was more frequently known in the Division) was transferred into this new group late in 1948 and was joined by Gerald Stanley, working under the direction of the Chief. The main lines of work concerned a tough problem, the nature of the physical deterioration of egg white during egg storage, and the group also studied a world-wide concern about the cause of the onset of 'pink whites'.

Studies on the first problem have continued almost to the present day. In the 'pink whites' study, Stan and his colleagues were the first to show that 'pink whites' were due to the ingestion by hens of two cyclopropenoid fatty acids which occur in certain weeds and in cotton-seed meal. The quality of the work was such that, without the seeking by the Division, the United States Department of Agriculture gave us a very substantial grant to finance further studies.

In the early stages of his time at the Division, Stan undertook the onerous task of studying mainly at night for the Associateship in Chemistry at the Sydney Technical College, which, in those days, was the equivalent of a university degree in science.

He made good progress through the various classification grades and became an Experimental Officer in 1958.

Stan continued a wide range of egg studies, including changes in baking quality during storage, in quality surveys in the domestic markets, and, in conjunction with Malcolm Smith, in the properties of egg whites.

There was no doubt that Stan became the leading authority in Australia on the quality of egg, a fact which was recognised in 1968 when he was invited by the British Egg Marketing Board to take part in an International Symposium on Egg Quality.

Stan's versatility was further evident in chemical studies on the separation and characterisation of polyunsaturated acids in lipids.

In the early 70's he joined the physical biochemistry group to continue his work on the properties of egg proteins and also on the membranes of avian erythrocytes.

In 1980, he transferred to the Food Structure Group and worked on a new range of egg problems, including biosynthesis of yolk and its colour; portomicrons in laying hens; foaming and gelling properties of white; ovo-mucin; and gas storage of eggs.

Stan has a record of over 30 publications on research and general reports.

Finally, reference should be made to the encouragement the Division gave Stan to consider matrimony. To this end, during the last 10 years at Homebush, we located him in a laboratory adjacent to the Library.

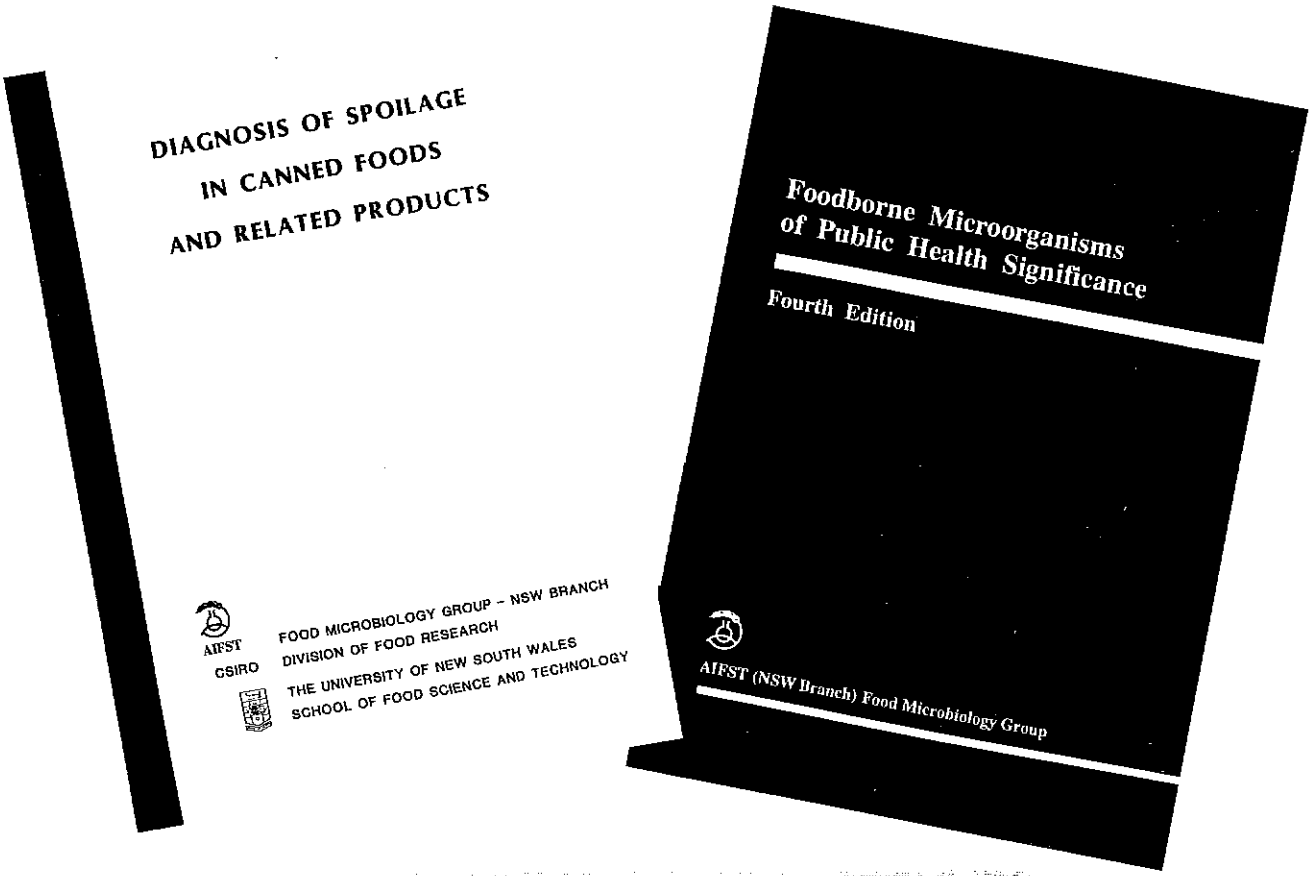
In due course, such propinquity was rewarded when Stan announced his engagement to a fine young lady, Jean Hicks, the Assistant Librarian.

Our very best wishes go to Stan in his retirement, which we hope will be long and happy.

Written by J.R. Vickery

Division of Food Processing Selected Publications

Selected publications available on application to Food Research Laboratory
PO Box 52, North Ryde, NSW 2113 Ph: (02) 887 8333



Selected publications available on application to Food Research Laboratory
PO Box 52, North Ryde, NSW 2113 Ph: (02) 887 8333