TECHNOLOGICAL ASPECTS OF MANUFACTURE OF HIGH, MEDIUM AND LOW CALCIUM COPRECIPITATE FROM BUFFALO MILK

THESIS
SUBMITTED TO THE KURUKSHETRA UNIVERSITY
KURUKSHETRA

FOR THE DEGREE
OF
DOCTOR OF PHILOSOPHY
IN THE FACULTY OF
DAIRYING, ANIMAL HUSBANDRY AND AGRICULTURE

By
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B.Sc. (D.T.) M.Sc. (D.T.)

DIVISION OF DAIRY TECHNOLOGY
NATIONAL DAIRY RESEARCH INSTITUTE
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DEDICATED TO MY PARENTS

Dr. C.A. Mulay
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Dated: 7th December, 1982.

I certify that the work reported in the dissertation entitled "Technological aspects of manufacture of high, medium and low calcium co-precipitate from buffalo milk" is a bonafide piece of work carried out under my guidance by Shri Rajbir Singh Mann towards the requirement for the degree of Doctor of Philosophy in Dairy Technology in the Faculty of Dairying, Animal Husbandry and Agriculture, Kurukshetra University, Kurukshetra.

(C.A. MULAY) 7/12/82

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R.S.Mann.

(R.S. MANN)

C O N T E N T S

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ABBREVIATIONS

CD	..	..	Critical difference
Cp	..	..	Centipoise
Cm	..	..	Centimeter
df	..	..	Degree of freedom
ED	..	..	Electrodialysis
EDTA	..	..	Ethylene diamine tetra acetic acid, di-sodium salt
g	..	..	Grams
IS	..	..	Indian Standards
Min	..	..	Minutes
ml	..	..	Millilitres
MSS	..	..	Mean sum of squares
NPU	..	..	Net protein utilization
OD	..	..	Optical density
PER	..	..	Protein efficiency ratio
ppm	..	..	Parts per million
RPM	..	..	Revolutions per minute
SD	..	..	Standard deviation
-SH	..	..	Sulphydryl groups
SMP	..	..	Skim milk powder
SS	..	..	Sum of squares
STPP	..	..	Sodium tripolyphosphate
TS	..	..	Total solids
%	..	..	Percent

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Milk solids-not-fat and milk fat are commonly preserved in the form of skim milk powder, unsalted butter or butter oil from surplus milk. Depending on demand, the other products are also manufactured to a certain extent. Amongst these, casein also finds its place due to its nutritional importance and is manufactured at times from sour milk as well. However, in the manufacture of casein, whey proteins which constitute about 0.8 per cent of milk, are lost.

It has been established that whey proteins are nutritionally important due to their sulphur containing amino acids and also reported to have 100 per cent net protein utilisation. It is, therefore, beneficial to recover the whey proteins rather than loading the sewers with additional Biological Oxygen Demand. But, as their contents are very low, it is not economical to isolate them as such.

It is well known that whey proteins are precipitated due to heat interaction between β-lactoglobulin and K-casein at temperature above 65°C. The co-precipitate has advantages over the casein and caseinates in being more nutritious and has a better flavour and emulsion stabilising property. This observation opened an avenue in the manufacture of co-precipitates in 1960s (Southward and Goldman, 1975). Studies were also conducted on its manufacture and its nutritional and functional properties in milk products and other food products manufacture.

Co-precipitates are formed by a two stage process: (i) heat treatment of milk or of a mixture of products which provide casein and whey protein, and (ii) precipitation of these proteins from the heated milk either by calcium chloride alone, or by acid precipitation or by combination of both.

Subsequently, low, medium and high calcium co-precipitates came in-to existence which are now being manufactured commercially in Australia, Newzealand and U.S.A. Their importance lies in terms of functional properties like viscosity, hydration, solubility, emulsion stability and whitening. This offered a wide scope for their use in food industry. Furthermore, the type of plant used for the batch or continuous manufacture can be adopted conveniently with little modification in the manufacture of co-precipitates. So far, no standards in respect of chemical,

physical or bacteriological have been laid down by any country or International Dairy Federation.

The daily Indian diet is short of 1250 Calories as compared to diets of people in developed countries. On an average, the Indian diet contains only 45 g proteins, mostly of vegetable origin, which are lacking in essential amino acids. Thus, when corrected for quality on the basis of essential amino acids, the actual protein intake works out to be 34 g, as compared to an optimum daily requirement of 55 g. Thus the protein starved population cannot afford to lose the whey proteins of milk. Consequently, conservation of proteins by all possible means assumes a special significance.

Nearly, 60 per cent of the milk produced in India comprises from buffalo species. The compositional differences between cow and buffalo milk specially in terms of protein and mineral matter calls for a suitable technology to be adopted in the manufacture of different types of co-precipitates from buffalo milk.

Very little work on the manufacture of co-precipitates from buffalo milk has been published till this date. It was, therefore, considered desirable to undertake the manufacture of low, medium and high calcium co-precipitates from buffalo skim milk, admixture of buffalo skim milk with buffalo cheddar cheese whey and electrodialysed skim milk on the lines as suggested for the manufacture of the above types of co-precipitates from cow milk, with

modifications, if needed. Further, it was also planned to investigate some important aspects of physico-chemical and functional characteristics of these co-precipitates. The influence on the whipping ability and quality of ice cream as a result of partial replacement of skim milk powder with co-precipitates was also proposed to be studied. The emulsifying activity and emulsion stability of the product was also planned to be included in this study.

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CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. BUFFALO MILK V/S COW MILK

The average composition of cow and buffalo milk reported by Sen and Dastur (1947) is given below:

Table 2.1. Average composition of milk (Per cent)

Constituents	Cow	Indian buffalo
Water	86.61	82.76
Fat	4.14	7.38
Protein	3.58	3.60
Lactose	4.96	5.48
Ash	0.71	0.78
SNF	9.25	9.86
Total solids	13.39	17.24

2.1.1. DISTRIBUTION OF PROTEINS IN COW AND BUFFALO MILKS

Protein is an important constituent of milk and it varies from species to species. Brahmachari (1933) reported that protein content of Murrah buffalo milk (10 samples analysed) varied between 3.28 and 4.65 per cent with an average of 3.81 per cent. Anantakrishnan *et al.* (1943)

gave the average protein value as 3.60 per cent for buffalo milk and 3.36 per cent for cow milk. Puri and Singh (1951) reported 3.37 to 4.06 per cent of protein in buffalo milk.

Schneider et al. (1948) presented detailed data on the protein constituents of cow and buffalo milk on the basis of analysis of 2,092 samples from farm and village animals. Their average values are presented below:

Table 2.2. Protein constituents of Indian cow and buffalo milk.
(Per cent)

Constituents	Cow	Buffalo
Crude protein	3.178	3.784
True protein	2.798	3.383
Casein	2.380	3.003
Albumin	0.402	0.383
Non-protein nitrogen	0.0542	0.0507

From a study of 700 samples of milk from 30 buffaloes over 40 lactations, the average protein content of buffalo milk was found to be 3.87 per cent (Asker et al. 1957). According to Ghosh and Anantakrishnan (1965), the average value for protein content of murrah buffalo milk was 3.91 per cent as compared to 3.33 per cent obtained for Indian cow milk. Other workers have reported higher values for protein content of buffalo milk i.e. 4.23 per cent

(Hamdy and Abd El-Aziz, 1961); 4.32 per cent (Merzametov, 1965).

Ghosh and Anantakrishnan (1963) reported 2.88 - 3.24 per cent casein in milk from Indian buffaloes during different seasons. The corresponding range for casein of cow milk was 2.54 - 2.59 per cent.

2.1.1.1. Structural differences in casein

Casein micelles of buffalo milk showed significant difference when compared with cow milk (Ganguli,1976). Proportion of micellar casein was more in buffalo milk but the soluble casein was found to be in very low proportion. Besides, the particle size of the buffalo micellar casein observed with electron microscope was found to be significantly larger (135 nm) compared to cow milk casein micelle (90 nm). Also, the calcium content of buffalo casein micelle was distinctly more (3.5 per cent) compared to that of cow casein micelle (2.8 per cent). Phosphorus content was similarly observed to be high in buffalo casein micelle.

Electrophoretic studies revealed (Ganguli, 1976) that buffalo milk casein had lower proportion of α_s-casein and higher proportion of β-casein compared to cow milk casein. Prodanski and Petrov (1962) from paper electrophoresis of casein observed the following proportions of α-, β- and γ caseins of different species: buffalo, 26.1, 50.5 and 23.4; and cow, 29.42, 48.3 and 22.28 per cent respectively. Dilanyan and Agababyan (1962, 1963) reported that buffaloes casein contained more α-casein and less

γ-casein than cow casein.

Ganguli and Bhalerao (1964) and Singhal and Ganguli (1965), who studied the separation and quantitative distribution of casein fractions by paper disk electrophoresis, observed that all the three components (α, β and γ) of buffalo milk casein had slower mobility than the corresponding components of cow milk casein. The relative concentrations (per cent of whole casein) of α-, β- and -γ fractions were 44.5, 52.4 and 3.1 in buffalo milk casein and 54.5, 39.1 and 6.4 in cow milk casein.

Ganguli (1963) reported that the amounts of inorganic phosphorus released by alkaline action on buffalo and cow, casein were similar but the rate of release was slower in the case of buffalo casein during certain stages of both chemical and enzymatic hydrolysis.

2.1.2. MINERALS

Buffalo milk is a richer repository in minerals (Ganguli, 1976). Calcium and phosphorus are distinctly higher (0.22 per cent and 0.13 per cent respectively) in buffalo milk compared to cow milk (0.12 per cent and 0.09 per cent respectively). The calcium phosphorus ratio is also high in buffalo milk (2.20) than in cow milk (1.96). In general, it is observed that cations (calcium and magnesium) are more in buffalo milk whereas anions (phosphate and citrate) are less in buffalo milk. Furthermore, the soluble forms of calcium and citrate are low in buffalo milk.

Puri and Parkash (1965) reported that the dissolved calcium content of buffalo, cow and goat milk was approximately 1/3 of the total calcium content, irrespective of the species or total calcium content.

The average phosphorus contents of buffalo and cow herd milk were 0.107 and 0.083 g/100 ml, respectively (Balba et al. 1958).

2.1.3. WHEY PROTEIN DISTRIBUTION IN RAW MILK

The serum or whey proteins constitute approximately 0.6 per cent of milk or 20 per cent of total milk proteins (Webb et al. 1974). The composition of the serum protein fractions, based on the electrophoretic analysis, is approximately 55 per cent β-lactoglobulin, 12 per cent α-lactalbumin, 10 per cent proteose peptone, and 10 to 15 per cent casein, the remainder being composed of the globulins and enzymes. Serum proteins are subject to more marked variation than casein and are particularly affected by the stage of lactation (Webb et al. 1974).

When milk is heated to 95°C (203°F) for 20 minutes about 80 per cent of the whey proteins co-precipitate with casein, these proteins were classified as heat labile whey proteins. Rowland (1938) reported that the 20 per cent heat stable protein remaining in solution is a separate protein which was called as "Proteose Peptone" fraction. The proteose-peptone can be separated from the non-protein nitrogen (NPN) by the addition of 12 per cent trichloro acetic acid (TCA). The heat-labile whey proteins were

separated into 'lactalbumin' and immunoglobulin fractions by salting out techniques applied to unheated whey.

Harland et al. (1955 a) reported on the serum protein content of skim milk based on 81 commercial bulk samples. They determined the serum protein content by Rowland (1938) and Harland Ashworth (1947) methods. According to them serum proteins varied between 0.82 to 1.48 and 0.62 to 0.91 mg/ml following Rowland and Harland-Ashworth methods, respectively.

The whey protein in 12 samples of buffalo milk was found to vary between 0.742 to 0.744 per cent compared with 0.525 - 0.530 per cent in cow milk (Girgis et al. 1966).

2.1.4. PROTEIN DENATURATION

According to Sorensen (1973) the term denaturation indicates that the proteins are no longer in their natural condition. There is a change in the native protein molecular configuration excluding the primary structure. Thus the denaturation process is confined to alteration in the secondary and tartiary structure of the protein molecule (O'Sullivan, 1971). Often the term 'denatured' also refers to proteins that have undergone changes that precede actual coagulation (Harland and Ashworth, 1945).

Native protein molecules are known to be folded into well defined, more or less rigid, three dimensional structures. For most proteins, this structure

is compact and globular as exemplified by lysozyme, α-lactalbumin and β-lactoglobulin. In some proteins the native structure is rod-like, or is a rod with globular appendages as in the case of myosin. The caseins, however, are known to be essentially random coils in comparison with other secondary protein structures.

The native structure of a protein remains stable over a fairly wide range of external conditions, but its internal organisation into α-helical or β-structures and/or disulfide bonds can be permanently disruped by changes in physical or chemical environment. This process is irreversible denaturation. However, the denaturation process may be reversible also in some cases (e.g. enzyme reactivation) but usually it is irreversible if the heat treatment is prolonged. Heat is probably the most important of the various physical and chemical denaturating agents.

Jenness and Patton (1959) pointed out that the molecules of globular proteins seem to consist of polypeptide chains coiled and folded in a manner specific for each protein. The molecules are maintained in their specific configuration by salt linkages and hydrogen bonds. The present concept of denaturation is that it involves the breaking of these bonds, allowing to unfold the α-helix structure of proteins into a random coil formation. No reaction occurs in the primary covalent bonds (such as

peptide linkages during denaturation, but the unfolding of the molecule often exposes groups which may undergo chemical reaction such as oxidation of sulphydryl groups by atmospheric oxygen). Therefore, the internal protein configuration of α-helical or β-structures and/or disulfide bonds can be permanently disrupted by the changes in physical or chemical environment.

Denaturation may be brought about a number of physical agents such as heat, sound waves, surface forces, pressure, ultra violet irradiation and ionizing radiations. It may also be produced by treatment with organic solvents such as alcohol and acetone and by such solutes as urea, guanidine, and ionic detergents. Apart from these, exposure to very high or very low pH condition can also bring about denaturation. Proteins are generally stable at room temperature over a pH range of 4 to 8 and some are stable over a wider range (Jenness and Patton, 1959).

Jenness and Patton (1959) and Tumerman and Webb (1965) and Roy (1964) enumerated the following major changes in the properties of whey proteins and milk on heat denaturation.

i) Decreased solubility at iso electric point.

ii) Increased reactivity of sulphydryl groups giving cooked flavour, reduced oxidation reduction potential, and development of anti-oxygenic properties.

iii) Reduced protein digestibility.

iv) Resistance to milk clotting by rennin.

v) Reduced curd tension.

vi) Loss of ability to form cream layer.

vii) Increase in reflectance.

viii) Increased heat stability of milk following concentration.

ix) Reduced colloidal stability of milk when frozen.

In the dairy industry heat and acidity are the two most important denaturing agents employed.

2.1.4.1 Heat denaturation of whey proteins

The rate of denaturation of serum or whey proteins in milk has been studied by a number of investigators. Rowland (1934) investigated the heat denaturation of albumin and globulin in cow milk in the temperature range of 63 to 80°C with holding time varying from 2½ minutes to 60 minutes. With minimum heat treatment, about 10.4 per cent of the total soluble proteins were denatured. Later the same study was extended to temperatures ranging from 75°C to 120°C. The denaturation of albumin and globulin in such samples was rapid at temperatures of 75°C and above.

Rowland (1937) observed the rate of disappearance of serum proteins from heated milk at pH 4.75 to 4.80. A maximum of about 75 per cent of the total soluble protein nitrogen is coagulable by heat. Larson et al. (1952) observed a higher value from serum protein denaturation when

precipitated by salt as compared to acid in milk systems subjected to temperatures of 63°C to 90°C. Harland et al. (1952 a) reported on the basis of several observation that the complete denaturation of whey proteins takes place at 77.5°C for one hour, at 80°C for 30 minutes or at 90°C for 5 minutes using salt-precipitation method. Harland et al. (1952 a) also observed that pasteurization caused no measureable serum protein denaturation. In most cases the extent of heat denaturation has been assessed by determining the amount of undenatured protein which does not precipitate in acid or salt medium. The changes that take place in whey proteins in heat treatment of milk are very fascinating. Destabilization or denaturation takes place in these proteins when milk is subjected to temperatures higher than that needed for H.T.S.T. pasteurization. The degree of denaturation depends on the time temperature combinations. In order to determine the heat denaturation of individual serum proteins, Larson and Rolleri (1955) indicated that all the whey proteins were denatured at 96°C when time of heating was 30 minutes. It was shown that immunoglobulins were the most heat sensitive, being completely denatured at 70°C for 30 minutes, lactalbumin was the least affected and was not completely denatured below 96°C. Serum albumin and β-lactoglobulin hold intermediate position, whereas proteose-peptones remain unchanged. Evaluation of the denaturation of whey proteins has been the subject of criticism because of the lack of

proper methodology. Larson and Rolleri (1955) reported that the heat treatment of skim milk at 70°C for 30 minutes denatured only 6 per cent lactalbumin, 32 per cent of β-lactoglobulin, 52 per cent of serum albumin and 89 per cent immunoglobulin. On the basis of paper electrophoresis Tarantola and Castino (1959)observed that 4 and 56 per cent of whey proteins were denatured in heat treatments involving pasteurization of milk at 63°C for 30 minutes and at 85°C for 30 minutes respectively. They also observed that β-lactoglobulin was much more sensitive to heat treatment than α-lactalbumin. The denaturation of whey proteins are presumed to begin at temperatures above 60°C. The degree of denaturation varies with the time and temperature combination of heat treatment.

Harland et al. (1955 a) demonstrated that when the raw milks were heated at 74.4°C (165°F) for 30 minutes the denaturation of serum proteins varied between 39.8 - 43.1 and 39.2 - 40.6 per cent using Rowland and Harland-Ashworth methods respectively. According to these investigators the Harland-Ashworth method always gave a lower result than the Rowland method, presumably salt precipitates a protein fraction that acid does not. In fact the difference in results by the two methods averages the same, and highly correlated with the amount of heat stable 'Proteose' fraction is least circumstancial evidence for the identity of the proteose with the fraction precipitated by salt and not by acid. However, if these fractions

are identical, one would expect that no protein would remain in the salt filtrate of boiled milk, in other words, all the serum protein determined by the Harland-Ashworth method would be heat labile. In this study an average of about 13 per cent of the Harland-Ashworth serum protein seem not be heat labile.

Shillam and Roy (1962) reported that indirect UHT sterilization at 135°C (275°F) caused 90 per cent denaturation of β-lactoglobulin, but only between 55 and 70 per cent of the total whey proteins. The percentage denaturation of the total whey proteins obtained in this experiment varied from 61.8 to 73.4 per cent. More and Josephson (1968) observed that the addition of calcium and N-ethylmalamide reduced the aggregation of whey proteins. Stephen (1973) reported that the total nitrogen of buffalo milk was higher than that of cow milk. In both samples of milk, heat treatment at 95°C and 100°C for periods of 10 minutes did not cause any change in the total nitrogen content; non-protein nitrogen content of both the species did not differ significantly. Although buffalo milk contained slightly higher non protein nitrogen than cow milk, this constituent was not affected by heat treatments. Heat treatments caused considerable reduction in the non casein nitrogen of milk of both the species. Total albumin nitrogen was higher in buffalo milk. Heat treatment reduced this nitrogen constituent drastically. Cow milk contained more β-lactoglobulin nitrogen than

buffalo milk. On heat treatment this fraction underwent considerable decrease. Proteose-peptone nitrogen was lower in buffalo milk than in cow milk. It was reduced considerably as a result of heat treatments. Residual albumin and globulin also underwent reduction as a result of heat treatment. Goel (1974) reported that the extent of heat denaturation of milk proteins is different in buffalo milk than in cow milk because of compositional differences. He also observed that whey proteins in buffalo milk are more susceptible to heat denaturation than in cow milk.

2.1.4.2. Effect of whey protein denaturation on solubility index

According to Sorenson (1973) denatured whey proteins do not precipitate from the milk, nor do they precipitate when milk powder is reconstituted unless the heat treatment has been in excess of the normal heat treatment used in drying of milk to powder. However, when roller dried milk is reconstituted the whey proteins will normally precipitate completely or partly due to excessive denaturation during drying. Denaturated whey protein will redisperse into stable suspension when the powder is reconstituted, unless the heat treatment is excessively high. However, coagulated casein will not form a stable suspension when the powder is reconstituted, but will appear as a sediment.

By analysis of the sediment obtained when

solubility test are made, it has been confirmed that the insoluble matter mainly consists of coagulated casein. Therefore, in the whole process of dried milk manufacture, it is of the greatest importance to avoid destabilization of the casein. It can be diffifult to make low heat powder of good solubility because a certain heat treatment of the milk is required to stabilize casein. However, too high a degree of denaturation of the albumins and the globulins may lessen the stability of the casein, although other factors such as pH and salt balance are decisive.

Sorenson (1973) also observed that denaturation of the whey protein does not normally affect the solubility of the powder. If that were the case it would not be possible to make high heat powder of good solubility. It is, of course not true that low heat powder is synonymous with powder of good solubility. There is no relationship between whey protein nitrogen and solubility index.

2.1.5. PROTEIN INTERACTION DURING HEATING

When milk is subjected to heat, the interaction between proteins takes place, besides their individual changes. McGugan *et al.* (1954) reported the formation of a complex between K-casein and β-lactoglobulin when heated together to above 85°C for 30 minutes. Zittle *et al.* (1962) observed that heating a mixture of K-casein and β-lactoglobulin at 90°C for 15 minutes resulted in an interaction between the two. Hunziker and Tarassuk (1965) reported an

interaction between α-lactalbumin and β-lactoglobulin on the basis of chromatographic study.

Sawyer et al. (1963) discussed the role of sulphydryl groups in the interaction between K-casein and β-lactoglobulin. Purkayastha et al. (1967) provided reasonable conclusive evidence on the presence of a complex of β-lactoglobulin and K-casein mixtures, using the reagent N-ethyl-maleimide for protecting sulphydryl (SH) group. They concluded that the complex between β-lactoglobulin and K-casein is formed by thio-disulphide interchange and that the principal chemical linkages responsible for stability of the complex is disulphide bond.

Mckenzie et al. (1971) found that when denatured β-lactoglobulin and K-casein were mixed at room temperature, no interaction took place. When the mixture was heated at 75°C for 7.5 hours about 50 per cent of the K-casein had interacted. Sawyer (1968) reported that thermo denaturation of β-lactoglobulin proceeds in two major steps. In the first stage, formation of small aggregates of four monomers takes place and is referred to as the primary denatured (aggregated) form. This aggregation involves sulphydryl groups and occur at room temperature at above 70°C. Above this temperature, the rate of formation of primary denatured form increases sharply and reaches a maximum at 80°C to 85°C. In the second stage, conversion of small aggregates to large ones

takes place. This process is referred to as secondary aggregation. Secondary aggregation does not involve sulphydryl groups and occurs at temperatures lower than those needed for primary forms. Mackenzie (1971) prefers the terms type I and type II aggregation instead of primary and secondary aggregations. He also outlined a third type of aggregation (type III) which occurs via the monomers and does not require SH/SS interchange and/or interaction.

Elfagm and Wheelock (1978) reported interaction of bovine α-lactalbumin and β-lactoglobulin during heating. According to them amount of β-lactoglobulin and α-lactalbumin recovered from solution by gel filtration was reduced after heating at 80°C for 20 minutes. Amount of α-lactalbumin recovered was reduced markedly by the presence of β-lactoglobulin during heating, but the amount of β-lactoglobulin was not affected by the presence of α-lactalbumin. The effect of β-lactoglobulin on the loss of α-lactalbumin increased with pH (range 6.4 to 7.2) and temperature (77 to 85°C, with α-lactalbumin being affected only after the appearance of the aggregated, form(s) of β-lactoglobulin at 77°C. N-ethyl-maleimide prevented the effect of β-lactoglobulin on α-lactalbumin on heating. These workers also indicated that the variation in pH has no effect in the thermo denaturation of α-lactalbumin. However, when β-lactoglobulin is present, the loss of α-lactalbumin increases as the pH is increased.

Since the denaturation of β-lactoglobulin increases with pH, it supports the view that α-lactalbumin interacts with the aggregates form of β-lactoglobulin.

2.1.6. KINETICS OF HEAT DENATURATION OF B-LACTOGLOBULIN

El-Shazly *et al.* (1978) reported that buffalo and cow β-lactoglobulin in various solutions were heated at 60, 70, 80, 90 or 100°C for 5 to 30 minutes, then cooled rapidly and the preci itated protein at pH 4.6 was removed by centrifugation. The residual protein content was determined spectrophotometrically by folin reagent. It was observed that buffalo β-lactoglobulin was more affected when in phosphate buffer than in milk salt solution or casein solutions. The heat denaturation followed a second order reaction. Heat of activation and free energy for cow and buffalo β-lactoglobulin in the different solutions were comparable.

2.2. WHEY

Whey is the serum or watery portion of milk that separates from the curd in the process of making cheese. In the manufacture of cheese, *Paneer*, *Chhana* and casein, approximately 80 per cent of milk appears as whey.

Much of the fluid sweet whey and practically all the fluid acid whey is diverted to streams or pumped to municipal sewer systems and lost from any further utilization by man. This is, in real sense, a tragedy in a

world becoming increasingly short of food, since whey is a rich source of nutrients (Kosikowski, 1958).

The use of whey products in food manufacture affords a much more efficient means for utilization of whey from the stand point of human nutrition than does feeding to the animals, followed by consuming the animals as food. Webb and Whittier (1948) and Webb (1970) stated that there was a world wide increased awareness of the need for stopping environmental pollution, caused by the dumping of whey. This, rather than a realization of the nutritional value of whey, has accelerated efforts to utilise whey and its components in new and improved food, feed or industrial products.

2.2.1. COMPOSITION

In India, comparatively small amount of whey is available as a by product of cheese and casein manufacture, from the organised sectors. However, the major source is from Chhana and Paneer manufacture. At present, milk processing through organised sectors is gaining momentum, as a number of plants are coming up and existing ones are expanding or trying to run at full capacity. Since, major share of milk used for manufacturing milk products is contributed by the buffalo milk, studies on conservation of whey solids from buffalo milk, for human consumption should be considered highly advantageous.

Composition of cheddar cheese whey from

different sources reported by different workers is given below:

Table 2.3 Composition of Cheddar cheese whey

Constituent	Srinivasan and Anantakrishnan (1964)	Ray and De (1953)	Oborn (1968)	Webb (1972)	Anon (1968)	Keay (1971)	Lampert (1965)
Fat, %	0.3	0.3	0.3	0.07	0.3	0.6	0.2
Protein,%	0.9	0.9	0.9	0.9	0.9	0.8	0.3
Lactose,%	4.9	4.9	4.9	5.0	4.7	4.7	4.8
Ash, %	0.6	0.6	0.5	0.56	0.6	0.5	0.5

Superiority of whey as a source of food, feed, protein and energy is beyond any doubt (Webb and Whittier, 1948; Mitchell and Block, 1949; Block et al. 1953; Watt and Merrill, 1963 and Kosikowski, 1967). Whey has been used by the physicians for stomach ailments, chronic diseases of heart and kidneys, as well as skin diseases.

Although whey contains less protein than skim milk, its proteins are of comparable biological value contain all the essential amino acids (Webb and Whittier, 1948). Tomarelli and Bernhart (1962), in their experiments on rats, observed that the whey protein and mixture of whey and milk protein, were superior to milk protein alone.

Wingered et al. (1970) found the PER of whey-protein concentrates (WPC) as 3.1 in comparison to 2.5 of casein.

Whey is also a good source of energy. One hundred g of dried whey supplies approximately 260 calories of energy (Watt and Merrill, 1963). Lactose increases the utilization of calcium, magnesium and phosphorus in young animals (Outhouse, 1936; French and Cowgill, 1937 and Mill et al. 1940). Unless fed in excessive quantities, lactose is more effective in accelerating the growth in young animals than other common carbohydrates (Whittier et al. 1935). It has also been found to favour the production of riboflavin and pyridoxine in the rat intestine (Morgan et al., 1938). Lactose has a tendency to encourage a gastro-intestinal motility and also has the property to discourage fat deposition in the body. In the young, galactose derived from lactose may be utilized in the synthesis of cerebrocides, important structural units in the brain and in the medullary sheaths of nerves (Jennes and Patton, 1959).

2.3. SULPHYDRYL GROUPS IN MILK

Flavour is an important attribute in the judging of dairy products. Thermal processing viz. pasteurization, preheating, sterilization, condensing and drying are indispensable processes in the dairy industry. As a result of heat application, a distinct cooked flavour is developed and the nutritive value of the product may

decrease because some essential nutrients are partly destroyed, the amount of destruction depending upon time and temperature of heat treatment exposure.

Proteins are important constituents of milk and milk products and are extremely complex nitrogen containing organic compounds. They contain besides nitrogen other constituent elements as sulphur in the sulphur containing amino acids like cysteine, cystine and methionine. The most universal application of heat as a means of thermal processing and sometimes sterilisation of milk has resulted in detailed investigation of the effect of such processing on the constituents of milk. Heat treatment brings about denaturation of proteins and "exposure" of sulphydryl compounds which play different roles in different products. At the same time low molecular weight sulphur compounds are liberated from the protein structure which play a great role in flavour chemistry. Sulphur in the form of disulphides or sulphydryl groups has unique and important roles in the properties and reactions of milk proteins. The sources of the sulphydryl groups and volatile sulphides in milk have been shown to be the serum material particularly, the 'Albumin and the Proteinaceous material associated with the fat globule membrane.' Further, β-lactoglobulin has been shown to be the principal reducing fraction of milk proteins and the primary source of sulphydryl groups in milk.

In the thermal processing of milk changes that take place in milk proteins present a number of problems especially during the manufacture and storage of various products like concentrated and dried milk. Thermal processing produces distinct cooked flavour and increased oxidative stability in milk and its products and at the same time is accompanied by the "exposure" of the sulphydryl groups. The low molecular weight sulphydryls, sulphide and disulphide compounds in combination with hydrogen sulphide are believed to be responsible for the presence of cooked flavours in thermally processed milk products. It is further believed that these sulphydrul groups impart oxidative stability to the milk system by themselves getting oxidized to disulphide compounds. The oxidative stability of milk products has often been correlated with the free sulphydryl groups present in them.

2.3.1 EFFECT OF HEAT

Koka et al. (1968) observed that the activation of -Sh groups in heated skim milk followed the kinetics of a first order reaction. Any increase in time of heating at 90°C produced no more -Sh groups. Prolonged holding for 15 minutes above 125°C resulted in a marked decrease of -SH groups. Rotkiswicz and Kizza (1972) reported that total sulphydryl groups content (moles/16 g nitrogen) decreased progressively with increase in severity

of time/temperature conditions from 0.523 and 0.509 respectively in raw milk and milk heated at 65°C for 15 seconds, to 0.331 in milk heated at 95°C for 30 minutes. Contents of free -SH groups increased progressively from 0 in raw milk to 0.026 in milk heated at 75°C for 15 minutes and to 0.350 at temperature of 90°C for 30 minutes. Seitov and Seitkaliev (1972) reported that raw milk contained 0.156 ± 0.06 moles disulphide groups per litre.

Kiermeir and Hamed (1963) followed the method of reduction of thiamine disulphide for the determination of sulphydryl groups in heated milk and milk products. Lyster (1964) estimated free and masked sulphydryl groups in heated milk by a new method using p-chloromercuribenzoate and 5,5' dithiobis (2-nitrobenzoate). The total sulphydryl content (i-e- the sum of masked and free -SH groups) were determined after adding a denaturing agent such as urea. The total sulphydryl content in milk from individual cows varied from 0.112 - 0.221 mM. Narang et al.(1967) reported that free sulphydryl contents of pasteurised cow and buffalo milk estimated by NEM method were respectively 0.193 and 0.098 mM moles per litre.

2.4 CO-PRECIPITATES

2.4.1. HISTORICAL BACKGROUND

The combination of casein and whey protein precipitated together by acidification of heated milk

was first referred to as "Co-precipitate" by Scott (1952). While this term has generally been confined to the proteins from milk, recent work has broadened the name "Co-precipitate" to cover combinations of proteins from milk and other biological systems. Coagulation techniques have also been extended to include salt precipitation.

The effects of heat on milk have been studied extensively, especially with regard to the heat stability of both evaporated and 'normal' (unconcentrated) milk (Rose, 1963). In co-precipitate manufacture, interest lies particularly in the effect of heat on unconcentrated milk, and on mixtures of milk with whey. Unconcentrated milk at its natural pH of about 6.6 is generally very stable to heat (Newstead, Sanderson and Conaghan, 1977), but it has been found that, when the pH is changed or a soluble calcium salt is added to the milk, the milk proteins become less stable and coagulation occurs more rapidly on heating (Rose, 1961).

In attempts to understand the change occuring in milk as a result of heat treatment, various workers have examined the effects of heat on different milk components either in milk or in model systems. Studies have thus been undertaken into the effect of various heat treatment, pH and added calcium chloride on 'Solutions' of caseins. (Zittle, Della monica and Custer, 1956) and whey proteins (Zittle, Della monica, Rudd and Custer, 1957).

The effect of heat treatment of milk, especially in the region of 65°C - 100°C, on the formation of the complex between β-lactoglobulin and K-casein has been reviewed by Sawyer (1969).

Whereas casein is precipitated from milk by acidification to its iso-electric point (pH 4.6), whey proteins are soluble at this pH unless they have been subjected to a heat treatment sufficient to heat denature them. Rowland (1937) found that the maximum precipitation of whey proteins occurred on boiling casein free whey at a pH of 4.75 - 4.80. He also observed that, in the precipitation of whey proteins with casein from heated milk by acidification to pH 4.7, a maximum of 76 per cent of the total soluble protein nitrogen was coagulable. This consisted mainly of albumin and globulin. Rowland (1938) considered the remaining soluble nitrogen fraction to be mainly "proteose peptone."

The study of the effect of heat treatment on whey protein led Harland and Ashworth (1945) to conclude that their method, involving salt precipitation techniques, yielded lower whey protein and higher casein values in milk than Rowland's (1938) method. This was confirmed in studies by Larson, Jenness and Geddes (1952) who studied whey protein denaturation in the temperature range 63 - 90°C, and by Puhan (1974).

The relationship between heat treatment of

skim milk in the temperature range 62 - 80°C and the extent of whey protein denaturation was studied by Harland, Coulter and Jenness (1952). They found that the variation of temperature with time was semilogarithmic for a given degree of whey protein denaturation. Normal milk pasteurization (71°C/15 sec) did not denature and significant proportion of whey protein. At higher ($>$80°C) temperatures, Harland, Coulter, Townley and Jenness (1953) found a slightly different time-temperature relationship for whey protein denaturation.

There has been considerable interest for many years in the extent of whey protein denaturation in milk powder, particularly for use in bread. Larson, Jenness, Geddess and Coulter (1951) found that only milk powder prepared from milk which had been subjected to a high heat treatment (69 - 74°C/30 min) was suitable for use in bread.

Sawyer (1969) reviewed the properties of the complex formed between β-lactoglobulin and K-casein in heated milk. He discussed the role of the complex in determining the heat stability and other properties of the milk. This heat induced interaction between β-lactoglobulin and K-casein which has been observed to occur at temperatures above 65°C (Sawyer, 1969) is of significance in co-precipitate manufacture. Following formation of the complex by heat-treatment of the milk, the casein is precipitated by addition of acid or soluble calcium to the

hot milk, and the complexed β-lactoglobulin is co-precipitated with the casein.

Studies into the effect of heat treatment of milk for cottage cheese manufacture (Morris, Coulter, Combs and Heinzel, 1951; McMeekin, 1952) showed that while yield was increased due to co-precipitation of the denatured whey protein with the acid casein, the properties of the cottage cheese could be adversely affected. These were probably the first investigations into the potential use of co-precipitate in curd form.

Casein, precipitated by either acid or rennet and dried, has been known and used for many years, mainly as an industrial chemical for general purposes in adhesives, paint and paper, in plastics, and for more specialized edible uses in dietary preparations. Co-precipitates, however, only became of particular value in the 1960s as the interest in the edible use of casein products began to increase. As noted by Muller (1971) in his review article, the main reasons for commercial development of co-precipitates were the increase in yield of co-precipitate from skim milk, compared with casein, the potential increase in the range of functional properties of milk proteins in foods, and the increase in nutritional value of the co-precipitates compared with casein.

The initial developments in the production and uses of co-precipitate occurred in U.S.A. and U.S.S.R.,

but it remained for the Australian Commonwealth Scientific and Industrial Research Organization (C.S.I.R.O.) to fully exploit the commercial potential of co-precipitates. This work has been reviewed by Genin (1966), Fox (1968), Nielsen (1969), Webb and Whittier (1970), Muller (1970, 1971) and Mann (1971). In a review on the use of milk protein products in food, Borst (1971 a, 1971 b) discussed the potential future for co-precipitates.

2.4.2 MANUFACTURE OF CO-PRECIPITATES

The initial publications relating to work in the U.S.A. were centred on the manufacture of acid co-precipitates from heated milk. Scott (1952) added alkali to the skim milk to reduce its acidity and prevent initial precipitation of protein when heating the milk to 90°C. The curd he produced by acidification of the heated milk was separated from the whey, washed and dried. Howard, Block and Sevall (1954) were granted a U.S. patent for a food product containing an acid co-precipitate produced in a manner similar to that described by Scott. The washed, centrifuged curd was subsequently mixed with lactalbumin curd, skim milk and water before being dissolved at pH 7 in alkali and dried to produce a protein-rich food product. The advantages of water-soluble co-precipitate were also recognized by Scott (1958) who described the production of a soluble milk protein by dissolving his "casein-lactalbumin fusion" in potassium hydroxide to neutral pH, increasing the pH to 8.0 - 8.5 by

addition of ammonia before heating it to 77 - 85°C and drying the solution. During drying ammonia was evaporated and the pH of the powder was reduced to 6.6 - 7.3.

The soluble co-precipitate described above by Scott was combined with skim milk and an emulsifier by Loewenstein (1961 a) to form a powder containing 40 - 85 per cent protein which had an improved solubility and dispersibility. Loewenstein (1961 b) also produced a co-precipitate product with a protein content similar to that described in his earlier patent but with a higher water binding capacity. This was achieved by adding hydrocolloids such as alginates to the milk concentrate. The product was claimed to be particularly suitable for use in ice cream. The manufacture of a soluble co-precipitate neutralized with lime to pH 7.0 - 7.4 was described by Loewenstein (1965). The acid co-precipitate was prepared by the method of Scott (1952), and following neutralization and addition of a calcium salt, the solution was spray dried. The product was claimed to have low water binding capacity and therefore, to be particularly suitable for use in baked goods.

In the U.S.S.R., D'yachenko, Vlodavets & Bogomolova (1953) were the first to describe the preparation of a co-precipitate product. They treated hot milk with a solution of calcium chloride, and precipitated a milk protein product which contained 94.9 per cent of the milk

proteins, i.e. caseins and whey proteins, and had a high calcium (2.65%) and phosphorus (1.49%) content. D'yachenko (1957) briefly described a continuous method for the manufacture of this product in which the calcium chloride was added to milk. This was then heated to precipitate the protein. The curd was subsequently separated from the whey by a continuous horizontal solid-bowl centrifuge. D'yachenko referred to the use of this edible milk protein in bread, particularly, and also in such foods as spaghetti.

Australian work on co-precipitates was introduced by Buchanan, Snow and Hayes (1965). They produced a co-precipitate by addition of calcium chloride to heated milk, as described by Arbatskaya et al.(1962), but modified the procedure to reduce losses of curd particles during processing. Manufacture was carried out on commercial acid casein equipment, the curd receiving one wash before drying. It was found that the co-precipitate curd could be dispersed in water containing sodium tripolyphosphate and spray dried. The use and production of one-wash, acid and calcium-precipitated co-precipitates were later discussed by Muller, Snow, Hayes and Buchanan (1966). They noted that the heat treatment required to precipitate most of the whey proteins by acid was much greater (15 min at 92°C) compared with that required when calcium chloride was used as the precipitant (5 min at 92°C).

The concept of controlling the calcium content of co-precipitates to produce a series of products with different physical (functional) properties was introduced by Muller, Hayes and Snow (1967), and the terms high, medium,and low calcium were used to define co-precipitates containing 2.5 - 3.0 percent, 1.5 percent and 0.5 - 0.8 per cent calcium respectively. In this process, the authors described a procedure suitable for commercial application which has since been patented in several countries (C.S.I.R.O. 1968, 1969 a, 1969 b, 1970; Muller, Hayes, Buchanan and Snow, 1970).

Schaap and Torenvliet (1969) manufactured a high calcium co-precipitate curd for subsequent use in a cheese spread.

A range of co-precipitates with different calcium contents were made by Kozhev et al. (1970). They gave the terms acid-, low-, medium- and high- calcium for co-precipitates containing 0.8 - 1.0 per cent, 1-2 - 1.5 per cent, 2.0 - 2.5 per cent and 3.5 - 4.5 per cent calcium respectively.

In Japan, a patent was issued to Wakodo Company Limited (1971) for a process in which polyphosphates were added to heated milk. The milk was subsequently acidified to produce a co-precipitate. Other work was carried out in Ireland (Anon, 1971) and in India (Hindustan Lever Ltd., 1971). Poznanski et al. (1971) described the preparation of

a high calcium co-precipitate curd for use in a food preparation.

Pijanowski (1973) reported the manufacture of co-precipitate from the skim milk, which was concentrated to twice the original solids content. Recovery of milk nitrogen was 85 - 90 per cent including 60 per cent of whey protein nitrogen.

Aird (1971) reported the manufacture of co-precipitates from combinations of whey with skim milk or casein products. A co-precipitate for baked products was prepared (Netherlands, Bedrijven van het Nederlands Institute voor Zuivelonderzoek, 1973) from a mixture of 4 parts whey and 1 part buttermilk or skim milk. The pH of the mixture was adjusted to 6.4 - 7.1 and heated in the presence of calcium chloride to produce a co-precipitate curd. After washing, the curd was mixed with a polyphosphate and dried.

2.4.3 HEAT TREATMENT OF MILK

Scott (1952) and Buchanan *et al.* (1965) preheated the skim milk to 90°C. Arbatskaya *et al.* (1962) described a two stage heating system in which skim milk was first heated by regeneration of the hot whey. Calcium chloride was then injected and the mixture of milk and calcium chloride was then heated by steam to 95°C - 97°C.

Engel and Singleton (1968, 1969) discussed the preparation of a co-precipitate from milk, heated between 78 - 99°C for a defined period, concentrated and subjected

to an ultra-high temperature heat treatment before being mixed with acid at 100°C to precipitate the co-precipitate curd. In a process given by C.S.I.R.O. (1969) the milk was preheated by regeneration to 70°C and heated further by steam injection to 90°C.

Surazynski et al. (1973) added 0.02 - 0.03 per cent calcium chloride to milk, heated mixture to 95°C and precipitated proteins by binding the pH to 4.5 with dilute hydrochloric acid. Rostrosa et al. (1974) also heated the milk to 95°C in their experiments.

2.4.4. HOLDING OF HEATED MILK

Buchanan et al. (1965) in their laboratory trials showed that the percentage of protein precipitated from skim milk after the addition of 0.2 per cent calcium chloride increased with temperatures upto 90°C and with holding time upto 1 minute. Under these conditions, upto 94 per cent of the proteins were precipitated.

Muller et al. (1966) noted that heat treatment required to precipitate most of the whey proteins by acid was much longer (15 min. at 92°C) compared with that required when calcium chloride was used as a precipitant (5 min. at 92°C).

A continuous process was developed by which co-precipitate of milk proteins could be manufactured with controlled calcium levels (Muller et al. 1967). For medium calcium products (calcium about 1.5 per cent)

heating the milk at 90.5°C for 10 - 12 minutes after addition of 0.06 per cent calcium chloride and precipitation at pH 5.3 - 5.4 was the optimum, for calcium levels of about 0.5 - 0.8 per cent (a low calcium product), 15 - 20 min. heating at 90.5°C in the presence of 0.03 per cent calcium chloride and precipitation at pH 4.6 gave satisfactory recovery. For a high calcium product the milk required only a short holding time at 90.5°C before precipitation by the addition of 0.20% calcium chloride, C.S.I.R.O. (1969), gave a process for the manufacture of milk proteins of low calcium co-precipitate, in which milk was held at 85 - 95°C for 10 - 30 min. (preferably 15 - 20 min.).

Pijanowski (1973) did the flash heating of the concentrated skim milk to 90°C, added 0.02 per cent calcium chloride and maintained the temperature for 10 minutes. Costin et al. (1974) heated skim milk to 91°C for 10 - 12 minutes after the addition of 0.06 per cent calcium chloride.

2.4.5. PRECIPITATION OF CO-PRECIPITATES

D'yanchenko (1957) briefly described a continuous method for the manufacture of this product in which the calcium chloride was added to milk. This was then heated to precipitate the proteins. A further more detailed description of the continuous process also described the addition of calcium chloride. (D'yachenko, 1961).

Buchanan et al. (1965) used calcium chloride for the precipitation of high calcium co-precipitate curd. Upto 96.5 per cent of the proteins were precipitated at calcium chloride levels of 0.3 per cent.

Rostrosa and D'yanchenko (1968) concluded from various measurements that the whey proteins and the casein calcium phosphate complex coagulated jointly at 90 - 95°C at a calcium chloride concentration above 0.83 g/ litre. Rostrosa et al. observed that upto 50 per cent of the calcium in the co-precipitate was derived from added calcium chloride when milk of low acidity was used, whereas when milk of high acidity was employed, the proportion of added calcium in the co-precipitate was reduced to a maximum of 25 per cent.

Southward et al. (1973) reported that the level of calcium chloride required for precipitation of milk to be inversely related to the precipitation temperature. At 90°C a concentration of 0.2 per cent calcium chloride in the milk was required for complete precipitation, whereas at lower temperature it was found necessary to use upto 0.3 per cent calcium chloride. This observation confirmed earlier work on the production of a high calcium casein (Lee, 1970) in which 0.31 per cent calcium chloride was required in the milk to precipitate the casein at a temperature of 65°C. This high calcium casein contained approximately 3 per cent calcium and was similar

in chemical and physical properties to rennet casein.

Acid (calcium content, 0.8 - 1.0 per cent), low calcium (1.2 - 1.5 per cent), medium calcium (2.0 - 2.5 per cent) and high calcium (3.5 - 4.5 per cent) milk protein co-precipitates were obtained (Kozhev et al., 1970) by heat coagulation of skim milk in the presence of different proportions of calcium chloride and hydrochloric acid.

In general, a low calcium product is precipitated by addition of hydrochloric acid to pH 4.6, a medium calcium product at a pH of about 5.3 by addition of calcium chloride and hydrochloric acid and high calcium product by addition of 0.2 per cent calcium chloride, of skim milk, without the use of any acid (Webb and Whittier, 1970).

Pijanowski (1973) precipitated co-precipitate curd by addition of 3.3 - 3.5 ml of 20 per cent hydrochloric acid solution/litre of milk to lower the pH to 6.0 - 6.1.

Surazynski et al. (1973) precipitated proteins by bringing the pH to 4.5 with dilute hydrochloric acid (concentrated hydrochloric acid diluted 1:5 with water) or acid whey.

Chojnowski et al. (1974 b) precipitated proteins in a cheese vat at 40°C, by addition of hydrochloric acid (diluted 1:6), lactic acid or acid whey

to bring the pH 4.5 - 4.7. Costin et al. (1974) added 0.06 per cent calcium chloride to the milk and acidified it to pH 5.3 for precipitation of high calcium co-precipitate curd. Dumount et al. (1974) added 0.2 per cent calcium chloride.

Chojnowski et al. (1975) co-precipitated proteins from milk at pH 4.5 - 4.6 by addition of hydrochloric acid or lactic acid.

2.4.6. WASHING OF CO-PRECIPITATES

Fairly through washing is described to reduce the lactose content. To obtain efficient washing, at least three separate washes with water of increasing purity is required, each for periods averaging 15 - 20 minutes (Hynd, 1975). It was found in practice that when more than one wash was given, the pH of the water rose at a rate depending on the calcium content of the curd. At pH values over 4.6 in the wash water, the curd tended to soften and partially redisperse, increasing losses and creating difficulties in pressing and drying. It was, therefore, necessary to add sufficient acid to the wash water to maintain a pH in the range of about 4.0 - 4.6 at the pressing stage. Sulphuric acid is preferred for this purpose because of the low solubility of casein in this acid. The quantity of acid required varies with the calcium content of the curd, being highest for the high calcium product. Such acid washing reduces the calcium content of the product (Muller et al.,1967).

Buchanan et al. (1965) gave one wash to high calcium co-precipitate curd and Muller et al.(1966) gave one wash to acid and calcium co-precipitates. In a process given by C.S.I.R.O. (1969), the co-precipitate curd was washed with water at 25 - 55°C depending on curd characteristics.

Pijanowski (1973) washed the precipitate once or twice with pasteurized water acidified to pH 5.3 - 5.4. Costin et al. (1974) washed the precipitated curd three times with water at 50°C, 30°C and 20°C respectively.

Chojnowski et al. (1974 a) washed co-precipitate twice with pasteurised water at 20 and 60 per cent respectively of the initial milk quantity, the pH of the wash water being maintained at 4.8 with hydrochloric acid. Chojnowski et al.(1974 b) washed the raw coagulam (3 times) with water in the ratio of 2.5 litres of water/litre of milk.

2.4.7. REDISPERSION AND DRYING OF CO-PRECIPITATES

Howard et al. (1954) mixed acid co-precipitate, washed, centrifuged curd with lactalbumin curd, skim milk and water before being dissolved at pH 7.0 in alkali and dried.

Scott (1958) described the production of a soluble milk protein by dissolving "casein-lactalbumin fusion" in potassium hydroxide to neutral pH, increasing the pH to 8.0 to 8.5 by addition of ammonia before heating it to 77 - 85°C and drying the solution. During drying

ammonia was evaporated and the pH of the powder was reduced to 6.6 - 7.3. Lowenstein (1965) described the manufacture of a soluble co-precipitate neutralized with lime to pH 7.0 - 7.4. Following neutralization and addition of a calcium salt, the solution was spray dried.

Buchanan et al. (1965) found that the co-precipitate curd was dispersed in water containing sodium tripolyphosphate (STPP) and spray dried. For spray drying, the wet curd was redispersed in water containing 2.0 per cent STPP calculated on the dry weight of the curd, and passed through a colloid mill. At 25 per cent total solids, the viscosity of the mixture was sufficiently low for it to be transported by centrifugal pumps, provided that the pH was adjusted to about 6.2. High inlet air drying temperatures upto 232.2^{o}C were successfully used during spray drying.

Smith and Snow (1968) showed that 4 to 6 per cent sodium tripolyphosphate (STPP) was required for maximum solubility of medium and high calcium co-precipitates.

Kozhev et al. (1970) added suitable quantity of STPP to the crude co-precipitates and dissolved in sodium hydroxide solution; the mixture was heated to 60^{o}C, passed through a colloid mill and an emulsifier, pasteurised at 85^{o}C for 10 minutes and dried in a laboratory anhydro drier.

Surazynski et al. (1973) brought wet co-precipitates in solution by stepwise addition of sodium

hydroxide and in the final step, ammonium hydroxide, but not allowing the pH to rise above 7.0. Solubilization process lasted approximately 50 minutes at temperatures rising from 60°C - 93°C. The solution was then spray dried.

Chojnowski et al.(1974 b) dissolved raw product containing about 20 - 25 per cent dry matter at 70 - 75°C under constant stirring by gradual addition of 20 per cent sodium hydroxide solution so as to achieve complete solution without exceeding pH 6.4 - 6.6. At the final stage, about 500 ml concentrated ammonium hydroxide solution were added per 5000 litres milk used to lower the solution viscosity and the solution was spray dried at 160 - 170°C inlet air temperature.

Alekseeva et al. (1974) solubilized wet co-precipitates by addition of 3 - 6 per cent (calculated on dry matter basis) pentasodium tripolyphosphate and water to approximately 13 - 15 per cent dry matter content, mixed it at 40 - 50°C, passed through a colloidal mill and dried on a Niro atomizer drier. The pH of the mixture was adjusted to 6.5 - 7.0 and heated in the presence of calcium chloride to produce a co-precipitate curd. After washing, the curd was mixed with a polyphosphate and dried.

Chojnowski et al. (1975) added sodium hydroxide in the form of 20 per cent solution to coagulum until the pH 6.4 - 6.8 was achieved. Then intensive stirring was done at 65 - 75°C. The solution was dried in a Niro atomizer

spray drying plant, without concentration.

2.4.8. COMPOSITION OF CO-PRECIPITATE

The curd formed by the co-precipitation of casein and whey protein has physical characteristics different from those of a casein curd. If casein is precipitated at 43.5 - 49°C at a pH above 4.6, the curd will be fibruns and sticky. A co-precipitate curd even at higher temperatures and wide range of pH is seldon fibrous and sticky (Whittier and Webb).

The composition of co-precipitates is affected by the extent of washing of the curd as indicated by Buchanan et al. (1965), who described the effect of washing high calcium co-precipitates in acid wash water on the ash and lactose content of the product, and compared it with the composition of acid casein treated in a similar manner.

The calcium content of a co-precipitate is determined mainly by its pH of precipitation (Muller et al. 1967). The calcium content of a low calcium co-precipitate can be increased, however, by neutralization with lime, as described by Loewenstein (1965). Neutralization with alkali of low- and medium- calcium co-precipitates yielded products which were substantially water soluble (C.S.I.R.O., 1968). The solubility of medium calcium co-precipitate was found to be increased by addition of STPP, and this was used on its own to disperse (2 per cent w/w of co-precipitate) or to render soluble (6 per cent w/w high

calcium co-precipitate) (C.S.I.R.O., 1968).

Dunkerley and Hayes (1967 a) described a technique for estimating calcium in co-precipitates, based on the method of Sawyer and Hayes (1961).

Average composition of co-precipitates from cow milk, manufactured by various earlier workers is presented in Table 2.4. It is apparent that maximum protein content was obtained by Pavlov et al. (1975).

2.4.9. PROPERTIES OF CO-PRECIPITATES

2.4.9.1. Colour: Smith and Snow (1968) observed that solution of co-precipitates were whitened to approximately the same capacity as skim milk by the addition of calcium chloride.

Hayes et al. (1969) reported that pH, calcium and polyphosphate content of co-precipitate solutions affected the whiteness of those solutions.

2.4.9.2. Flavour: Ramshaw and Dunstone (1969) found that off-flavour development was associated with non-enzymatic browning in the protein especially with a high (upto 0.85) lactose content.

Ramshaw and Dustone (1970) found that the development of "gluey" off flavours in low calcium co-precipitate was inhibited by the addition of 0.01 - 0.05 per cent sodium metabisulphate.

Table 2.4 Average composition of co-precipitates

Constituent	Buchanan et al. (1965) Granular form	Buchanan et al. (1965) Spray dried form	CSIRO (1969)	Chojnowski et al. (1974a)	Chojnowski et al. (1974b)	Costin et al. (1974)	Alekseeva et al. (1974)	Pavlov et al. (1975) (Granular form)
Protein %	82.0	83.0	83.5	79.4	84.0	79.0	80.1	85.1
Ash %	8.5	10.5	-	5.25	5.0	9.5	13.5	-
Calcium %	2.5	2.5	0.3	0.32	0.5	2.5	2.45	2.64
Lactose %	1.0	1.0	1.0	9.7	6.0	1.5	-	-
Fat %	1.5	1.5	1.5	0.47	-	1.5	2.0	1.13
Moisture%	7.0	4.0	-	4.25	5.0	8.5	8.0	10.15

Southward et al. (1978) studies the flavour characteristics of solutions of co-precipitates and found to have typically cooked milk or 'boiled egg' overtones, resulting from the relatively high heat-treatment given to the milk before precipitation of the co-precipitate curd. Granular high calcium co-precipitate, which had been subjected to two washes during manufacture, typically scored 5 - 6 on a scoring scale of 0 - 8 (8 = excellent, 0 = very strong off-flavour) when tasted after reconstitution in solution two weeks after manufacture. This co-precipitate tended not to develop the traditional 'musty' storage off-flavours associated with acid casein. These musty off-flavours were also observed to occur in acid co-precipitate. With the exception of the 'cooked' flavour the general flavour characteristics of high calcium co-precipitate were similar to those of rennet casein which has been shown (Walker, 1973) to be superior in flavour to acid casein. However, the intensity of the 'cooked' flavour was found to be enhanced in the finer (250 Um) fractions. The degree of washing also affected the intensity of the cooked flavour; more washes produced a co-precipitate with a less intense 'cooked ' off-flavour. The flavour of soluble co-precipitates prepared by dissolving fresh curd in alkali and/or phosphate, followed by spray drying of the solution, was generally superior to that of the respective granular co-precipitates.

2.4.9.3. Solubility: Buchanan et al. (1965) noted that

high calcium co-precipitate, unlike casein, would not dissolve at pH 6 - 7 without the addition of a calcium sequestering agent. Sodium tripolyphosphate (STPP) was effective in dissolving high calcium co-precipitate at levels of 4 - 6 per cent of the weight of co-precipitate, depending on its calcium content.

Smith and Snow (1968) reported that the solubility of low calcium co-precipitate in alkali over a range of pH values was similar to that ofcasein, but 4 per cent and 6 per cent STPP was required for maximum solubility of medium and high calcium co-precipitate.

Hayes et al. (1969) observed that the solubility of high calcium co-precipitate in water could be increased by raising the pH to 8 - 10 with alkali and adding STPP. The pH of the solution could then be lowered to about 7.0 without affecting the solubility. A similar technique was found to increase the solubility of medium calcium co-precipitate.

The solubility of co-precipitates has generally been considered in the pH range from 6 - 10. Hayes et al. (1970) demonstrated that co-precipitates can also be dissolved in several different acids such as phosphoric, hydrochloric and citric acid at pH 2 - 3.

2.4.9.4. Viscosity: Hayes et al. (1969) reported that low calcium co-precipitate had a viscosity similar to that of acid casein. The viscosity of medium and high calcium

co-precipitate solutions was found to be relatively high when the pH was about 7.0.

Towler and Aird (1970) observed that viscosity of solutions of low calcium co-precipitate was affected by the heat treatment of the milk from which it was made.

Southward et al. (1978) reported that the viscosity of high calcium co-precipitate was highest and that of acid (low) co-precipitate lowest, while medium calcium co-precipitate exhibited an intermediate viscosity.

2.4.9.5. Bulk density: The packing density (bulk density) of casein products such as co-precipitates can vary markedly depending on the method of manufacture. Granular, insoluble co-precipitates have a density of approximately 0.6 g/ml (Southward and Goldman, 1975), depending on particle size, whereas the density of spray dried high calcium co-precipitates can be much lower. The density of a spray dried high calcium co-precipitate containing 2 per cent STPP was reported 0.34 g/ml compared with 0.25 g/ml for sodium caseinate (Buchanan et al. 1965).

2.4.9.6 Emulsifying activity: Emulsion stabilizing capacity of soluble co-precipitates were reported by Southward et al. (1978). The soluble high and medium calcium co-precipitates both exhibited good emulsion stabilizing properties within the limits of the test. Soluble acid co-precipitate had the lowest emulsion stabilizing capacity of the five soluble co-precipitates examined but still

compared flavourably with commercial sodium caseinate.

2.4.9.7. Nutritional properties: While casein is recognised as a high quality protein, the whey proteins from milk are nutritionally superior. Demott (1972) and Muller (1971) suggested that one of the factors influencing the production of co-precipitates could be their superior nutritional properties when compared with casein.

Dumant and Julen (1973) prepared a high calcium co-precipitate which had a protein efficiency ratio of atleast 3.3.

Amino acid analysis of co-precipitates were determined by Muller et al. (1966) and Resimini et al. (1971).

Resmini et al. (1971) claimed the biggest change in amino acid analysis pattern from casein to co-precipitate was in the percentages of cysteine/cystine, alanine, proline and aspartic acid.

The nutritional quality of co-precipitates as measured by protein Efficiency Ratio was higher than that of casein (PER 2.5) and varied from a PER of 2.91 for high calcium to 2.72 for acid co-precipitate was 2.78 (Lohrey & Humphries, 1976). However, there was very little difference in amino acid composition between casein and the co-precipitates with the exception that the cystine content of the co-precipitate was higher (56 mg/g nitrogen) than that of casein (26 mg/g nitrogen).

2.4.10. APPLICATIONS OF CO-PRECIPITATES IN FOOD PRODUCTS

The use of co-precipitate as a meat replacer has been discussed (Southward and Goldman, 1975) and subsequent work has confirmed the value of co-precipitate as a meat extender and substitute. Thomas, Turner and Hyde (1976), for example, described the preparation of canned meat loaf products based on insoluble acid, dispersed and soluble high calcium and granulated medium calcium co-precipitates. Insoluble acid co-precipitate was the most suitable emulsifier. When used in combination with granulated co-precipitate, it gave a simulated meat product which after retorting, exhibited characteristics resembling those of meat in chewiness, 'sliceability', appearance and flavour. Czajka, Kardasz Wasilewska and Zalewski (1975) reported the successful use of soluble acid co-precipitate in meat cutlets when 30 per cent of the meat was substituted with co-precipitate. Insoluble co-precipitate was selected by Goldman (1974) for the preparation of meat substitute. Gwiaza *et al.* (1975) used insoluble calcium co-precipitate curd in frankfurters, sausages and pate sausages at levels of 2 - 10 per cent based on dry weight. Under these conditions, incorporation of the co-precipitate resulted in maintenance of quality and appreciable savings in costs.

Soluble co-precipitates could have wide applications when used as the non-fat milk solids ingredient

in dessert and confectionery foods. Their superior emulsifying properties, solubility and in the case of high and medium calcium co-precipitates, ability to form viscous solutions or gels, make co-precipitates useful functional additives to such good products.

The preparation of whipped toppings using soluble co-precipitates was described above. A similar formulation with reduced fat could be used to prepare ice cream and frozen desserts, such as cheese cakes and mousses. Soluble co-precipitates can also be employed as protein ingredients for coffee whiteners and dried coffee milk products where they can be used as emulsifying agents and for imparting 'body' and some whitening power to the product.

The ability of soluble high calcium co-precipitate to form gels can be utilized in the preparation of a variety of sauces, custards, pie-fillings, and gelled frozen desserts. A combination of 10 per cent soluble high calcium co-precipitate in liquid whole milk with the addition of 10 per cent sugar gave a gel of custard like consistency which was stable to sterilizing and freezing temperatures. Such products would be particularly suitable for dietetic purposes where an increased level of protein is desirable.

Some replacement of skim milk solids in yoghurt manufacture can be achieved with the addition of soluble co-precipitates although when replacement exceeds

30 per cent there is an apparent coarsening of the texture of the yoghurt (Humphries, personal communication).

Soluble and dispersed high calcium co-precipitates can also be used to replace the skim milk solids in sterilized, flavoured, low-fat beverages. In the case of chocolate beverages of this type, only partial replacement of the skim milk solids is possible since it is desirable to maintain a degree of whiteness in the product.

The emulsion stabilizing properties of soluble co-precipitates can also be utilized in the preparation of mayonnaise type sauces. A stable product (pH 4.6) was prepared from an emulsion of high or medium calcium co-precipitate (6 per cent), vegetable oil (33 per cent), water (45 per cent), vinegar (7 per cent), sugar (9 per cent), and seasonings. Soluble acid co-precipitate gave a less stable emulsion. Similar formulations can be used to prepare savoury sauces and dips.

The stability of high calcium co-precipitate gels to sterilizing temperatures permits their use in the preparation of canned creamed soups and savoury sauce products.

In bread, co-precipitates can either be used to replace milk solids in a milk loaf formulation or at higher levels to produce a high protein bread. Regulations for the required amount of milk solids in milk bread vary

from country to country with a maximum limit of milk solids of 6 per cent of flour weight. Co-precipitates may be used alone to replace the milk solids in flour at 3 per cent of flour weight provided lactose is not an essential ingredient for the description of the loaf as 'milk bread.' Alternatively, co-precipitates may be used to form the high protein ingredient in skim milk replacer products. Skim milk replacers have become accepted alternatives for skim milk solids in baked goods as a result of the increase in price for skim milk powder in several countries.

In experiments using skim milk replacers in cake mixes at New Zealand Dairy Research Institute as described by Goldman (1976), it has been found that dispersed high calcium co-precipitate is suitable for this purpose.

A high protein biscuit containing 17 per cent insoluble acid co-precipitate was described by Humphries and Goldman (1975). The biscuit had a total protein content of 22 per cent and a PER of 2.8. It is also possible from a similar high protein biscuit dough. The baked biscuits can be sandwiched together with a cream filling containing dispersible or soluble co-precipitate, thus giving a supplementary or total meal product of high nutritional value.

The application of co-precipitates in food products will be successful only if the protein used has

satisfactory functional (physical) properties in the food system in which it is incorporated so that texture, etc. are not adversely affected; has no undesirable organoleptic properties which may affect the flavour or odour of the consumer product; and is nutritionally equivalent, or superior, to the original protein.

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CHAPTER 3.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

This chapter deals with the methods and materials used for standardising the process for the manufacture of low, medium and high calcium co-precipitates and the methods used for the analyses of co-precipitates and its raw materials. Only analytical and food grade reagents were used.

The following raw materials were used for the manufacture of co-precipitates:

(a) Fresh buffalo skim milk.

(b) Buffalo skim milk from which calcium has been removed partially.

(c) Buffalo skim milk mixed with four parts buffalo cheddar cheese whey.

Buffalo skim milk used for the manufacture of co-precipitates was obtained from the Experimental Dairy, N.D.R.I., Karnal.

Buffalo milk cheddar cheese whey was obtained during the manufacture of cheddar cheese, at the Experimental Dairy, National Dairy Research Institute, Karnal. The whey was drained from the cheese vat and passed through muslin cloth to remove any suspended curd particles. This strained

whey was used in part (c) above.

3.1. STANDARDISATION OF METHOD OF MANUFACTURE FOR LOW, MEDIUM AND HIGH CALCIUM CO-PRECIPITATES

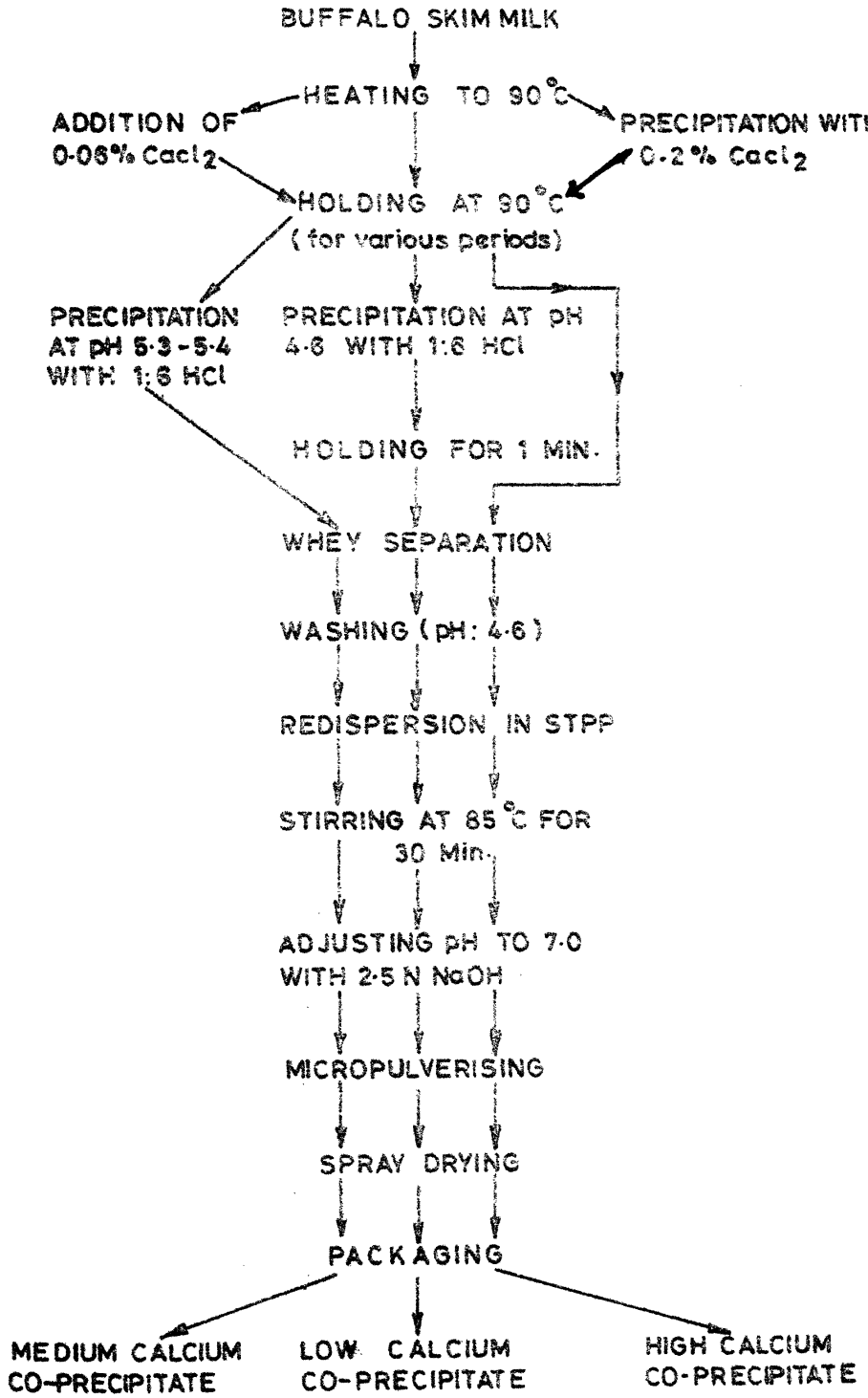
Low, medium and high calcium co-precipitates were manufactured based on the procedure as outlined by Muller et al. (1967) for cow milk (Fig 1)

In order to standardise the method of manufacture for low, medium and high calcium co-precipitates, trials were conducted using various parameters such as different periods of holding, pH of precipitation, number of washings so as to arrive at the most acceptable product as given below in the manufacture of different types of co-precipitate manufacture. The standardised product after spray drying was utilised in the manufacture of ice cream in different proportions, which was later judged by a panel of six selected judged from Dairy Technology Division, N.D.R.I., Karnal. High calcium co-precipitate was selected at levels of 10, 30, 50, 70 and 100 per cent levels, as a replacement for SMP, for making ice cream using Giusti, London, ice cream freezer.

3.1.1. LOW CALCIUM CO-PRECIPITATE

For low calcium co-precipitate, the heated buffalo skim milk was held with constant stirring at 90°C for 15, 20 or 25 minutes. These holding times were selected because of the findings of Muller et al. (1967), that for low calcium co-precipitates 15 - 20 minutes

FIG. 1. FLOW DIAGRAM FOR THE MANUFACTURE OF LOW, MEDIUM AND HIGH CALCIUM CO-PRECIPITATES



heating at 90°C in the presence of 0.03 per cent calcium chloride and precipitation at pH 4.6 gave good recovery and satisfactory processing conditions. Calcium chloride was not added in the experiments because buffalo skim milk already has more calcium than cow skim milk. Following the holding stage, 18.0 to 19.0 ml of 1:6 hydrochloric acid/litres of milk were added slowly with constant stirring to lower the pH to 4.6. It was again held for 1 min. before draining the whey.

3.1.2. MEDIUM CALCIUM CO-PRECIPITATE

To obtain medium calcium co-precipitate curd, 0.06 per cent calcium chloride (as 5 per cent w/v aqueous solution) was added slowly while constantly stirring the heated milk, and held for 10, 12 or 15 min. These holding times were selected on the basis of findings of Muller et al. (1967), that heating the milk at 90°C for 10 - 12 min. after addition of 0.06 per cent calcium chloride and precipitation at pH 5.3 - 5.4 was optimum for medium calcium product. Following the holding stage 8.0 to 10.0 ml of dilute hydrochloric acid (1:6)/litres of milk was added slowly with uniform mixing, to lower the pH to 5.3 - 5.4.

3.1.3. HIGH CALCIUM CO-PRECIPITATE

For high calcium co-precipitate, 0.2 per cent calcium chloride (as a 5 per cent w/v aqueous solution) was added to the heated milk, slowly with

continuous stirring. Following thorough mixing, holding of heated milk for 1,2 or 5 min. was done as per investigations of Buchanan et al. (1965).

3.1.4. REMOVAL OF WHEY

After the precipitation of the co-precipitate curd, it was allowed to settle and the whey removed after filtering through the stainless steel strainer covered with muslin cloth. Filtration was done to avoid loss of fine particles

3.1.5. WASHING OF CO-PRECIPITATES

The co-precipitate curd was given 2, 3 or 4 washings with the acidulated water (pH 4.6) at 30 - 35°C with 15 min. holding time for each washing. One litre of wash water/litre of processed milk was used for each washing and the pH of the wash water was maintained at pH 4.6 by addition of 1:20 sulphuric acid. Sulphuric acid in wash water was preferred because of the less solubility of casein in this acid.

3.1.6. DISPERSION OF CO-PRECIPITATES

For spray drying, the wet curd of low, medium and high calcium co-precipitates were dispersed in water containing 2, 4 and 6 per cent STPP respectively, calculated on the dry weight basis of the curd. The mixture was heated in a water bath to about 85°C and continuous stirring was done for about 30 min. This heat treatment gave a pasteurization treatment to the co-precipitates.

Gradually 2.5 N sodium hydroxide solution were added to the curd with constant stirring in such a way that the pH did not rise to more than 7.0. The mixture was then passed through the mini-pulveriser in place of colloid mill as recommended by Smith et al.(1968). The material was then filtered through muslin cloth. The pH of the dispersion was adjusted to 7.0 at this stage.

3.1.7. SPRAY DRYING OF CO-PRECIPITATES

The material obtained was spray dried in an anhydro spray dryer (DENMARK) with an inlet air temperature of 195 ± 5°C and outlet air temperature of 105 ± 2°C. The revolutions of the atomiser was controlled at 25,000 ± 1,000 rpm.

3.1.8. PACKAGING OF CO-PRECIPITATES

The spray dried co-precipitate was collected in 300 gauze polythene bags which were heat sealed later.

3.2 ELECTRODIALYSIS OF BUFFALO MILK

Electrodialysis is an electrochemical process for modifying the mineral composition of liquids through the use of direct current electricity and in selective membranes. The latter are sheets of ion exchange resin mechanically reinforced with synthetic fabrics. Electrodialysis is not an ion exchange process but depends upon the ability of ion-selective membrane to conduct electricity by the movement of either anions or cations depending upon the type of membrane used.

Demineralisation was carried out in a pilot scale electrodialyser equipment (Ionics, Inc., Watertown, Mass., U.S.A.).

For every experiment, effective surface area was increased from 0.3 to 1.5 sq.m. at regular increments of 0.3 sq.m. All the ED experiments were carried out under ambient temperature (about 30°C). The membrane stack contained 18 cell pairs of 22 cm x 25 cm size membranes. For every set of experiment product tank was charged with feed and pumped at the rate of 90 lit./hr. through the membrane stack. The product stream, brine stream and electrode stream were turned on simultaneously. The flow rate in all these was increased slowly to predetermined values so as to avoid the excessive pressure differential and accidental intermixing of product stream and concentrating streams. Electrical potential was maintained at 3 V/cell pair during this set of experiment.

Using an ion modification unit, patented by Ionic Incorporated Co., Inc., (1966), calcium content in buffalo milk was reduced by 8.0 per cent, at normal pH. Low calcium co-precipitate was prepared from this milk as per the method standardized for buffalo skim milk, a flow diagram of which has already been given.

3.3. LOW CALCIUM CO-PRECIPITATE FROM A ADMIXTURE OF BUFFALO SKIM MILK + FOUR PARTS OF BUFFALO CHEDDAR CHEESE WHEY

Low calcium co-precipitate was prepared

utilising an admixture of skim milk with 4 parts of whey, as per the standardised conditions for low calcium co-precipitate from buffalo skim milk.

Wakodo Company Limited (1969) was issued a patent for the preparation of co-precipitates from mixtures of skim milk and whey.

3.4. ANALYTICAL METHODS

3.4.1. RAW SKIM MILK

The analytical procedures adopted for examination of raw buffalo skim milk were as detailed below:

3.4.1.1. Sampling: Sample of buffalo skim milk was prepared as per AOAC method (1975).

3.4.1.2. Fat: As per IS 1224 (Part-I) - 1977.

3.4.1.3. Total solids: Mojonnier method (Laboratory Manual, 1959) was adopted for T.S. determination.

3.4.1.4. Titratable acidity: As per IS 1479 (Part-I)-1960.

3.4.1.5. Alcohol test: Alcohol test was carried out as per IS 1479 (Part-I) - 1960.

3.4.1.6. Ash: Total ash was estimated as per IS 1479 (Part-II) - 1961.

3.4.1.7. Calcium: Calcium was estimated as per the method standardized by Ntailianas and Whitney (1964), using calcein as an indicator as below:

Reagent:

(i) Standard EDTA solution: Ten gram of disodium

dihydrogen ethylenediamine tetra acetate dihydrate (EDTA) (AR) and 2 g of sodium hydroxide pellets were dissolved in water and diluted to 1 litre with water. This solution had approximately 0.05 meq of EDTA/ml and was standardized against the standard calcium solution.

(ii) Standard calcium chloride solution: Calcium carbonate (AR) was dried overnight at 100°C and about 2.5 g (exactly weighed) of it was dissolved in a minimum of hydrochloric acid, transferred to a litre volumetric flask and made up to the volume with water. This solution contained approximately 0.05 meq. Ca/ml.

$$\frac{g\ CaCO_3 \times 2}{100.09} \quad \text{meq. Ca/ml.}$$

(iii) Potassium hydroxide (AR): 8 N solution.

(iv) Calcein indicator solution: 0.02 g of bis N, N. di (carboxymethyl amino methyl fluorescein (obtained from Sigma Chemical Company, U.S.A.) in 25 ml of 0.1 N sodium hydroxide (AR) solution was dissolved and diluted to 100 ml with water. The solution was stored in dark.

Procedure

Transferred 25 ml of milk into 500 ml volumetric flask and diluted the volume with water. Added 5 ml of standard EDTA solution to 50 ml aliquot of the diluted milk in 125 ml Erlenmeyer flask.

The pH of the above solution was raised to 13.0 by the addition of 1.5 to 2.0 ml of 8N potassium hydroxide solution. Three drops of indicator solution were added to it. The colour of the sample became pink. It was back titrated with standard calcium chloride solution (from a microburette of 5 ml capacity, calibrated in 0.01 divisions) to a definite and permanent green colour.

To determine the end point accurately the Erlenmeyer flask, containing the sample was rested on a black background.

3.4.1.8. Protein

(i) Total protein: Total protein was estimated as per the method of McKenzie (1970) using 0.05 to 0.1 ml of milk as follows:

Powdered potassium sulfate (1.5 g) was added to the bottom of a dry 30 ml Kjeldahl flask through a long funnel. Sulphuric acid (1.5 ml), mercuric sulphate solutions (0.5 ml), and a sample containing, preferably, 0.2 - 1.0 mg nitrogen (although 0.1 - 2.0 mg may be used) were transferred to the flask. The walls of the flask were washed down with a little ammonia-free water. The flask was transferred to the digestion rack and heated, observing the time when fuming ceased and good refluxing took place. The clearing time was approximately 5 minutes. The total digestion time (from end of fuming and good reflux) was 20 minutes.

At the end of the digestion the flasks were removed from the rack, allowed to cool for a few minutes, and each digest diluted with a few millilitres of water. The digest material was transferred quantitatively to the micro Kjeldahl distillation apparatus with several washings of ammonia-free water so that the total volume did not exceed 25 ml. The 10 ml. of the sodium hydroxide-thiosulphate solution was added and the mixture steam distilled (with distilled water containing a trace of acid and indicator in the boiler) in the following manner: The tip of the condenser was immersed in 5 ml of the boric acid indicator solution contained in a 50 ml of Erlenmeyer flask that had been marked to indicate levels of 15, 20 and 35 ml. Distillation was continued until 10 ml had come over, and the boric acid solution was lowered from the tip until a further 5 ml had distilled. The condenser was rinsed with a few milliliters of ammonia-free water and distillation stopped. The content of the flask was titrated with biiodate solution to the graylilac end point, making the final volume approximately 35 ml (adding water if necessary). (Blank determinations were carried out through the whole procedure). The contents of the distillation apparatus were removed by suction after each determination, and the apparatus was rinsed several times with distilled water. The apparatus was steamed out each day and cleaned occassionally with chromic acid.

1 ml of 0.01 MKH (I 03) 2 ≡ 0.1401 mg N

After determining the total percentage nitrogen content, according to the above procedure, the total percentage nitrogen content was multiplied by the factor 6.38 which gave the total protein content of the product.

3.4.1.9. Non protein nitrogen

Into a 50 ml graduated flask, pipetted and weighed 10 ml of milk, diluted to the mark with 15 per cent trichloriacetic acid solution and mixed immediately. When the precipitate had settled, leaving clear supernatant liquid, filtered it using Whatman No.40 filter paper into a dry flask. Pipetted 2.0 ml of the filtrate into a 30 ml Kjeldahl flask and estimated the nitrogen as per the method used for the total nitrogen (McKenzie, 1970).

3.4.2. BUFFALO CHEDDAR CHEESE WHEY

Composite samples of whey were analysed for total solids, total nitrogen, non-protein nitrogen, fat, lactose and ash contents.

3.4.2.1. Total solids (TS)

The TS content of whey was obtained by taking approximately 10 ml of accurately weighed whey and drying over a water bath and later at $100^{\circ} \pm 1^{\circ}C$ for 3 hours to a constant weight (IS 1479 - 1961).

3.4.2.2. Total nitrogen (TN)

TN in cheese whey was determined as per the method of McKenzie (1970) using 0.05 to 0.1 ml of whey

weighed in a 30 ml micro-kjeldahl flask and digested and distilled as recommended. A conversion factor of 6.38 was used to obtain crude protein content.

3.4.2.3. Non-protein nitrogen (NPN)

NPN was determined by the method Rowland (1938).

3.4.2.4. Fat

The fat in cheese whey was determined gravimetrically as per ADMI (1965), wherein approximately 10 ml of whey was weighed and used.

3.4.2.5. Lactose

Lactose in cheese whey was determined by Lane-Eynon method of ISI (IS 1479, Part-II - 1961).

3.4.2.6. Ash

Ash content in cheese whey was estimated according to (IS 1547 - 1968). Ten ml of whey accurately weighed was dried in a silica crucible over a water bath then gently on a flame and finally ashed in a muffle-furnance, at 550 $\pm$ 20°C to a constant weight. Ash content was then calculated as under:

$$\text{Total ash (percent by weight)} = \frac{100\ (W_2 - W)}{W_1 - W}$$

where,

W_2 = Weight in g of the dish with ash

W = Weight in g of the empty dish

W_1 = Weight in g of the dish with the sample.

3.4.3. REDISPERSED CO-PRECIPITATES

3.4.3.1. Total solids

Total solids were determined by using the Mojonnier method (Laboratory Manual, 1959).

3.4.3.2. pH

Redispersed co-precipitate solution was taken in a 100 ml beaker and pH was recorded at room temperature (20 ± 2°C) on a digital pH meter type DPH 500.

3.4.4. SPRAY DRIED CO-PRECIPITATES

3.4.4.1. Total solids/moisture of the sample

3.4.4.2. Protein

Total proteins in co-precipitate powder were estimated as per McKenzie (1970) using 5 to 7 mg of sample.

3.4.4.3. Fat

Fat in dried co-precipitate was determined by the Rose Gottlieb method (Mojonnier modification) as described in Laboratory Manual (1959).

3.4.4.4. Total ash

Total ash was determined as per B.S. 1417: 1955.

3.4.4.5. Calcium

Calcium in co-precipitates was determined by the method of Dunkerley and Hayes (1967).

Reagents:

(i) EDTA solution 0.05 M: Dissolved 18.613 g

of disodium EDTA (AR) in 1 litre glass distilled water (1.0 ml equivalent to 2.0 mg of calcium).

(ii) Standard calcium solution: 0.05 M - prepared by dispersing 5.005 g calcium carbonate (AR) as a slurry in distilled water and neutralising with hydrochloric acid (AR) (Approx. 20 ml of conc. Hcl was added slowly until all the carbonate was dissolved) and made upto 1 litre with glass distilled water.

(iii) Sodium hydroxide (AR): 0.6 N.

(iv) Hydroxy naphthal blue (HNB): powder form obtained from Sigma Chemical Company, U.S.A.

Procedure:

Weighed 2 g of dry co-precipitate accurately into each of two suitable beakers. For the range 5 to 10 mg/g of calcium, 5 ml of EDTA solution were added to one beaker and 10 ml of the other and the co-precipitate was allowed to soak for 30 min. with occasional stirring. Then 25 ml of the 0.6 N sodium hydroxide solution were added, with stirring for about 20 min. to dissolve the sample as completely as possible. Added one or more charges from the indicator dispenser to give a distinctive colour.

For the higher range of calcium in medium and high calcium co-precipitates, the quantity of EDTA solution added was appropriately increased. For example, 10, 15 and 20 ml were added for the medium calcium range, and 20, 25 and 30 ml were added for high calcium range.

For the approximate quantitative determination, a back titration technique was adopted using a graduated 5 ml burette. The back titration was conducted on the beaker with the minimum excess of EDTA, and the colour changed from blue to red.

Calculation:

$$Ca^{++} \text{ mg per g} = \text{ml EDTA} \times 2/4 \times \frac{90}{100 - \text{Moisture}}$$

The result from this formula was expressed on the basis of product containing 10 per cent moisture.

3.4.4.6. Phosphorus

The phosphorus content in the product was determined as per the method of Fiske and Subaraw (1925).

3.4.4.7. Lactose

Lactose was determined on the protein free filtrate of co-precipitates by a copper reduction method, and followed the method of Folin and Wu. (1926).

One g of co-precipitate was taken in a 100 ml volumetric flask followed by addition of 2 ml of 10 per cent sodium tungstate, while 2 ml of 0.66 N H_2SO_4 were added gradually. The contents were mixed well and allowed to stand for 5 minutes. The volume was made to 100 ml with water and filtered. Took 1 ml of the filtrate and added 1 ml of water in a Folin Wu tube. In other tube, took 2 ml of standard lactose solution. Added 2 ml Folin Wu Alkaline copper solution and heated in a boiling water bath for 8 mts.

Cooled and added 4 ml of acid molybate reagent. After one minute added acid molybate solution to 25 ml mark. Mixed well and read in a photometer at 420 mμ setting the instrument at 0 against water blank.

3.4.4.8. pH

pH in powdered co-precipitate was determined by the method as given in B.S. 1416 - 1962. Three g of powdered co-precipitate were weighed into a 100 ml stoppered vessel, added 30 ml of freshly boiled and cooled water, shook well and allowed to stand for 20 min. pH was recorded at room temperature (20 ± 2°C) on a Global digital DPH-500 pH meter.

3.4.4.9. Colour

The colour of the low, medium and high calcium co-precipitates was determined using a Lovibond Tintometer (The Tintometer Ltd., England). The sample was placed in a glass cell and the matching colour reading was obtained with appropriate combinations of various intensities of red, yellow and blue colour glasses against the standard background of magnesium carbonate block. Whenever sample was brighter than the nearest combination of any two colours, neutral rack was introduced in front of sample to dull it. Results were expressed in terms of units of red, yellow and blue.

3.4.4.10. Flavour

The flavour of the co-precipitate samples

was judged as per the method suggested by Roeper et al. (1978) with some modifications as follows:

Spray dried co-precipitate was dissolved in water at 60°C, using mechanical stirring, to produce 10 per cent (w/v) co-precipitate solution.

A trained panel of six judges was used to ensure unbiased results. The samples to be tested were cooled and presented in random order to each tester at a temperature of about 40°C. The tasters assessed each sample under suitably controlled environmental conditions (Amerine et al., 1965). Salted water was used between each sample to remove any lingering impression from the mouth.

Description of the off-flavours detected were recorded in the flavour evaluation score sheet. (Fig. 2) in the appropriate columns (serious and not serious) together with their intensity (absent, threshold, slight, moderate or strong). To assist the taster in describing off-flavours, the score sheet included a list of suggested off-flavour descriptions (serious: astringent, bitter etc., most serious: acidic, caramel etc.). The tasters were asked to give an overall score (scale 0-8, where 8 = excellent, 6 = good, 3 = poor and 0 = extremely objectionable) to each sample based on the type and intensity of off-flavour. A guide for relating the type and intensity of off-flavours to the overall score was also given.

Fig. 2

FLAVOUR EVALUATION OF MILK PROTEIN PRODUCTS

Evaluator_____Date_____Product_____

Desirable Flavour: Bland Flavour

Suggested Off-flavours:

Serious		Not Serious	
Astringent	(Ast)	Acidic	(AC)
Bitter	(Bit)	Caramel	(Car)
Puckery	(Puc)	Cereal	(Cer)
Burnt (cooked)	(But)	Milky	(Mlk)
Card board	(Cbd)	Nutty	(Nut)
Fishy	(Fsh)	Sweet	(Swt)
Metallic	(Met)		
Mouldy	(Mol)		
Gluey	(Gln)		
Putrid	(Put)		
Rancid	(Ran)		
Salty	(Sal)		
Soapy	(Spy)		
Stale	(St)		
Storage	(Sto)		
Whey	(Wh)		

Guide for overall score (8-0)

Off-Flavour intensity	Serious off-flavour	Non serious off-flavour
Absent (Abs)	8	8
Threshold (Thr)	7	7
Slight (Sl)	5	6
Moderate (Mod)	3	5
Strong (Str.)	1	3

Note: Type of off-flavour must be described.

Sample No.	Serious off-flavours		Not serious off-flavour		Over-all score
	Off-flavour	Intensity	Off-flavour	Intensity	
1					
2					
3					
4					
5					
6					
7					
8					

Time of Evaluation _____AM/PM Signature of the Evaluator

3.4.4.11 Sulphydryl groups

Chapman and Mc Farlane (1945) published a method for determining reducing substances in milk and its products by heating it with potassium ferricyanide under specific conditions. Singh et al. (1970) used this method with slight modifications. The same method was used in the present study.

Acid ferricyanide is reduced by the sulphydryl compounds to ferrocyanide and at the same time sulphydryl compounds are oxidised to disulfide compounds.

$$2\ -SH \xrightarrow[\text{Ferricyanide}]{\text{Acid}} -S.S.$$

Acid ferricyanide reacts with ferric chloride solution to give prussian blue colour which can be estimated spectrophotometrically at 660 mu. The method adopted is as follows:

To 0.1 g of co-precipitate powder was added 9.9 ml of water and 5 ml of 0.2 M phosphate buffer of pH 7.4 to get a final pH of 6.6. Five ml of 1 per cent potassium ferricyanide solution was added. The contents were heated for 20 min in a water bath thermostatically controlled at 70°C and then cooled in ice bath for 30 min. Five ml of 10 per cent trichloro acetic acid solution was added and after mixing, filtered through Whatman filter paper No.40. Five ml of the filterate was mixed with 5 ml of distilled water and 1 ml of freshly prepared 0.1 per cent

ferric chloride solution. After 10 min. readings were taken in a sepctrophotometer against a reagent blank at 660 mu. Cysteine HCl was used as a reference standard.

Reagents:

One per cent potassium ferricyanide solution was prepared in distilled water and kept in a refrigerator, but not more than a week.

Phosphate buffer was prepared according to AOAC (1955). Prepared a solution with potassium dihydrogen orthophosphate (13.6 g dissolved in 500 ml - 'A') and sodium hydroxide (4 g dissolved in 500 ml - 'B'). Fifty ml of solution 'A' was mixed with 39.35 ml of solution 'B' to get 7.4 pH. Final volume was made upto 200 ml.

3.4.4.12. Solubility index

Solubility index was determined by the method of ADMI (1965) which measures the volume of sediment obtained by centrifugation of a specific volume reconstituted co-precipitate. Five g of dried co-precipitate was reconstituted in 100 ml of distilled water at 25°C by blending in a mixer for 90 sec. After standing for a while the foam was removed and 50 ml of the liquid were centrifuged in a conical graduated tube for 5 min. Supernatant fluid upto 15 ml replaced with equal quantity of distilled water and centrifuged again for 5 min. The volume (in ml) of sediment was designated as "Solubility Index."

3.4.4.13. Viscosity

The viscosity of the reconstituted co-precipitates was determined by Hoppler viscometer (type BH, manufactured by Veb Prufgeratewerk Medingen/Dresden). Ten g sample was dissolved in 100 ml of water in a mixer (solubility index mixer) and the temperature was kept at 30°C prior to determining dynamic viscosity. The temperature was kept constant to 30 ± 1°C with the help of Colora Ultrathermostat (Germany). After levelling the Hoppler Viscometer, the measuring tube was filled with the sample and ball No.2 (15.627 mm dia) inserted through the open end of the tube (15.937 - 15.938 mm dia). Time required for the ball to travel 100 mm distance was recorded for 4 such events and averaged.

The specific gravity of sample was also determined at 30°C using a specific gravity bottle.

The dynamic viscosity of the sample was calculated by the following formula (based on Stokes law):

$$\eta = F\ (SK - Sf)\ K$$

where,

η = dynamic viscosity in centipoise

F = fall time of the ball in seconds (angle of inclination 80°)

SK = specific gravity of the ball (2.409)

Sf = specific gravity of the sample, and

K = ball constant (0.07338).

3.4.4.14. Bulk density

Bulk density was determined by Sjollema's (1963) method. A 100 ml graduated cylinder of tared weight was taken. The funnel was placed at the mount of cylinder and the powder was allowed to flow freely to 100 ml mark. The net weight was obtained and results expressed as g/ml (loose bulk density).

To determine the packed bulk density the cylinder with the powder was tapped on a rubber mat until the volume was reasonably constant. The volume of powder was read in ml and density recorded in g/ml.

3.4.4.15. Emulsifying activity (EA) and emulsion stability (ES)

EA and ES were determined by the method of Yasumatsu *et al.* (1972) with some modifications as follows:

0.7 g of co-precipitate was added to 10 ml of water and dispersed at 6,000 rpm in a beaker. Groundnut oil (10 ml) was then added and the blending was resumed for 2 min. The emulsion thus formed was divided equally into two 12 ml centrifuge tubes and centrifuged at 1300 x G (3200 rpm) for 5 min.

EA was expressed as the (height of emulsified layer/the height of total contents in the tube x 100.

ES was measured similarly to that of EA except that emulsion in the centrifuge tube was initially heated in water bath (80^{o}C) for 30 min. and subsequently cooled

to 15°C before centrifugation. Emulsion stability was measured as the (height of the emulsified layer after heating/the height of total content in the tube) x 100.

3.4.4.16. Protein efficiency ratio (PER)

Protein efficiency ratio of co-precipitates was determined according to the procedure of AOAC (1980). The various materials and animals used in this study are described as under:

Reference casein:

The casein fed as control diet to rats was prepared by the method of Newport (1967) from buffalo milk, in the Experimental Dairy, National Dairy Research Institute, Karnal.

Salt mixture:

The salt mixture included in the diet of all animals was prepared by grinding, to fine powder, and thorough mixing of salt mixtures given below:

Sodium chloride,	NaCl	139.3	g
Potassium Iodide,	KI	0.79	g
	(Grind the above together)		
Potassium phosphate,	KH_2PO_4	389.000	g
Magnesium sulphate,	$MgSO_4$	57.300	g
Calcium carbonate,	$CaCO_3$	381.400	g
Ferrous sulphate,	$FeSO_4 . 7H_2O$	27.000	g
Manganese sulphate,	$MnSO_4 . H_2O$	4.010	g

Zinc sulphate,	$ZnSO_4.7H_2O$	0.548	g
Copper sulphate,	$CuSO_4.5H_2O$	0.477	g
Cobalt chloride,	$CoCl_2.6H_2O$	0.023	g

The prepared salt mixture was kept in screw capped polythene bottles.

Vitamin mixture:

Since B complex vitamins were not available individually, a vitamin premix was obtained from Roche Laboratories, Bombay. The final mixture used contained the following vitamins in proportions mentioned against them.

	mg/100 g ration
Vitamin A (Dry, stabilized)	2000 I.U.
Vitamin D (Dry, stabilized)	200 I.U.
Vitamin E (Dry, stabilized)	10 I.U.
Menadione	0.5
Choline	200
P-Aminobenzoic acid	10
Niacin	4
Calcium D-pantothenate	4
Riboflavin	0.8
Thiamine hydrochloride	0.5
Folic acid	0.2
Biotin	0.04
Vitamin B_{12}	0.003
Glucose, to make	1000

Vegetable oil:

Commercially available Dalda brand hydroganated vegetable oil, manufactured by Hindustan Lever Ltd., was used in the study.

Cellulose:

Powdered cellulose was obtained from Vallabhai Patel Chest Institute, Delhi University, Delhi.

Composition of basal diet:

Sample x*

$$X^* = \frac{1.60 \times 100}{\% \text{ nitrogen of sample}}$$

$$\text{Cottonseed oil} = e - \frac{x^{**} \times \% \text{ ether extract}}{100}$$

$$\text{Salt mixture} = 5 - \frac{x^* \times \% \text{ ash}}{100}$$

Vitamin mixture = 1

$$\text{Cellulose} = 1 - \frac{x^* \times \% \text{ crude fiber}}{100}$$

$$\text{Water} = 5 - \frac{x^* \times \% \text{ moisture}}{100}$$

Sucrose or corn starch, to make 100

As far as possible, all the diets contained approximately the same proportions and contents of nitrogen, fat, ash, moisture and crude fibre.

Experimental animals:

Weaned albino rats (male) bred and brought up by the Human Nutrition and Dietetics Division, National Dairy Research Institute, Karnal, were used.

Weanling male albino rats weighing 30-40 g from the small animal house, stock colony maintained at the Institute, were used for the experiment. Animals were distributed randomly into four groups, taking care that sum total of the four groups did not exceed 5 g. Each group consisted of ten animals. Animals were housed individually in anodized aluminium cages. They were fed ad libitum for a period of 28 days and had free access to water. Daily food intakes were recorded and animals were weighed every seventh day. On the basis of these data, protein efficiency ratio was calculated as under:

$$\text{PER} = \frac{\text{g weight gain}}{\text{g protein intake}}$$

3.4.4.17. Whipping ability of ice cream mix

An apparatus designed by Prof. Mohr for determining the whipping ability of ice cream was used with slight modifications. A pair of resistances was connected in series to the whipping machine which is equipped with two six edged cylindrical whippors made of wire 1.5 mm in diameter. The whipping machine is connected

to an ammeter which in turn is connected to rectifier. The resistances are adjusted so that in idle running the whippers make 300 rpm. A nickel metal cup which is kept in another container holding a mixture of ice and water, serves as whipping container.

For the experiment the motor was run idle for 30 minutes to avoid variations in speed of the motor due to voltage fluctuation while the performance was recorded.

Ice cream mix was tampered at 20°C, 100 ml of the ice cream mix (at 20°C) was taken in the nickel cup. The nickel cup was then surrounded with a mixture of ice and salt and temperature of the mix adjusted to 0°C. Immediately on reaching this temperature, the motor was stopped and the nickel metal cup and the container placed on the platform of the machine. The whippors were than attached to the spindle and allowed to revolve for 5 minutes. The volume of the whipped mix was determined after measuring in a graduated cylinder.

The percentage overrum was calculated from the formula:

$$100 \times \frac{\text{Volume of ice cream mix after whipping} - \text{Volume of ice cream mix before whipping}}{\text{Volume of ice cream mix before whipping}}$$

3.4.4.18. Sensory evaluation of ice cream

All the control samples and the samples utilizing co-precipitates, in the manufacture of ice cream, were subject to sensory evaluation by a panel of six judges. The ice cream score card approved by the American Dairy Science Association (1941) and recommended by Nelson and Trout (1951) was used as follows:

Attribute	Score	Remarks
Flavour	45	
Body and Texture	30	
Bacteria	15	
Melting quality	5	
Colour and Package	5	
Total score	100	

Perfect scoring was allowed for Bacteria, Melting quality, Colour and package. Major emphasis was stressed on flavour, body and texture.

3.5. STATISTICAL ANALYSIS

The data obtained in this investigation were subjected to analysis of variance and critical difference by 'F' value, wherever, necessary.

CHAPTER 4

RESULTS & DISCUSSION

4. RESULTS AND DISCUSSION

4.1. LABORATORY TRIALS

Laboratory trials were conducted to standardize the process for the manufacture of low, medium and high calcium co-precipitate from buffalo skim milk, based on the studies of Muller et al.(1967), for cow milk and Vijay Kumar (1976) for buffalo milk. The conditions for the manufacture of co-precipitates were standardised so as to have a minimum ash and calcium content and highest protein recovery.

4.1.1. EFFECT OF HOLDING TIMES OF HEATED MILK ON PROTEIN RECOVERY, ASH AND CALCIUM CONTENT

Holding times were varied between 15, 20 and 25 min., 10, 12 and 15 min., and 1, 2 and 5 min. for low, medium and high calcium co-precipitates respectively. The number of washings in each case was kept constant at 3. The result of this study on the protein recovery, ash and calcium content in the product have been given in tables 4.1, 4.2 and 4.3. The statistical analysis of the data have been reported in tables 4.4, 4.5 and 4.6.

4.1.1.1. Low calcium co-precipitate

It is evident from Table 4.1 that the protein recovery increased with the increase of holding time. The

Low calcium co-precipitate

Table 4.1 Percent protein recovery, ash and calcium contents at different periods of holding at 90°C

Trial No.	15 min.			20 min.			25 min.		
	Protein recovery	Calcium	Ash	Protein recovery	Calcium	Ash	Protein recovery	Calcium	Ash
1	91.30	2.1	8.37	93.50	2.00	8.28	94.16	1.95	8.21
2	91.80	1.9	8.22	94.20	1.85	8.17	94.24	1.90	1.85
3	91.70	2.0	8.34	93.70	2.00	8.15	94.44	1.85	8.17
Average	91.60	2.0	8.31	93.80	1.95	8.20	94.28	1.90	1.95

Medium calcium co-precipitate

Table 4.2 Percent protein recovery ash and calcium contents at different periods of holding at 90°C

Trial No.	10 min.			12 min.			15 min.		
	Protein recovery	Calcium	Ash	Protein recovery	Calcium	Ash	Protein recovery	Calcium	Ash
1	92.70	2.90	10.66	94.90	2.82	9.92	95.10	2.81	9.82
2	92.55	2.90	10.05	94.82	2.78	9.78	94.90	2.77	9.68
3	92.46	2.90	9.74	94.67	2.80	9.88	94.85	2.80	9.84
Average	92.57	2.90	10.15	94.79	2.80	9.86	94.95	2.79	9.78

Table 4.3 Percent protein recovery, ash and calcium contents at different periods of holding at 90°C

Trial No.	1 min.			2 min.			5 min.		
	Protein recovery	Calcium	Ash	Protein recovery	Calcium	Ash	Protein recovery	Calcium	Ash
1	93.90	3.70	11.78	95.12	3.61	11.48	95.20	3.60	11.42
2	93.80	3.61	11.60	95.02	3.59	11.38	95.12	3.55	11.32
3	93.82	3.59	11.68	94.95	3.57	11.45	95.18	3.52	11.34
Average	93.84	3.63	11.69	95.03	3.59	11.44	95.17	3.56	11.36

average protein recovery with holding times 15, 20 and 25 min. was observed to be 91.60, 93.60 and 94.28 per cent respectively. Holding time of 20 min. significantly increased the protein recovery compared to that at 15 min. holding time. However, further increase to 25 min. did not have significant effect on the protein recovery.

Ash and calcium contents in the product were found to be unaffected statistically with varied holding times. This may be probably due to the similar type of co-precipitate's characteristics formed with different holding periods. The average calcium content varied from 1.90 to 2.0 per cent and ash content from 8.18 to 8.31 per cent. Vijay Kumar (1976) reported the ash content varying from 7.33 to 7.70 and calcium content from 1.55 to 1.60 per cent.

On the basis of these findings, the holding period of 20 min. was selected to be optimum for the preparation of low calcium co-precipitate on large scale.

4.1.1.2. Medium calcium co-precipitate

Protein recovery increased with the increase of holding times in medium calcium co-precipitates, also (Table 4.4). On critical analysis the recovery was observed to be of the same order in case of 12 and 15 min. of holding period, but holding time of 12 min. significantly increased the protein recovery over 10 min. holding period.

Ash and calcium content were found to

Table 4.4. Analysis of variance for protein recovery of low, medium and high calcium co-precipitates at different holding periods.

Source	d.f.	M.S.S.		
		Low Calcium co-precipitate	Medium calcium co-precipitate	High calcium co-precipitate
Period	2	6.6808**	5.3229**	1.5541**
Error	6	0.08005	0.01528	0.0053
Total	8			

** Significant at 1 per cent level of significance

CD for low calcium co-precipitate = 0.56528

CD for medium calcium co-precipitate = 0.2469

CD for high calcium co-precipitate = 0.1454

Table 4.5. Analysis of variance for ash content of low, medium and high calcium co-precipitate at different holding periods

Source	d.f.	M.S.S.		
		Low calcium co-precipitate	Medium calcium co-precipitate	High calcium co-precipitate
Period	2	0.0147	0.1137	0.1053
Error	6	0.00397	0.0773	0.4581
Total	8			

Table 4.6. Analysis of variance for calcium content of low, medium and high calcium co-precipitate at different holding periods

Source	d.f.	M.S.S.		
		Low calcium co-precipitate	Medium calcium co-precipitate	High calcium co-precipitate
Period	2	0.0075	0.00895	0.00445
Error	6	0.0067	0.00334	0.00182
Total	8			

unaffected with the change in holding periods as was the case with low calcium co-precipitates (4.1.1.1.). Keeping in view the maximum protein recovery and good quality of the product in terms of low ash and calcium contents, the optimum holding period was observed to be 12 min. for large scale manufacture.

4.1.1.3. High calcium co-precipitate

As is revealed from Table 4.3, protein recovery was found to be affected significantly with different holding periods. 2 min. holding period significantly increased the protein recovery over that with 1 min. holding time. However, further increase to 5 min. of holding period did not significantly improve the protein recovery.

Ash and calcium contents were observed to be unaffected with different levels of holding times as was also the case with low and medium calcium co-precipitates (4.1.1.1. and 4.1.1.2.).

The optimum holding period giving the maximum protein recovery without affecting the quality of the product was observed to be 2 min.

4.1.2. EFFECT OF NUMBER OF WASHINGS ON PROTEIN RECOVERY, ASH AND CALCIUM CONTENT

With the results obtained from the study on effects of holding period of heated milk, it was found that 20, 12 and 2 min were the optimum holding times for the manufacture of low, medium and high calcium co-precipitates respectively.

The observations on the effect of number of washings on low, medium and high calcium co-precipitates have been reported in the Tables 4.7, 4.8, 4.9, 4.10, 4.11, 4.12 and 4.13. The statistical analysis for the same have been reported in Table 4.14, 4.15 and 4.16.

4.1.2.1. Low calcium co-precipitate

The data regarding the effect of number of washings with different pH of precipitation, on protein recovery, ash and calcium content of low calcium co-precipitate have been presented in Tables 4.7, 4.8, 4.9 and their statistical analysis have been reported in Tables 4.14, 4.15 and 4.16.

It has been observed that as the number of washings were increased, there was no significant difference in protein recovery, but there was a significant decrease in ash and calcium contents with the increase in the number of washings. Buchanan *et al.* (1965) also observed that there was a progressive decrease in the ash and calcium contents with the increased number of washings.

It was also found that the reduction in ash and calcium contents with 3 or 4 washings to be non-significant.

4.1.2.2. Medium calcium co-precipitate

The data has been presented in Tables 4.10, 4.11 and 4.12. The number of washings were observed to have

Table 4.7. Effect of washings on the protein recovery, ash and calcium content at 20 min of holding, at pH of precipitation (4.6)

Trial No.	Number of washings								
	2			3			4		
	Protein recovery %	Ash %	Calcium %	Protein recovery %	Ash %	Calcium %	Protein recovery %	Ash %	Calcium %
1	93.64	8.20	2.45	93.66	6.85	2.10	93.48	6.74	2.10
2	93.48	8.24	2.35	93.92	7.15	2.00	93.60	6.85	2.90
3	94.52	8.30	2.40	93.88	7.42	2.0	93.66	7.18	2.00
Average	93.88	8.24	2.40	93.82	7.14	2.03	93.58	6.92	2.03

Low calcium co-precipitate

Table 4.8. Effect of washings on the protein recovery, ash and calcium content at 20 min holding (pH of precipitation (4.8)

Trial No.	Number of washings								
	2			3			4		
	Protein recovery %	Ash %	Calcium %	Protein recovery %	Ash %	Calcium %	Protein recovery %	Ash %	Calcium %
1	94.37	8.52	2.42	93.28	7.32	2.22	93.10	7.13	2.22
2	93.68	8.32	2.30	93.10	6.88	2.17	92.95	6.62	2.18
3	93.84	8.44	2.35	93.14	7.10	2.18	92.88	6.95	2.19
Average	93.96	8.42	2.36	93.17	7.10	2.19	92.98	6.90	2.19

Table 4.9. Effect of washing on the protein recovery, ash and calcium content at 20 min. holding at pH of precipitation (5.0)

Trial No.	Number of washings								
	2			3			4		
	Protein recovery %	Ash (%)	Calcium (%)	Protein recovery %	Ash (%)	Calcium (%)	Protein recovery %	Ash (%)	Calcium (%)
1	94.10	8.80	2.48	93.86	7.65	2.19	93.62	7.48	2.18
2	93.88	8.70	2.45	93.72	7.35	2.15	93.42	7.12	2.16
3	94.61	8.60	2.42	93.45	7.32	2.10	93.28	7.10	2.10
Average	94.20	8.70	2.45	93.68	7.44	2.15	93.44	7.23	2.15

Medium calcium co-precipitate

Table 4.10. Effect of washings on the protein recovery, ash and calcium content at 12 min. of holding, at pH of precipitation (5.4)

Trial No.	Number of washings								
	2			3			4		
	Protein recovery %	Ash (%)	Calcium (%)	Protein recovery %	Ash (%)	Calcium (%)	Protein recovery %	Ash (%)	Calcium (%)
1	94.57	9.89	3.25	94.25	8.73	3.15	93.87	7.98	3.16
2	94.51	9.75	3.15	94.69	9.04	3.05	94.45	8.86	3.00
3	94.84	9.80	3.20	94.62	8.92	3.10	94.16	8.66	3.11
Average	94.64	9.79	3.20	94.52	8.89	3.10	94.16	8.50	3.09

Medium calcium co-precipitate

Table 4.11. Effect of washings on the protein recovery, ash and calcium content at 12 min. holding (pH of precipitation 5.5)

Trial No.	Number of washings								
	2			3			4		
	Protein recovery %	Ash (%)	Calcium (%)	Protein recovery %	Ash (%)	Calcium (%)	Protein recovery %	Ash (%)	Calcium (%)
1	94.66	10.12	3.35	94.51	9.12	3.15	94.10	8.88	3.14
2	94.67	10.06	3.25	94.48	8.89	3.20	93.98	8.12	3.22
3	94.82	10.00	3.15	94.52	8.67	3.10	93.86	8.00	3.11
Average	94.71	10.06	3.25	94.50	8.89	3.15	93.98	8.33	3.15

Medium calcium co-precipitate

Table 4.12. Effect of washings on the protein recovery, ash and calcium content at 12 min. of holding (pH of precipitation 5.6)

Trial No.	Number of washings								
	2			3			4		
	Protein recovery %	Ash (%)	Calcium (%)	Protein recovery %	Ash (%)	Calcium (%)	Protein recovery %	Ash (%)	Calcium (%)
1	94.92	10.22	3.33	94.62	9.10	3.19	94.18	8.98	3.18
2	94.68	10.32	3.34	94.38	9.17	3.17	94.11	9.00	3.18
3	94.66	10.66	3.36	94.14	9.42	3.20	93.91	9.23	3.20
Average	94.75	10.40	3.34	94.38	9.23	3.19	94.06	9.05	3.19

Table 4.13. Effect of washings on the protein recovery, ash and calcium content at 2 min. of holding (pH of precipitation 6.4)

Trial No.	Number of washings								
	2			3			4		
	Protein recovery (%)	Ash (%)	Calcium (%)	Protein recovery (%)	Ash (%)	Calcium (%)	Protein recovery (%)	Ash (%)	Calcium (%)
1	95.80	16.34	4.15	95.13	13.18	3.65	94.96	11.98	3.58
2	95.72	14.96	4.0	95.10	11.45	3.70	94.98	10.96	3.73
3	95.88	16.10	4.15	94.92	12.93	3.75	94.82	11.32	3.78
Average	95.80	15.80	4.10	95.05	12.52	3.70	94.92	11.42	3.69

Table 4.14. Average recovery of protein, ash, and calcium at different number of washings

Number of washings	Protein recovery (%)	Ash (%)	Calcium (%)
	Low calcium co-precipitate (pH of precipitation 4.6)		
2	93.88	8.24	2.40
3	93.82	7.14	2.03
4	93.58	6.92	2.03
CD	-	0.4257	0.1113
	Low calcium co-precipitate (pH of precipitation 4.8)		
2	93.96	8.42	2.36
3	93.17	7.10	2.19
4	92.98	6.90	2.19
CD	0.4497	0.4085	0.0799
	Low calcium co-precipitate (pH of precipitation 5.0)		
2	94.20	8.70	2.45
3	93.68	7.44	2.15
4	93.44	7.23	2.15
CD	-	0.3443	0.0799

Table 4.15. Average recovery of proteins, ash and calcium at different number of washings

Number of washings	Protein recovery (%)	Ash (%)	Calcium (%)
	Medium calcium co-precipitate (pH of precipitation 5.4)		
2	94.64	9.69	3.20
3	94.52	8.89	3.10
4	94.16	8.50	3.09
CD	-	0.5679	N.S.
	Medium calcium co-precipitate (pH of precipitation 5.5)		
2	94.71	10.06	3.25
3	94.50	8.89	3.15
4	93.98	8.33	3.15
CD	0.1970	-	-
	Medium calcium co-precipitate (pH of precipitation 5.6)		
2	94.75	10.40	3.34
3	94.38	9.23	3.19
4	94.06	9.05	3.19
CD	0.3614	0.3780	0.0282

Table 4.16. Average recovery of proteins, ash and calcium at different number of washings

Number of washings	Protein recovery (%)	Ash (%)	Calcium (%)
2	95.60	15.80	4.10
3	95.05	12.52	3.70
4	94.92	11.42	3.69
CD	0.2247	1.4975	0.1666

no significant effect on the protein recovery. However, there was a progressive decrease in the ash and calcium contents as for low calcium co-precipitate (4.1.2.1.).

4.1.2.3. High calcium co-precipitate

The data has been presented in Table 4.13. The increased number of washings had no significant effect on the protein recovery but there was a progressive decrease in ash and calcium contents as in case of low and medium calcium co-precipitates (4.1.2.1. and 4.1.2.2.).

pH of wash water was maintained at 4.6 with the help of 1:20 H_2SO_4 in all the three types of co-precipitates because pH values over 4.6 in the wash water the curd tends to soften and partially redisperse, increasing losses and creating difficulties in pressing and drying (Muller et al. 1967).

It was also observed that as the pH of precipitation was raised there was a corresponding increase in ash and calcium content of low and medium calcium co-precipitates. Southward and Aird (1978) also observed that with the increase in pH, there was a corresponding increase in the calcium content of the curd.

On the basis of this study it can be concluded that for low calcium co-precipitates the optimum conditions were pH of precipitation 4.6 and 3 washings. Corresponding values for medium and high calcium co-precipitates were 5.4, 3 washings and 6.4, 3 washings, respectively.

4.2. PLANT TRIALS

Based on laboratory trials, it was observed that the optimum conditions for the manufacture of low, medium and high calcium co-precipitates were holding time 20, 12 and 2 min; pH of precipitation 4.6, 5.4 and 6.4 and three washings each respectively.

Utilising the above study, low, medium and high calcium co-precipitates were manufactured on a large scale and spray dried.

4.2.1. LOW CALCIUM CO-PRECIPITATE

The composition of low calcium co-precipitate has been presented in Table 4.17. Moisture content ranged from 2.82 to 3.04 per cent. Fat content in the product varied from 1.48 to 1.57 per cent. Buchanan et al. (1965) reported a fat percentage of 1.0 to 2.0 per cent in case of co-precipitate. The more percentage of fat of co-precipitates as compared to acid casein, prepared from the same skim milk is attributed to the fat protein complexes formed during the manufacturing process (Buchanan et al. 1965).

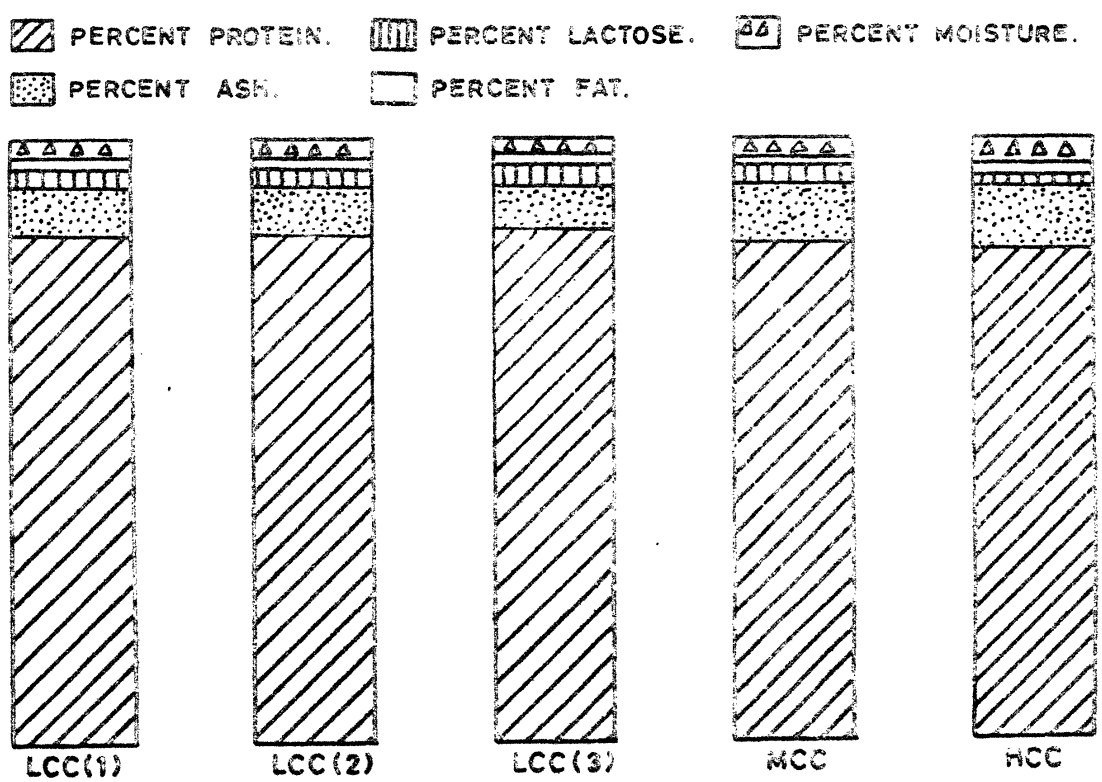
Southward and Aird reported 0.9 per cent fat in low calcium co-precipitate. The ash content was observed in the range of 7.98 to 8.45 per cent. Buchanan et al. (1965) reported an ash content of 10.0 to 11.0 per cent. Southward and Aird (1978) reported 4.1 per cent ash in low calcium co-precipitate. The calcium content was

Table 4.17. Compositional analysis of spray dried low calcium co-precipitate (20 min.of holding)

Trial No.	Moisture (%)	Fat (%)	Ash (%)	Calcium (%)	Phospho-rus (%)	Lactose (%)	pH	Protein (%)	Protein recovery (%)
1	3.04	1.48	8.45	1.95	1.05	2.95	7.0	83.98	93.82
2	2.55	1.51	7.98	2.10	1.11	3.13	6.9	84.63	94.12
3	2.82	1.57	8.17	2.13	1.20	3.49	7.1	83.75	93.78
Average	2.80	1.52	8.20	2.06	1.12	3.19	7.0	84.12	93.90

FIG.3. CHEMICAL COMPOSITION OF DIFFERENT TYPES OF CO-PRECIPITATES.

LCC(1)—LOW CALCIUM CO-PRECIPITATE FROM BUFFALO SKIM MILK.
LCC(2)—LOW CALCIUM CO-PRECIPITATE FROM ELECTRODIALYSED BUFFALO SKIM MILK.
LCC(3)—LOW CALCIUM CO-PRECIPITATE FROM AN ADMIXTURE OF BUFFALO SKIM MILK AND WHEY.
MCC——MEDIUM CALCIUM CO-PRECIPITATE FROM BUFFALO SKIM MILK
HCC——HIGH CALCIUM CO-PRECIPITATE FROM BUFFALO SKIM MILK.



observed to be in the range of 1.95 to 2.13 per cent. Muller et al (1967) reported a calcium content of 0.5 - 0.8 per cent in case of low calcium co-precipitate from cow milk. Southward and Aird (1978) also reported a calcium content of 0.5 per cent in low calcium co-precipitate. The higher per cent of calcium in the low calcium co-precipitate may due to higher percentage of calcium in buffalo milk phosphorus content was found to range between 1.05 to 1.20 per cent. D'yachenko (1953) reported a phosphorus content of 1.49 per cent in co-precipitates.

Lactose per cent was observed to vary between 2.95 to 3.49 per cent. Buchanan et al. (1965) reported 0.5 - 1.5 per cent lactose in co-precipitate.

The pH of the product ranged from 6.9 to 7.1.

Protein content in low calcium co-precipitate was observed to be 83.75 to 84.63 per cent. Southward and Aird (1978) reported 94.0 per cent proteins in low calcium co-precipitate. Buchanan et al. (1965) reported 82 - 84 per cent protein in co-precipitate.

Protein recovery was observed to range between 93.78 to 94.12 per cent. Southward and Aird (1978) reported a recovery of milk proteins in low calcium co-precipitates to be 92.7 per cent (Fig 4)

4.2.2. MEDIUM CALCIUM CO-PRECIPITATE

The average gross chemical composition of

FIG. 4. PROTEIN RECOVERY AND CALCIUM CONTENTS IN DIFFERENT TYPES OF CO-PRECIPITATES.

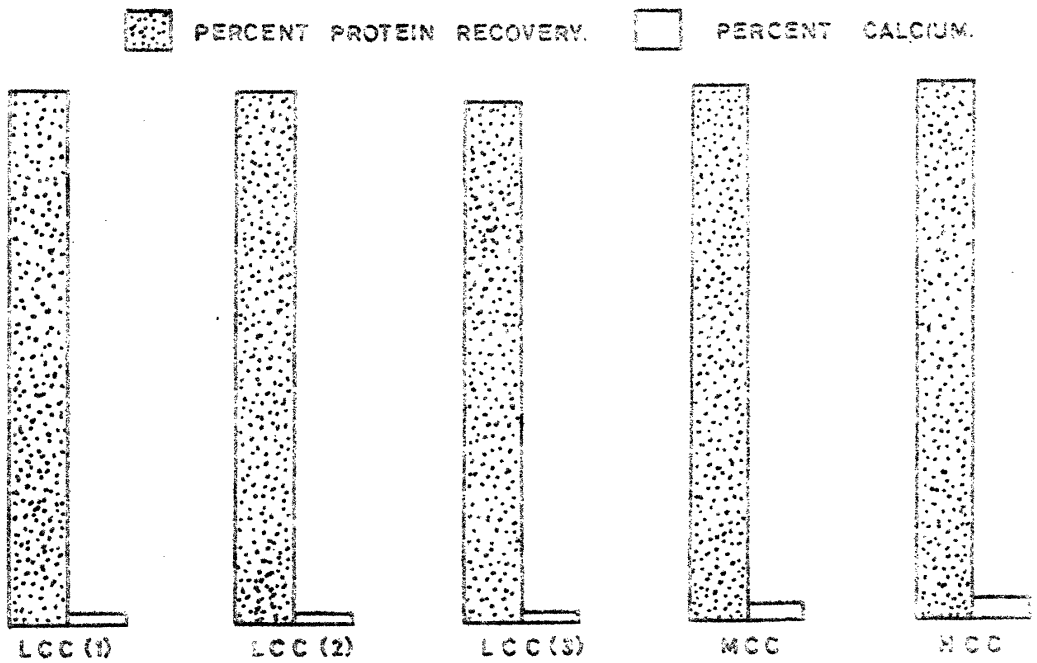
LCC(1)—LOW CALCIUM CO-PRECIPITATE FROM BUFFALO SKIM MILK.

LCC(2)—LOW CALCIUM CO-PRECIPITATE FROM ELECTRODIALYSED BUFFALO SKIM MILK.

LCC(3)—LOW CALCIUM CO-PRECIPITATE FROM AN ADMIXTURE OF BUFFALO SKIM MILK AND WHEY.

MCC——MEDIUM CALCIUM CO-PRECIPITATE FROM BUFFALO SKIM MILK.

HCC——HIGH CALCIUM CO-PRECIPITATE FROM BUFFALO SKIM MILK.



medium calcium co-precipitate has been presented in Table 4.18 and Fig 3.

The moisture content varied between 3.00 to 3.65 per cent.

Fat content in the product was observed to vary between 1.38 to 1.46 per cent. Southward and Aird (1978) reported 0.7 per cent fat in medium calcium co-precipitate.

Ash content was observed to vary between 9.61 to 10.15 per cent. Buchanan et al.(1965) reported 10.0 to 11.0 per cent ash in co-precipitate. Southward and Aird (1978) reported 5.0 per cent ash in medium calcium co-precipitate.

Calcium content was observed to range between 2.83 to 2.95 per cent. Muller et al. (1967) reported a calcium content of 1.5 per cent in medium calcium co-precipitate. Buchanan et al.(1965) reported calcium content of 2.0 - 3.0 per cent for co-precipitate. Southward and Aird (1978) reported a calcium content of 1.2 per cent in medium calcium co-precipitate. The higher percentage of calcium in the co-precipitates is due to higher per cent of calcium in buffalo milk.

Phosphorus content in medium calcium co-precipitate was observed to vary between 1.20 to 1.37 per cent. D'yachenko et al. (1953) reported a phosphorus content of 1.49 per cent in co-precipitates.

Table 4.18. Compositional analysis of spray dried medium calcium co-precipitate (12 min. of holding)

Trial No.	Moisture (%)	Fat (%)	Ash (%)	Calcium (%)	Phospho-rus (%)	Lactose (%)	pH	Protein (%)	Protein recovery (%)
1	3.00	1.46	10.15	2.95	1.37	2.28	7.0	82.95	94.66
2	3.65	1.38	9.82	2.92	1.33	2.52	7.1	82.46	94.84
3	3.00	1.42	9.61	2.83	1.20	2.16	7.2	83.59	94.92
Average	3.21	1.42	9.86	2.90	1.30	2.32	7.1	83.00	94.80

Lactose per cent was observed to vary between 2.16 to 2.52 per cent. Buchanan et al. (1965) reported 0.5 - 1.5 per cent lactose in co-precipitates.

The pH of the product varied from 7.0 to 7.2.

Protein content in medium calcium co-precipitate was observed to vary between 82.46 to 83.59 per cent. Southward and Aird reported 93.0 per cent proteins in medium calcium co-precipitate. The lower per cent of protein in the buffalo milk co-precipitates may be due to higher ash content in the product.

Protein recovery was observed to vary between 94.66 to 94.92 per cent. Southward and Aird (1978) reported 94.1 per cent recovery of proteins in medium calcium co-precipitates (Fig 4)

4.2.3. HIGH CALCIUM CO-PRECIPITATE

The composition of high calcium co-precipitates has been reported in Table 4.19 and Fig 3.

Moisture content in various samples varied from 3.50 to 4.0 per cent.

The fat content of the product on dry basis ranged from 1.44 to 1.63 per cent. Buchanan et al. (1965) reported a fat per cent of 1.0 - 2.0 per cent in co-precipitates. Southward and Aird reported 0.6 per cent fat in high calcium co-precipitate.

Table 4.19. Compositional analysis of high calcium co-precipitate (2 min. of holding)

Trial No.	Moisture (%)	Fat (%)	Ash (%)	Calcium (%)	Phospho-rus (%)	Lactose (%)	pH	Protein (%)	Protein recovery (%)
1	4.00	1.44	11.75	3.70	1.62	1.55	7.2	81.16	95.65
2	3.50	1.58	11.18	3.52	1.56	1.43	7.1	82.06	95.32
3	3.53	1.63	11.42	3.58	1.56	1.46	7.3	81.76	95.76
Average	3.67	1.55	11.45	3.60	1.58	1.48	7.2	81.66	95.57

Total ash content of high calcium co-precipitate was observed to range from 11.18 to 11.75 per cent, approximately 1½ times the ash content of low calcium co-precipitate. Buchanan et al. (1965) reported an ash content of 10.0 - 11.0 per cent in co-precipitates. Southward and Aird (1978) reported 13.5 per cent ash in high calcium co-precipitate. A higher content of ash may be due to the presence of higher per cent of sodium tripolyphosphate, (6 per cent w/w).

The calcium content of the product ranged from 3.52 to 3.70 per cent. Buchanan et al. (1965) reported 2.5 - 3.0 per cent calcium in high calcium co-precipitate. Southward and Aird (1978) reported 2.9 per cent calcium in high calcium co-precipitate.

Phosphorus content of high calcium content of high calcium co-precipitates ranged from 1.56 to 1.62 per cent. D'yachenko et al. (1953) reported a phosphorus content of 1.49 per cent in co-precipitates.

Lactose per cent ranged from 1.43 to 1.55 per cent in high calcium co-precipitate. Buchanan et al. (1965) reported 0.5 - 1.5 per cent lactose in co-precipitates. High calcium co-precipitate was observed to contain minimum percentage of lactose as compared to the other two types.

The pH of the product varied from 7.1 to 7.3.

The protein content varied from 81.06 to 82.06 per cent. Buchanan et al. (1965) reported a protein content of 82.0 - 84.0 per cent for co-precipitates. Southward and Aird (1978) reported 84.5 per cent protein in high calcium co-precipitate.

The high ash content of high calcium co-precipitate lead to a consequential reduction of about 3 per cent in the protein content of the product as compared to low calcium co-precipitate. Southward and Aird (1978) reported a reduction of 3 - 5 per cent in the protein content of the high calcium co-precipitate as compared to low calcium co-precipitate.

Protein recovery was observed to vary between 95.32 to 95.76 per cent. Southward and Aird (1978) reported 95.8 per cent recovery of proteins in the product. The higher protein recovery may be due to the increased co-precipitation of whey proteins due to higher pH (6.4) of precipitation (Fig 4)

The protein recoveries in all the three types of co-precipitate was almost similar to the recovery reported by Muller et al. (1967), Southward and Aird (1978), which indicates a satisfactory recovery through the standardized procedure from buffalo milk.

4.2.4. LOW CALCIUM CO-PRECIPITATE FROM ELECTRODIALYSED MILK

The optimum conditions for the manufacture of low calcium co-precipitate from buffalo skim milk were

utilised in the manufacture of low calcium co-precipitate from electrodialysed buffalo skim milk. The compesition of the product has been presented in Table 4.20.

The product was observed to have moisture 3.0 - 3.13 per cent, fat 1.47 - 1.48 per cent, ash 7.95 - 7.97 per cent, calcium 1.83 - 1.85 per cent, phosphorus 1.0 to 1.05 per cent, lactose 3.0 - 3.15 per cent, protein 84.10 - 84.48 per cent and protein recovery from 93.65 - 93.84. The product was observed to have a pH of 7.0.

The product had almost similar composition to that of low calcium co-precipitate from buffalo skim milk. However, there was a reduction in the amount of calcium per cent to the extent of 8 per cent and the ash content was also lower by about 3 per cent. The purpose of this study was to have a low calcium co-precipitate having calcium content at par with cow milk co-precipitate. Bu the reduction in calcium content could not be achieved at par with low calcium co-precipitate from cow milk. Also electrodialysis of milk for the manufacture of low calcium co-precipitates being quite cumbersome and expensive is not recommended for large scale manufacture.

4.2.5. LOW CALCIUM CO-PRECIPITATE FROM SKIM MILK + FOUR PARTS OF BUFFALO CHEDDAR CHEESE WHEY

A co-precipitate was prepared (Netherlands, Bedrijven van het Netherlands Institute Voor Zuivelonderzoek, 1978) from a mixture of 4 parts of whey and 1 part skim milk.

Table 4.20. Compositional analysis of spray dried low calcium co-precipitate (20 min. holding) using electrodialysed milk

Trial No.	Moisture (%)	Fat (%)	Ash (%)	Calcium (%)	Phospho-rus (%)	Lactose (%)	pH	Protein (%)	Protein recovery (%)
1	3.00	1.48	7.96	1.84	1.05	3.10	7.0	84.26	93.65
2	3.13	1.47	7.97	1.85	1.00	3.00	7.0	84.48	93.84
3	3.04	1.48	7.95	1.83	1.02	3.15	7.0	84.10	93.66
Average	3.06	1.47	7.96	1.84	1.02	3.08	7.0	84.28	93.82

Same proportion has been kept in this study.

Low calcium co-precipitate from an admixture of buffalo skim milk and buffalo cheddar cheese whey, was prepared as per the standardised conditions for low calcium co-precipitate from buffalo skim milk. The standardised conditions for the manufacture of low calcium co-precipitate were also reported to be suitable for the manufacture of co-precipitates from mixtures of milk and whey (Southward and Aird, 1978).

Table 4.21, gives the composition of low calcium co-precipitate from an admixture of buffalo skim milk and 4 parts of buffalo cheddar cheese whey. The moisture content was observed to range from 3.18 - 3.62 per cent, fat 1.44 - 1.46 per cent, ash 7.10 - 7.20 per cent, calcium 1.49 - 1.51 per cent, phosphorus 0.92 - 0.98 per cent, lactose 2.65 - 2.88 per cent, pH 7.0, protein 84.88 - 85.10 per cent and protein recovery 91.34 - 92.41 per cent.

The composition of this type of co-precipitate was almost similar to that of one prepared from skim milk except that it contain 25 per cent less calcium then the one prepared from skim milk or electrodialysed skim milk. Also there was a reduction in ash content to the extent of 13 per cent. This can possibly be due to the difference in the structure of co-precipitate and proportionately more loss of ash and calcium in the whey after precipitation.

Table 4.21. Compositional analysis of spray dried low calcium co-precipitate (20 min. holding) using an admixture of skim milk and four parts of whey

Trial No.	Moisture (%)	Fat (%)	Ash (%)	Calcium (%)	Phospho-rus (%)	Lactose (%)	pH	Protein (%)	Protein recovery (%)
1	3.18	1.44	7.20	1.49	0.98	2.88	7.0	85.10	92.41
2	3.40	1.45	7.15	1.51	0.95	2.65	7.0	85.14	91.88
3	3.62	1.46	7.10	1.50	0.92	2.76	7.0	84.88	91.34
Average	3.40	1.45	7.15	1.50	0.95	2.76	7.0	85.04	91.67

The reduction in calcium content of co-precipitate does not justify the qualification of this method on a large scale utilization due to increased quantity of whey going into drain even though to a certain extent the whey proteins are recovered.

4.2.6 PHYSICAL CHARACTERISTICS OF SPRAY DRIED CO-PRECIPITATES

Some important physical characteristics like colour, flavour, -SH groups, solubility index, viscosity, bulk density of different types of co-precipitates were studied. Also the above properties were studied for low calcium co-precipitates prepared from electrodialysed buffalo skim and an admixture of buffalo skim milk with 4 parts of cheddar cheese qhey. The values have been presented in Tables 4.22, 4.23, 4.24, 4.25, 4.26 and 4.27.

4.2.6.1. Colour:

Colour of different types of co-precipitates has been depicted in Tables (4.22, 4.23, 4.24, 4.25 and 4.26) in terms of matching red, yellow and blue colour units of Tintometer. It may be observed from the over all matching colour units of different types of co-precipitates that there were little lower values of matching colour units with higher calcium co-precipitate as compared to low and medium types of co-precipitates. This may be due to higher percentage of STPP in the case of high calcium co-precipitate which increases the whiteness. However,

Table 4.22 Physical properties of low calcium co-precipitate from fresh skim milk

Trial No.	Total -SH (mg/g)	Colour			Solubility index (ml)	Viscosity (cP)	Bulk density (g/ml)	
		R	Y	B			Loose	Packed
1	1.67	0.3	0	0	8.1	9.22	0.19	0.30
2	1.68	0.3	0	0	8.0	9.10	0.20	0.31
3	1.69	0.3	0	0	8.2	9.38	0.21	0.32
Average	1.68	0.3	0	0	8.1	9.23	0.20	0.31

Table 4.23 Physical properties of medium calcium co-precipitate from fresh skim milk

Trial No.	Total -SH (mg/g)	Colour			Solubility index (ml)	Viscosity (cP)	Bulk density (g/ml)	
		R	Y	B			Loose	Packed
1	1.52	0.2	0	0	10.1	10.68	0.24	0.32
2	1.48	0.2	0	0	10.0	10.72	0.25	0.33
3	1.44	0.2	0	0	10.2	10.14	0.26	0.34
Average	1.48	0.2	0	0	10.1	10.51	0.25	0.33

Table 4.24. Physical properties of high calcium co-precipitate from fresh skim milk

Trial No.	Total -SH (mg/g)	Colour			Solubility index (ml)	Viscosity (cP)	Bulk density (g/ml)	
		R	Y	B			Loose	Packed
1	1.36	0.1	0	0	11.9	20.84	0.29	0.43
2	1.38	0.1	0	0	12.0	20.65	0.30	0.41
3	1.40	0.1	0	0	12.1	20.88	0.28	0.42
Average	1.38	0.1	0	0	12.0	20.79	0.29	0.42

Table 4.25 Physical properties of spray dried low calcium co-precipitate utilising electrodialysed milk.

Trial No.	Total -SH (mg/g)	Colour			Solubility index (ml)	Viscosity (cP)	Bulk density (g/ml)	
		R	Y	B			Loose	Packed
1	1.67	0.3	0	0	7.5	9.09	0.22	0.35
2	1.68	0.3	0	0	7.5	9.12	0.21	0.36
3	1.67	0.3	0	0	7.5	9.15	0.20	0.34
Average	1.67	0.3	0	0	7.5	9.12	0.21	0.35

Table 4.26 Physical properties of low calcium co-precipitate prepared from skim milk and four parts of whey

Trial No.	Total -SH (mg/g)	Colour			Solubility index (ml)	Viscosity (cP)	Bulk density (g/ml)	
		R	Y	B			Loose	Packed
1	1.70	0.3	0	0	7.5	9.64	0.21	0.30
2	1.68	0.3	0	0	7.6	9.44	0.22	0.29
3	1.66	0.3	0	0	7.7	9.15	0.20	0.31
Average	1.68	0.3	0	0	7.6	9.41	0.21	0.30

Table 4.27 Average flavour score of low, medium and high calcium co-precipitate

Judges	Low			Medium	High
	Skim milk	Electro-dialysed Skim milk	Skim milk + whey		
1	6.2	5.9	6.1	6.5	7.4
2	6.0	6.1	6.0	6.6	7.5
3	6.1	6.0	5.9	7.0	7.3
4	6.0	5.8	6.0	6.8	7.5
5	6.1	6.1	6.0	6.7	7.5
6	6.2	6.1	6.2	6.0	7.2
Average	6.1	6.0	6.03	6.6	7.4

all the three types of co-precipitates looked white on visual observations.

Hayes et al. (1969) also reported that pH, calcium and polyphosphate content of co-precipitate solutions were the factors which particularly affected the whiteness of co-precipitates.

4.2.6.2. Flavour:

The data has been presented in Table 4.27. The flavour characteristics of spray dried co-precipitates subjected to 3 washes during manufacture, scored 6 - 7.4 highest score being for high calcium co-precipitate on a scoring scale of 0 - 8 (8 = excellent, 0 = very strong off flavour). The judging was done as per the method of Roeper et al. 1978).

Flavour characteristics of co-precipitate were assessed by a taste panel, members of which had been trained in evaluating casein flavour. In considering the effects of whey protein on the flavour characteristics of co-precipitates, it may be concluded that the only obvious contribution which they made appears to be in the cooked over-tones traditionally associated with boiled milk. There are generally attributed to liberation of volatile sulphides (and hydrogen sulphide in particular) from -SH groups which are achieved by heat denaturation of β-lactoglobulin. (Jennes and Patton, 1952). The degree of washing also affected the intensity of cooked

flavour, increased washings produced a co-precipitate with less intense cooked off flavour, (Southward and Goldman, 1978).

With the exception of the cooked flavour, the general flavour characteristics of high calcium co-precipitate were similar to those of rennet casein which has been shown to be superior in flavour to acid casein (Walker, 1973). Granular high calcium co-precipitate, which had been subjected to two washes during manufacture, typically scored 5 - 6 on a recovery scale of 0 - 8. The flavour of soluble co-precipitates prepared by dissolving fresh curd in alkali and/or phosphate, followed by spray drying of the solution, was generally superior to that of the respective gramular co-precipitates (Southward and Goldman, 1978).

4.2.6.2.1. Total -SH groups:

Tables 4.22, 4.23, 4.24, 4.25 and 4.26 give the values of total -SH group expressed as mg cysteine HCL per g of co-precipitate. Total -SH group was highest in case of low calcium co-precipitate (1.68 mg/g) and lowest in case of high calcium co-precipitate (1.38 mg/g). This difference may be attributed to higher period of holding at 90°C in case of low calcium co-precipitate. Even for the low calcium co-precipitate from an admixture of skim milk and whey did not show noticeable difference from the -SH values those from similar type of co-precipitates prepared from skim milk alone.

These values of -SH groups are very low than the spray dried skim milk powder which had an average value of 5.57 mg cysteine Hcl per g. (Joginder Singh 1976).

4.2.6.3. Solubility index:

As per tables 4.22, 4.23 and 4.24, the solubility index values for low, medium and high calcium co-precipitate were found to be 8.1, 10.1 and 12.0 ml respectively. These values were found to be 7.5, 7.6 ml for low calcium co-precipitate only prepared from electrodialysed milk and skim milk + four parts of whey, respectively (Tables 4.25 and 4.26).

It has been observed that the spray dried high calcium co-precipitate had the least solubility as compared to low and medium types of co-precipitates. High calcium co-precipitate with 6 per cent STPP was less soluble than the low and medium calcium co-precipitates which contain 2 and 4 per cent STPP respectively. The lower solubility of spray dried co-precipitates is due to the presence of the heat denatured whey proteins which are incorporated with the caseins during precipitation. Traditional lactalbumin, for instance, produced by heat precipitation of the protein from acid casein whey, is almost completely insoluble at pH 7 (Robinson et al. 1976).

Buchanan et al. (1965) found that high calcium co-precipitate, unlike casein would not dissolve at pH 6 - 7

without the addition of a calcium requestering agent. Sodium tripolyphosphate was effective at levels of 4 - 6 per cent of the weight of co-precipitate, depending on its calcium content. When lower quantities of phosphate are used, the co-precipitate becomes partly soluble, with very little change in the pH of the solution.

The solubility characteristics of the co-precipitates are generally similar to those of the corresponding caseins. Thus, rennet casein (with a high calcium content) may be dissolved completely in STPP at pH 7.5 to produce solutions (Towler, 1974), which, in contrast to acid casein, do not exhibit the adhesive characteristics traditionally associated with that product. Co-precipitate, even in the spray dried form contains denatured whey proteins and therefore, cannot be dissolved in the same way as sodium caseinate as true solution. It disperses in water more easily than sodium caseinate, and a stable suspension of 10 per cent total solids can be prepared in 4 per cent STPP (Buchanan et al. 1965)

4.2.6.4. Viscosity:

It has been observed that high calcium co-precipitate had almost twice as much (20.79 cP) as that of medium (10.51 cP) and low calcium co-precipitate (9.23 cP). The data has been presented in Tables 4.22, 4.23, 4.24, 4.25 and 4.26.

Southward and Goldman (1978) reported that

the viscosity of high calcium co-precipitate was highest, and that of low calcium co-precipitate lowest, while medium calcium co-precipitate exhibited and intermediate viscosity.

The viscosity shear rate characteristics of the co-precipitates show patterns similar to those of various caseins which were measured by Towler (1974). Thus, the viscosity shear rate plots for high calcium co-precipitate and rennet casein, both of which have a relatively high calcium content (nearly 3 per cent) are very similar (Southward and Goldman, 1978). Furthermore, the low calcium co-precipitate and sodium caseinate have similar but lower viscosity profiles, which was also noted by Hayes (1969). As observed earlier, medium calcium co-precipitate has intermediate viscosity characteristics. From these results, therefore, it appears that the 'Occluded' calcium content of co-precipitates and caseins is the prime factor causing the variations observed in the viscosity of these milk protein products at similar temperatures and concentrations, while the co-precipitated whey proteins appear to have little or no effect.

4.2.6.5. Bulk density:

It has been observed as per Tables 4.22, 4.23, 4.24, 4.25 and 4.26 that the high calcium co-precipitate had the highest bulk density (0.42 g/ml) as

compared to low (0.31 g/ml) and medium types of co-precipitates (0.33 g/ml). The medium type co-precipitate had a intermediate value for bulk density. These results agree with the findings of Southward and Aird (1978) who reported the values as 0.30, 0.26 and 0.24 g/ml for high, medium and low calcium co-precipitate respectively.

Bulk density of the co-precipitates, which had been dried in a granular form and sieved to pass 180 um was found to be slightly greater than commercial lactic casein of similar particle size range. The bulk density of the spray dried soluble and water dispersible co-precipitates was similar to, or slightly lower than, that of sodium caseinate. Generally, the bulk density of the soluble co-precipitate powders was affected by the total solids content of the feed solution to the drier, and this was somewhat lower than that used in producing commercial sodium caseinate (Southward and Aird, 1978).

4.2.6.6. Emulsion stabilizing capacity

Emulsion stabilizing capacity of spray dried co-precipitates is shown in Tables 4.28, 4.29 and 4.30. All the three types of co-precipitates including the ones prepared from electrodialysed milk and skim milk + whey had excellent emulsion stabilising properties within the limits of the test, retaining 100 per cent of emulsified oil.

Table 4.28. Emulsion stabilizing capacity of co-precipitates

Tyoe of co-precipitate	pH	Emulsified oil (%)			
		I	II	III	Average
High Calcium	7.20	100	100	100	100
Medium calcium	7.10	100	100	100	100
Low calcium	7.00	100	100	100	100

Table 4.29 Emulsion stabilizing capacity of low calcium co-precipitate prepared from electrodialysed milk

Trial No.	pH	Emulsified oil (%)
1	7.0	100
2	7.0	100
3	7.0	100
Average	7.0	100

Table 4.30 Emulsion stabilizing capacity of low calcium co-precipitate, prepared from skim milk and whey

Trial No.	pH	Emulsified oil (%)
1	7.00	100
2	7.00	100
3	7.00	100
Average	7.00	100

Southward and Goldman (1978) reported that soluble high and medium calcium co-precipitate appeared to stabilize emulsions more completely than the soluble low calcium co-precipitates and were certainly better than sodium caseinate for this purpose. Using the same test method, Chojnowski et al. (1975) found that the emulsion stability of soluble acid "proteinates" (co-precipitates) was influenced by the alkali used in the neutralization of the proteinate. Calcium milk proteinate (0.98 per cent calcium content) had the highest stability, retaining 100 per cent of emulsified oil, whilst ammonium and sodium calcium milk proteinate retained 45 - 48 per cent and 12 per cent respectively, and sodium caseinate only 10 per cent. The co-precipitates which are most effective in stabilizing emulsions of oil appear, therefore, to be those containing 1 per cent or more of calcium in a semi colloidal form with the protein.

4.2.7. PROTEIN EFFICIENCY RATIO OF CO-PRECIPITATES

The mean value of protein efficiency ratio obtained with low, medium and high calcium co-precipitates have been reported in Table 4.31.

The nutritional quality of co-precipitates as measured by Protein Efficiency Ratio was observed to be higher than that of casein (PER 2.5) and varied from a PER of 2.95 for high calcium to 2.75 for low calcium co-precipitate. The PER of medium calcium co-precipitate was 2.82.

Table 4.31 Protein efficiency ratio of co-precipitates*

Group	Initial weight of rats (g)	Total weight gained (g)	Total protein intake (g)	PER**
Casein	35.7	108.45	43.38	2.5
Low calcium co-precipitate	33.7	112.65	40.96	2.75
Medium calcium co-precipitate	35.7	115.45	40.94	2.82
High calcium co-precipitate	35.7	125.32	42.48	2.95

* Average value for 10 male weaning albino rats

** Protein efficiency ratio = $\frac{\text{g wt. gain}}{\text{g protein intake}}$

The nutritional superiority of co-precipitates over casein is probably due to the higher cystine content of the former (Lohrey et al. 1974). They reported that the PER was highest in co-precipitates with the highest proportion of whey protein i.e. high calcium co-precipitate (mean 2.91) and somewhat lower for low calcium co-precipitate (PER 2.72) which contained the lowest amount of additional whey protein. The PER of medium calcium co-precipitate was 2.78. This agrees very well with our results.

4.2.8. UTILIZATION OF CO-PRECIPITATES IN ICE-CREAM MAKING

4.2.8.1. Whipping properties of ice cream mix

The whipping properties of ice cream mix utilizing different percentage of high calcium co-precipitate have been given in Table 4.32. It has been observed as the per cent of co-precipitate increased in the ice cream mix there was a reduction in the over run of ice cream mix. The co-precipitate per cent were used as a substitute for skim milk powder at levels of 10, 30, 50, 70 and 100 per cent (Fig 5)

Southward and Aird (1978) reported that soluble co-precipitates, when whipped alone or with sugar, gave lower over runs than fresh egg albumen when whipped under the same conditions. They have also reported that the soluble co-precipitates have lower over runs than sodium caseinate with or without sugar. Anne - Marie Bech

FIG. 5. EFFECT OF DIFFERENT LEVELS OF HIGH CALCIUM CO-PRECIPITATE AS PERCENT OF SMP ON THE OVER-RUN OF ICE-CREAM MIX.

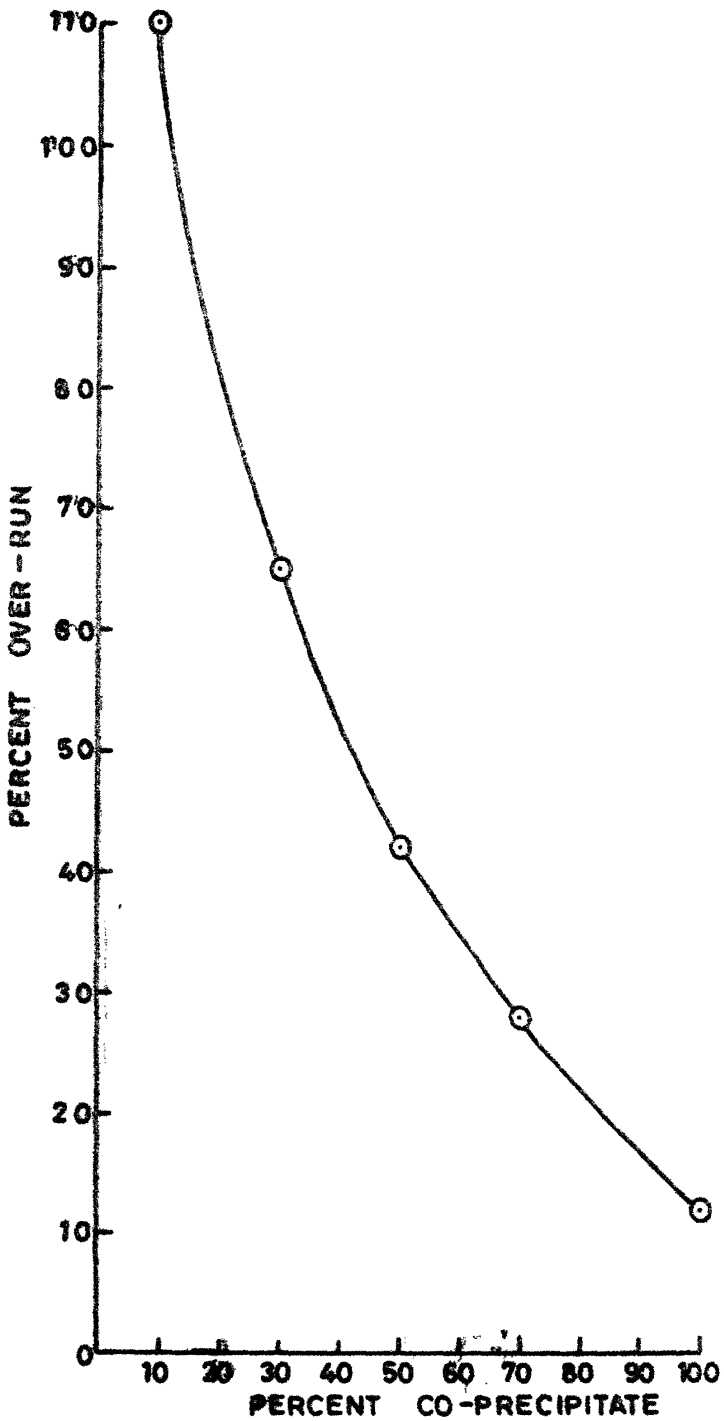


Table 4.32 Effect of different levels of high calcium co-precipitate (as per cent of SMP) on the over run of ice cream mix

Percent co-precipitates	Per cent over run
10	110
30	65
50	42
70	28
100	12

(1981) while working on the physical and chemical properties of whey proteins reported that a maximum level of whippability is reached when the whey proteins are heated to 55 - 60°C. At higher temperatures whippability decreases considerably.

4.2.8.2. Sensory evaluation of ice cream

Results of total score, flavour, body and texture have been given in Tables 4.33, 4.34, and 4.35.

A statistical analysis of grading done by six judges with respect to flavour, body and texture and total score (Tables 4.36, 4.37 and 4.38), put treatment (10:90) superior to other combinations. The treatment effects were found to be significantly different in all the cases. Critical difference test shows that body and texture of 5 (100:0) was less favourable as compared to other types. Though the liking of other combinations were of the same order in statistical terms, however, average scores obtained by combination 1, (at 10 per cent replacement of SMP with high calcium co-precipitate) were highest. This analysis indicated that ice cream prepared by first combination (10:90) can be considered best.

So on the basis of these findings it can be concluded that high calcium co-precipitate can be utilized in ice cream making to the extent of 10 per cent as a substitute for SMP, without having any adverse effect on the quality of ice cream.

Table 4.33 Evaluation of ice cream with different per cent of high calcium co-precipitate as a substitute for SMP on the basis of total score (perfect score 100)

Judges	Co-precipitate:SMP					Control
	10:90	30:70	50:50	70:30	100:0	
J1	93	90.5	88.5	93.0	81.5	90.5
J2	93	91	88	90	88	93
J3	93	89	83	85.5	81	92
J4	94	93	85	90.5	88	93
J5	94	93	92	92	85	93
J6	94	87	97	90	79.5	91
Average	93.5	90.58	88.91	90.16	83.83	92.08

Table 4.34 Evaluation of ice cream with different per cent of high calcium co-precipitate as a substitute for SMP, on the basis of flavour score (per cent score 45)

Judges	Co-precipitate:SMP					Control
	10:90	30:70	50:50	70:30	100:0	
J1	40	39	35	40	33.5	40
J2	39	38	35	37.5	35	40
J3	40	36	41	35	35	39
J4	40	40	34	40	37	39
J5	40	40	40	40	36	40
J6	39	40	44	38	33	40
Average	39.66	38.83	38.16	38.41	34.91	39.66

Table 4.35 Evaluation of ice cream with different per cent of high calcium co-precipitate, as a substitute for SMP, on the basis of body and texture score (perfect score 30)

Judges	Co-precipitate:SMP					Control
	10:90	30:70	50:50	70:30	100:0	
J1	28	27.5	29	29	25	27
J2	29	28	28.5	28	28	29
J3	28	28	28	26.5	24	28.5
J4	29	28	26	28	27	27.5
J5	29	28	27	27	25	28
J6	29	28	28	26	23	28
Average	28.66	27.91	27.75	27.41	25.33	28.00

Table 4.36 Analysis of variance : Sensory evaluation

Total score

Source	d.f.	S.S.	M.S.S.	F
Treatment	5	331.39	66.27	8.38**
Error	30	227.80	7.59	
Total	35	559.19		

CD = 3.248

Table 4.37 Analysis of variance : Sensory evaluation
Flavour score

Source	d.f.	S.S.	M.S.S.	F
Treatment	5	92.97	18.594	4.299**
Error	30	129.75	4.325	
Total	35	222.75		

CD = 2.452

Table 3.38 Analysis of variance : Sensory evaluation
Body and Texture Score

Source	d.f.	S.S.	M.S.S.	F
Treatment	5	39.28	7.856	6.971**
Error	30	33.80	1.127	
Total	35	73.08		

CD = 1.252

CHAPTER 5

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

5.1. Manufacture and utilization of co-precipitate afford, to a certain extent, a solution to the problem of malnutrition in India. No work on the manufacture of co-precipitates from buffalo milk, which comprises 60 per cent of the total milk production in India, has been reported. Hence the study was conducted on the manufacture of co-precipitates, on lines of work commonly adopted in Australia, for cow milk, with modifications as needed.

5.2. Laboratory trials were conducted on the manufacture of low, medium and high calcium co-precipitate from buffalo skim milk. The steps adopted in preparation of co-precipitate were: separation of skim milk, heating to 90 ^{o}C, holding, precipitation, washing and drying in an oven. As the buffalo milk contains more calcium than cow milk, no calcium chloride was added in the manufacture of low calcium co-precipitate. The effect of different pH of precipitation for high calcium co-precipitate was not studied as there acid was not added during its manufacture.

5.3. All the three types of co-precipitates were studied under three different variables, namely (i) holding time of heated milk (ii) different pH of precipitation and (iii) number of washings given to the co-precipitate.

5.4. Holding time of co-precipitates at 90°C was varied between 15, 20 and 25 minutes; 10, 12 and 15 minutes; 1, 2 and 5 minutes for low, medium and high calcium co-precipitates respectively.

5.5. Holding time of 20 minutes, pH of precipitation 4.6 and three washings with acidulated water (1:20 H_2SO_4) having a pH of 4.6 for wash water were observed to be optimum in the manufacture of low calcium co-precipitate.

5.6. Holding time of 12 minutes pH of precipitation 5.4 and three washings with acidulated water (1:20 H_2SO_4) having pH 4.6 were observed to be optimum in the case of medium calcium co-precipitate.

5.7. Holding time of 2 minutes pH of precipitation 6.4 and three washings with acidulated water (1:20 H_2SO_4) having a pH of 4.6 were observed to be the optimum conditions for the manufacture of high calcium co-precipitate.

5.8. The protein recovery was observed

to be 93.82, 94.52 and 95.05 per cent for low, medium and high calcium co-precipitate respectively.

5.9. The calcium content was observed to be 2.03, 3.10 and 3.70 per cent for low, medium and high calcium co-precipitate, respectively which was on little higher side as compared to corresponding calcium contents as reported for cow milk co-precipitates.

5.10. Plant trials were conducted on the basis of laboratory experiments and the co-precipitates were spray dried instead of oven drying at an inlet air temperature of 195 $\pm$ 5^{o}C and an outlet air temperature of 105 $\pm$ 2^{o}C. All the three types of co-precipitates from buffalo skim milk were spray dried.

5.11. Large scale prepared low calcium co-precipitate was observed to have moisture, 2.80 per cent, fat 1.52 per cent, ash 8.20 per cent, calcium 2.06 per cent, phosphorus 1.12 per cent, lactose 3.19 per cent and protein 84.12 per cent with protein recovery of 93.90 per cent.

5.12. Large scale prepared medium calcium co-precipitate had a moisture content 3.21 per cent, fat 1.42 per cent, ash 9.86 per cent, calcium 2.90 per cent, phosphorus 1.30 per cent, lactose 2.32 per cent and protein 83.0 per cent with protein recovery of 94.80 per cent.

5.13. Large scale prepared composition of high calcium co-precipitate was observed to be; moisture 3.67 per cent,

fat 1.55 per cent, ash 11.45 per cent, calcium 3.60 per cent, phosphorus 1.58 per cent, lactose 1.48 per cent and protein 81.66 per cent with protein recovery of 95.57 per cent.

5.14. Spray dried low calcium co-precipitate was also prepared utilising electrodialysed buffalo skim milk. The product was observed to have the following composition: moisture 3.06 per cent, fat 1.47 per cent, ash 7.96 per cent, calcium 1.84 per cent, phosphorus 1.02 per cent, lactose 3.08 per cent and protein 84.28 per cent with protein recovery of 93.82 per cent.

5.15. Spray dried low calcium co-precipitate was prepared utilising an admixture of skim milk with four parts of buffalo milk cheddar cheese whey. The product had a moisture 3.40 per cent, fat 1.45 per cent, ash 7.15 per cent, calcium 1.50 per cent, phosphorus 0.95 per cent, lactose 2.76 per cent and protein 85.04 per cent with protein recovery of 91.67 per cent.

5.16. With the same number of washings given to all the three types of co-precipitates, the ash content of high calcium co-precipitate was approximately 1½ times, the ash content of low calcium co-precipitate. The lactose content of the high calcium co-precipitate was the lowest as compared to the other two types of co-precipitates.

5.17. The lactose content in the low calcium co-precipitate was about two fold as compared to the lactose content in high calcium co-precipitate. The medium calcium co-precipitate had a lactose content of 1½ times that of high calcium co-precipitate.

5.18. It was observed that the low calcium co-precipitate had the least flavour score (6.1) as compared to medium (6.6) and high calcium co-precipitates (7.4).

5.19. The colour of the high calcium co-precipitate was observed to be slightly whiter in comparison to the other two types of co-precipitates.

5.20. The solubility index was found to be highest in case of high calcium co-precipitate (12.0 ml) followed by medium (10.1 ml) and low calcium co-precipitates (8.1 ml), indicating highest solubility for low calcium co-precipitate as compared to the other two types.

5.21. The viscosity of high calcium co-precipitate was found to be highest in case of high calcium co-precipitate (20.79 cP)followed by medium calcium co-precipitate (10.51 cP) and low calcium co-precipitate (9.23 cP).

5.22. It was observed that the high calcium co-precipitate had a bulk density of 0.29 and 0.42 g/ml as loose and packed density respectively. The corresponding values for medium and low calcium co-precipitate were 0.25, 0.33 g/ml and 0.20, 0.31 g/ml respectively.

5.23. All the three types of co-precipitates were found to stabilise emulsions completely. They had highest emulsion stabilising capacity, retaining 100 per cent of emulsified oil.

5.24. The nutritional quality of co-precipitates as measured by Protein Efficiency Ratio was observed to be higher than casein (PER 2.5). The average PER values being 2.95, 2.82 and 2.75 for high, medium and low calcium co-precipitate respectively. This establishes the nutritional superiority of co-precipitates over casein.

5.25. The spray dried high calcium co-precipitate was utilised in the manufacture of ice cream. Replacement of skim milk solids upto 10 per cent with high calcium co-precipitate in the manufacture of ice cream resulted in satisfactory flavour, body and texture. The score for this type was almost at par with the conventionally prepared ice cream utilising SMP. With the increase in the quantity of high calcium co-precipitate, for replacing SMP, the ice cream scored low on flavour, body and texture.

5.26. Over-run studies of ice cream mix were carried out, utilising different percentage of high calcium co-precipitate replacing SMP, at 10, 30, 50, 70 and 100 per cent levels. At 10 per cent replacement level over run of 110 per cent was achieved. There was observed to be progressive decrease in the per cent over run as the concentration of co-precipitate increased.

5.27. On the basis of this study, it has been established that low, medium and high calcium co-precipitates can be manufactured from buffalo skim milk and these co-precipitates are almost similar in physico-chemical properties and nutritional value, as compared to the co-precipitates prepared from cow milk.

5.28. On the basis of our findings it may be concluded that, whenever, the skim milk is to be converted to casein manufacture, the most ideal course is to go for the manufacture of co-precipitates.

5.29. Further research is suggested on the use of marginally sour milk in the manufacture of co-precipitates. The utilisation of functional properties in different products need to be elaborately studied. Also large scale trials are recommended for laying down the standards as in case of different milk products.

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A P P E N D I X

Appendix 1: Quality of buffalo skim milk used in the manufacture of low, medium and high calcium co-precipitates

Type of co-precipitate	Trial No.	Particulars		Fat (%)	SNF (%)	Total solids (%)	True proteins (%)	NPN (%)	Ash		Acidity (%) L.A.	Alcohol test
		Holding time (min.)	Number of washings						Total ash (%)	Calcium (%)		
Low calcium co-precipitate	1	20	3	0.05	9.78	9.83	4.00	0.22	0.82	0.184	0.14	-ve
	2	20	3	0.05	10.00	10.05	3.95	0.19	0.80	0.168	0.15	-ve
	3	20	3	0.05	9.40	9.45	4.20	0.22	0.81	0.182	0.15	-ve
Average				0.05	9.73	9.78	4.05	0.21	0.81	0.178	0.146	-ve
Medium calcium co-precipitate	1	12	3	0.05	9.88	9.93	4.0	0.26	0.79	0.184	0.15	-ve
	2	12	3	0.05	9.80	9.85	4.0	0.25	0.80	0.184	0.16	-ve
	3	12	3	0.05	10.00	10.05	4.15	0.27	0.82	0.182	0.15	-ve
Average				0.05	9.89	9.94	4.05	0.26	0.80	0.183	0.153	-ve
High calcium co-precipitate	1	2	3	0.05	10.16	10.21	4.22	0.26	0.80	0.165	0.14	-ve
	2	2	3	0.05	10.00	10.05	3.98	0.25	0.78	0.186	0.15	-ve
	3	2	3	0.05	9.88	9.93	3.98	0.24	0.76	0.184	0.15	-ve
Average				0.05	10.01	10.06	4.06	0.25	0.78	0.178	0.146	-ve

Appendix 2: Quality of buffalo skim milk used in the manufacture of low calcium co-precipitate from electrodialysed skim milk, and an admixture of buffalo skim milk + whey.

Type of co-precipitate	Trial No.	Particulars		Fat (%)	SNF (%)	Total solids (%)	True proteins (%)	NPN (%)	Ash		Acidity (%)L.A.	Alcohol test
		Holding time (min.)	Number of washings						Total ash (%)	Calcium (%)		
Low calcium co-precipitate (Electrodialysed milk)	1	20	3	0.05	10.12	10.17	4.10	0.22	0.74	0.172	0.14	-ve
	2	20	3	0.05	9.86	9.91	3.90	0.20	0.75	0.170	0.14	-ve
	3	20	3	0.05	9.48	9.53	3.95	0.21	0.73	0.168	0.14	-ve
	Average			0.05	9.82	9.87	3.98	0.21	0.74	0.170	0.14	-ve
Low calcium co-precipitate (Quality of skim milk used with whey)	1	20	3	0.05	9.88	9.93	4.0	0.20	0.80	0.184	0.14	-ve
	2	20	3	0.05	9.78	9.83	3.98	0.23	0.78	0.186	0.14	-ve
	3	20	3	0.05	9.52	9.57	3.90	0.20	0.76	0.178	0.14	-ve
	Average			0.05	9.72	9.77	3.96	0.21	0.78	0.182	0.14	-ve

Appendix 3: Chemical composition of buffalo milk cheddar cheese whey

Constituent	Percent			Average
	I	II	III	
Total nitrogen g/100 g	0.134	0.158	o.134	0.142
Crude protein (N x 6.38) %	0.85	1.01	0.75	0.87
Ash, %	0.55	0.65	0.63	0.61
Fat, %	0.31	0.42	0.35	0.36
Lactose, %	4.82	4.98	4.96	4.92
Non-protein nitrogen mg/100 ml	31.20	33.50	32.65	32.45

Appendix 4: Production data for the low, medium and high calcium co-precipitate

Type of co-precipitates	Precipitation				Washing		Dispersion			Spray drying		
	Final temp. of heated milk maintained	Precipitant (Hcl: H_2O)	pH of precipitation	Characteristics of curd	pH of washing	Acidulent of wash water (H_2SO_4 : H_2O)	STPP added (%)	pH	TS (%)	Speed of atomizer (rpm)	Inlet air temp. (^oC)	Outlet air temp. (^oC)
Low	90°C	1:6	4.6	Hard & Lumpy	4.6	1:20	2	7.0	19±1	25000 ± 1000	195±5	105±2
Medium	90°C	0.06% $Cacl_2$ as 5% solution + 1:6 Hcl	5.4	Rubbery & Lumpy	4.6	1:20	4	7.0	16±1	25000 ± 1000	195±5	105±2
High	90°C	0.2% $Cacl_2$ as 5% solution	6.4	Soft & Non-plastic	4.6	1:20	6	7.0	15±1	25000 ± 1000	195±5	105±2