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PROTEIN - PECTIN INTERACTIONS IN COTTAGE CHEESE WHEY

BY

(C) KAPILA DEVKOTA

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND
RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
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THE UNDERSIGNED CERTIFY THEY HAVE READ, AND RECOMMEND TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH FOR ACCEPTANCE, A THESIS ENTITLED **PROTEIN-PECTIN INTERACTIONS IN COTTAGE CHEESE WHEY** SUBMITTED BY **KAPILA DEVKOTA** IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF **MASTER OF SCIENCE** IN **FOOD PROCESSING.**

.....

Dr. Pavel Jelen (Supervisor)

.....

Dr. Dimitri Hadziyev

.....

Dr. Arnost Horak

41K Jan 1991

To my mother, Phanendra K. Devkota
and
the sweet memories of my father, M. N. Devkota.

ABSTRACT

Interactions of pectin with whey proteins were investigated in various model systems and in mixtures of fruit juices and cottage cheese whey to study the effect of pectin in sedimentation of whey proteins in fruit juice-based whey beverages.

Different levels of pectin (0.05%-0.3%) were added to 1% whey protein concentrate (WPC) solutions or cottage cheese whey (CCW), and the solutions were adjusted to three different pH levels (3.4, 3.7 and 4.0).

Upon addition of pectin, visual sedimentation was observed in 1% WPC solutions or CCW at all levels of pH studied. Amount of sediment increased and protein content of the supernatant decreased with the increasing levels of the pectin added. Analysis of sediment showed the presence of pectin and protein indicating the ability of pectin to interact with whey proteins in 1% WPC or CCW resulting in the formation of insoluble complexes.

Effect of natural pectin present in a fruit juice was observed by mixing various fruit juices (apple, cranberry, grapefruit, lime, rhubarb or tomato) separately with cottage cheese whey in a ratio of 1:9 and then heating at 90°C for 15 minutes. Turbidity development was noted in all cases except for cranberry which may have been due to the pretreatment of the pectin present in this commercially processed juice. Highest turbidity development was found in tomato juice and whey mixture. Presence of pectin and protein in the

sediment obtained from (1:1) tomato juice-CCW mixture as well as elimination of the turbidity development after heating in the 1:9 tomato juice-CCW mix by using tomato juice treated with the enzyme polygalacturonase strongly indicated the pectin protein interaction to be responsible for the turbidity development or sedimentation in fruit juice based whey beverages.

The mixture of rhubarb and CCW showed immediate sedimentation even without heating due to formation of calcium oxalate as a result of interaction between calcium present in the whey and oxalic acid present in the rhubarb juice.

The prototype whey drink made by mixing commercially available tomato juice and CCW in 1:1 ratio showed similar acceptability in various parameters (color, consistency, taste and overall liking) as compared to commercially available tomato based juice, clamato. No visual sedimentation was observed in the prototype and the turbidity that might have developed during heat treatment, was totally masked because of the intense red colour and presence of suspended particles in the commercial tomato juice.

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Whey protein concentrate.....WPC

Cottage cheese whey.....CCW

HIgh methoxy pectin.....HM pectin

Low methoxy pectin.....LM pectin

1. INTRODUCTION

1.1 Potential for Cheese Whey Utilization

Whey, the by-product of cheese making, contains more than half the solids present in original whole milk (Hayes 1985). The composition of whey varies depending upon whether it is acid whey or sweet whey as shown in Table 1.1.

Table 1.1: Approximate composition of sweet and acid whey.

Components (% by weight)	Sweet whey (pH 5.9-6.3)	Acid whey (pH 4.3-4.6)
Water	93.65	93.50
Total solids	6.35	6.80
Fat	0.30	0.04
Protein	0.80	0.75
Lactose	4.65	4.90
Minerals	0.50	0.80
Lactic acid	0.05	0.40

Adapted from Hayes (1985)

In the old days, whey was considered a waste product to be disposed of by the cheapest possible method. However, the steady

increase of the volume of cheese production every year, in the last three to four decades, has resulted in the production of higher amount of whey, creating a big problem of whey disposal in the dairy industry.

Disposal of whey to water bodies such as lakes and rivers causes water pollution due to high biological oxygen demand of the whey (Hill, 1982). Therefore, in this era of increased awareness of environmental pollution, there is a great pressure to find a satisfactory solution for the utilization of surplus whey. Besides the problem of water pollution, the presence of various valuable nutrients in the whey makes the disposal of it as waste product highly undesirable.

In addition to lactose and proteins, the two common nutrients provided by whey, several vitamins and minerals required in minor quantities for adequate human nutrition can be obtained from whey (Glass and Hedrick, 1976).

Among these minerals, calcium is the most important nutrient present in the whey. In many developing countries like India and Nepal, where the diet of most native people is deficient in calcium, whey products can play an important role. Rangappa and Achaya (1974) have pointed out that daily consumption of 200 mL of whey for three days by people in one of the villages in India with poor rice diet, was enough to raise the calcium balance.

The protein present in whey is considered to be of high nutritional quality (Forsum, 1974) because of the presence of high

quantities of essential amino acids. The amino acid composition of dried whey protein is given in Table 1.2.

Table 1.2: Amino acid composition of dried whey proteins.

Amino acids	g/100 g
Lysine	10.3
Histidine	2.3
Arginine	2.8
Tryptophan	2.4
Aspartic acid	10.2
Threonine	4.9
Serine	4.7
Glutamic acid	18.4
Proline	6.4
Glycine	1.7
Alanine	4.1
Cystine	2.2
Valine	5.2
Methionine	1.8
Isoleucine	5.4
Leucine	10.5
Tyrosine	3.1
Phenylalanine	3.7

Adapted from Glass and Hedrick (1976).

There seem to be several ways available for the utilization of whey. Dry whey powder, lactose or whey protein concentrates can be

produced from whey, and these can be incorporated into human food or animal feed. However, for one or another reason, in spite of the extensive research being carried out, a satisfactory solution for the problem of the utilization of surplus whey has yet to be found.

In recent years, the production of whey protein concentrates (WPC) has received much attention, because of the presence of high quality protein in the whey. But the expensive technology involved in the production of WPC makes several other products consisting of whey, contemplated for human consumption, of nutritional interest (Forsum and Hambraeus, 1976).

The simplest and most logical use of whey in the human diet seems to lie in the production of whey beverages due to high water content of the whey. According to Prendergast (1985), the utilization of whey as a drink has a twofold advantage in that whey can be used directly from a cheese vat avoiding the problem of disposal, and the requirements for processing equipment are simple.

A great deal of effort has been exerted in the area of utilization of unprocessed or modified whey as the basis of a drinkable product (Holsinger *et al.*, 1974; Kravchenko, 1987).

From a nutritional point of view, the utilization of unprocessed whey in the formation of whey-based drinks is desirable. Whole whey drinks are characterized by their high nutritional value because of the presence of carbohydrates, proteins and mineral components (Kravchenko, 1987). However, the heat instability of the whey proteins could interfere in the quality

maintenance of the whey-based drinks due to the thermal sedimentation of the whey proteins resulting from heat processing. This problem can be overcome by adjustment of the pH of the product to a range of 3.4 - 3.8, where whey proteins are quite stable to heat coagulation (Jelen and Buchheim, 1984). The presence of higher amounts of calcium in acid whey increases the resistance of whey proteins to heat induced denaturation, compared to the proteins in sweet whey (Patocka *et al.*, 1986). Thus acid whey seems more suitable for production of the whey-based drinks.

The unpleasant insipid taste of whey becomes a barrier in the production of a beverage consisting entirely of whey (Webb, 1938). Hence addition of a proper fruit or vegetable juice having a pleasant flavour is essential to make the drink palatable. However, finding a compatible flavour could be a difficult task (Jelen, 1990). The beverage produced by mixing of a fruit juice and whole whey combines the benefit of having fruit based vitamins and dairy based calcium and whey proteins.

Despite the simple technology involved in the production of fruit juice-based whey drinks, satisfying proper quality criteria is very difficult. The problem of sedimentation is often observed in these kinds of products thus resulting in frequent market failures (Jelen, 1990). This seems to be the reason for the scarcity of truly successful fruit juice-based whey beverages and subsequent disappearance of several whey drinks from the market in the past, in spite of major marketing efforts (Jelen, 1990).

Jelen *et al.*, (1987) investigated the sedimentation in various commercial whey drinks. Sedimentation was found only in the products which contained whey proteins. This indicates that proteins appear to be the primary reason for sedimentation in these products. Because the pH of the drinks in which sedimentation was observed was below 3.4 (a level where whey proteins should be heat stable), the sedimentation was most likely not the result of the heat sensitivity of the whey proteins. Hence there must be some alternative reasons for the sedimentation.

A prototype fruit juice-based whey drink made at the University of Alberta, showed high turbidity development and substantial amount of sedimentation following ultra high temperature (UHT) processing (Jelen, personal communication). The sediment thus obtained showed presence of 55% protein on a dry weight basis (Devkota, unpublished data). This confirms protein to be one of the components responsible for the sedimentation. However, because the drink was processed within a pH range at which whey proteins are heat stable, the sedimentation should not have resulted from the heat coagulation of the the whey proteins alone. This leads to the possibility of the sedimentation of whey proteins due to interaction with various fruit juice components such as tannins and pectins.

Because of the presence of pectin in almost all fruit juices and its ability to interact with proteins (Serov *et al.*, 1985), pectin was suspected to be the fruit juice component responsible for the

interaction with whey proteins resulting in the sedimentation. In view of the scarcity of information regarding the interactions of whey proteins with pectin, it was decided to study the role of pectin in the precipitation of whey proteins in more detail.

1.2 Research Objectives

The main objective of this project was to investigate the interactions of pectin with whey proteins and the effect of the interaction on the sedimentation of whey proteins in fruit juice-based whey beverages. The specific objectives included the following aspects:

(1) Interactions of high and low methoxy pectins with whey proteins in model systems using whey protein concentrate (WPC) at pH levels 3.4, 3.7 and 4.0.

(2) Interactions of an industrial pectin preparation with whey protein in cottage cheese whey (CCW) at pH 3.4, 3.7, and 4.0.

(3) Interactions of natural pectins present in fruit juices with whey proteins using mixtures of various fruit juices and CCW.

(4) Effects of pectin added as a stabilizer in fruit juices-CCW prototype whey drinks.

(5) Evaluation of a prototype tomato juice based whey drink and its acceptance in comparison with a similar commercially available non whey-based drink.

2. LITERATURE REVIEW

2.1 Whey

Whey is the yellowish green serum that separates from curd during cheese making or casein manufacture (Kosikowski, 1979). Whey contains lactose, water soluble proteins, mineral salts and traces of fat.

Protein present in the whey is one of the best proteins available for human nutrition (Webb, 1972). Presence of calcium and lactose makes whey a good source of mineral and energy. Whey is also rich in vitamins particularly those belonging to the B group (Evans, 1980).

Depending upon the way coagulum has been separated from the milk, whey can be divided into two types, i. e. sweet and acid whey. Sweet whey is obtained when the rennet type enzymes are used to get the coagulum whereas the acid whey is obtained when the coagulum is formed by acidification (Zall *et al.*, 1979). Sweet whey has an approximate pH value of 5.9 - 6.3 and is produced during hard cheese manufacture. Acid whey, on the other hand, is obtained during the manufacture of soft cheese such as cottage cheese or quarg or industrial casein and has a pH value of 4.3 - 4.6 (Hayes, 1985). Besides pH the main difference between acid whey and sweet whey lies in the content of calcium and phosphate ions. Acid whey contains almost twice the level these ions as compared with sweet whey

(Muller, 1981). Moreover, there is more lactic acid, and less lactose present in acid whey than in sweet whey (Kosikowski, 1979).

2.1.1 Availability

About 600-900 gram of whey is obtained from every kilogram of milk used for cheese, cottage cheese or industrial casein production (Jelen, 1979). According to International Dairy Federation (Milk Production and Processing Statistics), about 90 million metric tons of whey was produced world wide in 1983. However there has been an increase in whey production in recent years as a result of increase in world wide cheese production to fulfill the demands of the increasing population (Marwaha and Kennedy, 1988).

Despite the great deal of research in the area of whey utilization, effective utilization is still at very low level and more than 50 % of the whey is dumped as waste world wide.

Considering the various valuable nutrients present in the whey, dumping of whey is a great loss of potential food in addition to the creation of a water pollution problem. Owing to the presence of lactose and to a much lesser degree proteins, whey shows a high biological oxygen demand (BOD) value of 30,000-50,000 ppm on waste water treatment plant or on the land (Marwaha and Kennedy, 1988). So if whey has to be dumped in lakes or other water bodies, it needs to be treated in order to reduce the BOD to less than 15 ppm (Hill, 1982). This leads to the requirement of additional money of

about 0.6-4.4 cents per pound of cheese produced (Marwaha and Kennedy, 1988). Therefore instead of creating a water pollution problem by dumping the valuable nutrients present, utilization of whey in human nutrition, in some form, would be highly desirable.

2.1.2 Utilization

About three decades ago, the utilization of whey was almost totally limited to animal feed (Mann, 1987). However, today, because of the recent developments in the processing technology (such as ultrafiltration, reverse osmosis, new drying methods, fermentation and protein fractionation), whey has become the most versatile by-product in the food industry (Hayes, 1985).

There are many potential uses for whey. It could be utilized as whole whey or through the fractionation to its constituents (Coton, 1985). Whey protein and lactose are two major fractionation products of whey.

Whey protein can be recovered from the whey by various processes such as heat coagulation and membrane separation. Since heating results in denaturation of the whey proteins, usually membrane processes like ultrafiltration (UF) are used to obtain high quality whey protein concentrate (Texieira *et al.*, 1983). The whey protein concentrate can be incorporated into various foods such as meat products, bakery products and baby foods.

Another well established fractionation process is the lactose crystallization from the whole whey or UF permeate. Lactose is generally used in baby foods and in pharmaceuticals. But the market demand for lactose is such that a very small percentage of the total whey production can fulfill it. Lactose hydrolysis may be a way to expand the utilization of lactose present in the whey, as a sweetener (Shukla, 1975). Lactose can also be used as sweetener after chemical modification to lactitol, which is a polyol and can be obtained from lactose at a reasonable price (Booy, 1987). Being non cariogenic and well tolerated by human beings including diabetics, lactitol can be used as a bulk sweetener in various food products.

The whole whey can be used as is in animal feed or as a basis for different kinds of beverages. Whole whey may also be converted to dry whey powder. Generally, dry whey powder is recovered from whole whey by concentration and drying by large industrial whey processors (Jelen, 1979). The whey solids can be used as an ingredient for human foods or animal feed. However the high water content of whey makes the condensation and/or the drying processes expensive. Moreover, for small whey producers, purchasing of the equipment for further processing of whey may be economically prohibitive (Cripper and Jeon, 1984). Thus, the use of whey as a basis for beverage production seems to be the simplest, inexpensive and most practical outlet for the return of whey into the human diet.

Despite the pressure for whey utilization because of the environmental pollution problem and the simple technology involved

in production of whey beverages, even today, whey drinks are not commercially available in many countries (Jelen 1979). Dodds (1989) suggested that the major problem with the utilization of whey in North America is the lack of enough research and commercial development for a successful whey beverage product.

2.2 Whey Beverages

Use of whey as a beverage in human nutrition is not a new idea as there is a record of drinking whey particularly for therapeutic reasons already in the ancient Greek period (Prendergast, 1985). Use of whey in middle ages and as late as 1940's for the treatment of various diseases such as tuberculosis, arthritis, gout, liver disease and dyspepsia has also been reported (Holsinger *et al.*, 1974).

Whey drinks seem to have many advantages. Whey has been found to be a good thirst quencher. According to Prendergast (1985), whey drinks are light and refreshing and are less acidic as compared to many fruit juices. Being rapidly assimilable as compared to milk, whey seems to be an ideal metabolic substrate. Moreover, whey drinks contain many beneficial components such as calcium, phosphate, vitamins B1, B2, B6 and amino acids, if whey proteins are included.

Both alcoholic and non alcoholic drinks can be made from whey. Some whey based beverages are listed in the Table 2.1.

Table 2.1: Examples of some commercially available whey based drinks

Name	Characteristics
Rivella	Clarified whey fermented with lactic microorganisms and concentrated. Carbonated after addition of Swiss alpine herbs extracts for typical flavor.
Way-Mil	Homogenized, concentrated, clarified whey with flavour and stabilizer added.
O-Way	Concentrated whey and orange juice (4:1).
Bodrost	Whey with raisins, beet sugar and caramel. Fermentation as in Kefir.
"Whey Champagne"	Clarified whey, sugar and caramel. Yeast used for fermentation.
Whevit	Deproteinated fat free whey with sugar, citric acid and colour. Fermented to low alcohol content using yeast.

Adapted from Robinson and Tamime (1978).

Whole whey or deproteinized whey can be used to produce whey drinks. Deproteinized whey is used to avoid the sedimentation of the whey proteins during thermal processing and/or to give clearness to the products. Rivella, the second most popular soft drink in Switzerland after coke, is produced from deproteinated whey (Annonymous,1985; Annonymous,1960). Whey is usually deproteinated by heating and removing sediments by filtration or centrifugation or by using membrane processes. However, protein retention in whey drinks would be highly desirable owing to the higher biological value of the whey proteins as compared to other proteins such as whole egg proteins, milk protein, and beef proteins (Werner, 1981). Such a preparation also eliminates one processing step i.e. the removal of the protein by heating or ultrafiltration.

Whole whey beverages are of sufficient nutritive and biological value because of their protein, carbohydrate, and mineral components (Kravchenko, 1987). Thus the beverages made from whole whey have been recommended especially for the people with increased vital energy expenditure (Crippen and Jeon, 1984; Kravchenko, 1987). This could be the reason for adaptation of a whey drink as the official drink for the 1984 winter olympics at Sarajevo (Prendergast, 1985).

Non alcoholic drinks made from whole whey could be carbonated or non carbonated and are usually made by mixing with various fruit juices for the purpose of desirable flavor profile development.

2.2.1 Characteristics of fruit juices

Fruit juice, extracted from fully developed mature fruit, contains about 80-90% of water with various chemical components suspended or solubilized in it. Besides water, the most common chemical components present in fruit juices are starch, sugars, organic acids, vitamins, minerals, pectin and tannins (Charley and Harrison, 1950).

The simple carbohydrates present in juices are mainly glucose, sucrose or fructose, all of which are rapid sources of energy. Organic acids such as citric, malic, tartaric, which are present in fruit juices, influence the flavour of juices and can also have stimulating effect on digestive glands (Charley and Harrison, 1950).

Fruit juices represent a good source of vitamins such as vitamin A, riboflavin, nicotinic acids, pantothenic acid, and ascorbic acid (Pollard, 1950). Even though all the fruit juices are considered to be a good source of vitamin C, the amount varies considerably with the type of juice as shown in the Table 2.2.

Beside vitamins, most fruit juices contain adequate supplies of mineral salts needed for the metabolic requirements of a human body (Charley and Harrison, 1950).

The amount of pectin present in juices varies from juice to juice. Pectin present in juices are highly responsible for the viscosity, texture and appearance of the juices (Reid, 1950). Tannins

are also found in fruit juices in varying amounts, and may have an influence on the flavour of the juices (Charley and Harrison, 1950).

Table 2.2: Vitamin C content of some fruit juices.

Juice	Vitamin C content (mg/100 mL)
Orange	30-65
Grape fruit	37-50
Lemon	30-55
Lime	8-62
Apple	2-34
Tomato	16-33
grape	1-3
Loganberry	10-20

Adapted from Pollard (1950)

From a nutritional point of view, fruit juices, when mixed with whey, not only give the pleasant addition to the taste but also provide various important nutrients such as vitamins and minerals.

2.2.2 Fruit juice based whey beverages

Various fruit juices can be mixed with whey to produce delicious drinks. This kind of drink can be used instead of fruit juice as breakfast drink, provided that proper quality of the drink is maintained.

Many previous workers in this field have tried making prototype fruit juice-based whey drinks by mixing whey with various fruit juices such as orange, grapefruit, mango, passionfruit, peach, pineapple, guava and tomato (Gagrani *et al.*, 1987; Brunner *et al.*, 1969; Nelson and Brown, 1969; Holsinger *et al.*, 1974; Webb, 1938). All these drinks have been reported to have good acceptability among the test panelists, but most of them seem to be found only in the research papers and not in every day markets.

Despite the simplest technology and great deal of research involved in the production of the fruit juice based whey drinks, very few have established good market presence in the past due to the difficult commercialization of the product in the competition with other beverages (Jelen, 1990). The main reason for this seems to be the fact that consumption of beverages is usually based on their sensory or physiological appeal with nutrition being only the secondary consideration (Towler, 1982). However, a dramatic increase in the production of fruit juice whey drinks has been reported in some European countries probably due to increasing awareness of the modern consumers regarding nutritional value of foods (Iverson, 1984; Jelen *et al.*, 1987). The obvious reason for this

is the availability of calcium and whey proteins from the whey component and different vitamins from the juice component (Jelen, 1990). Some of the fruit juice based whey beverages recently available on the European markets are listed in the Table 2.3.

Table 2.3: Some commercially available fruit juice based whey drinks.

Country	Commercial Name of the Product	Type of Fruit Juice
Germany	Frusighurt	Apple, lemon (10%)
	Frucht-molke	Peach, maracuya, passion fruit, apple
Switz-erland	Fit	Mango or grapefruit (25 %)
Austria	Latella	Mango, maracuya, grape-fruit, lime
France	Morea	mix. of mango, guava, kiwi, passion fruit (40%)
Holland	Taksi	Tropical fruit juice conc (6.3%) + colouring
Finland	Hedelmatarha	Mango or tropical fruit juice mixture with lactose hydrolyzed whey

Adapted from Jelen, 1990.

In spite of all those listed and many more whey beverages found on European markets, hardly any whey beverage is available in North America. Thus one could say that whey beverage technology is still at infancy level and needs innovation and development. "Thumbs Up" and "Nature's Wonder" are the only two commercially prepared beverages available in the United States of America at the present time and neither of them has been particularly commercially successful (Dodds, 1989).

"Nature's wonder", made from blend of orange, pineapple and passion fruit juice and whey, was also introduced in Canada with great hope of success but failed to become a successful commercial venture (Annonymous, 1983). This could be because of the lack of consumer appeal for whey as a drink. However "Nature's Wonder" is still available in Edmonton Safeway Stores.

Most of American consumers "think of whey as pig food and nothing else" (Dodds, 1989). This could be the reason why Rivella, one of the most popular soft drinks in Switzerland, could not gain any popularity in Quebec when it was tried in a large scale test (Jelen, personal communication).

Nevertheless as suggested by Dodds (1989), because of the increased awareness about health and environmental pollution, the right time has come in America to produce a whey beverage. He concludes: "The knowledge and technology is there, all that is needed is a little innovation, ingenuity, hard work and some luck". However,

even though whey beverage technology looks very simple, in real practice, it could encounter various technological problems.

2.2.3 Processing of fruit juice based whey beverages

The use of the right kind of fruit juice to mix with whey is the first essential step in production of fruit juice based whey drinks. Not all fruit juices will be compatible with whey due to their peculiar flavour or other reasons. The fruit juice that is to be mixed with whey should have a flavour that blends well with whey flavour. Citrus juices, in particular the orange flavour, have been reported as the most suitable for whey (Brunner, *et al.*, 1969; Holsinger *et al.;* 1974).

The high lactose content of the whey may also hinder the successful commercialization of the fruit juice based whey drinks. Since lactose is much less sweet than sucrose, sweeteners are required to be added in such products. This high calorie content may create a problem for the diet conscious consumers. However, use of intense sweeteners such as aspartame and acesulfame-K together with hydrolysis of lactose into its monosaccharide units glucose and galactose, could solve the problem to some extent (Beukema, 1990).

Heat instability of the whey protein seems to be a major problem in the utilization of whole or unprocessed whey for the production of fruit juice based whey drinks. This problem could be minimized by proper adjustment of pH before the thermal

processing. Whey proteins are stable to heat coagulation at pH below 3.8 (DeWit, 1981; Jelen and Buchheim, 1984; Patocka *et al.,* 1986). Hence adjustment of the products to pH between 3.4 and 3.7, a pH range suitable for the thirst quenching drinks, could avoid the problem of loss of product quality due to heat sedimentation of whey proteins alone.

However, sedimentation has been reported in fruit juice based whey drinks even if they are processed at pH below 3.8 at which heat sedimentation of whey proteins should not occur. Jelen *et al.,* (1987) reported sedimentation upon centrifugation in fruit juice whey drinks even at pH levels as low as 3.1 and 3.0. In our preliminary investigations, heat induced turbidity was observed at pH 3.4, when mixtures of fruit juice and whey were heated (Devkota, unpublished data). The turbidity did not develop when fruit juice or whey were heated separately, or when fruit juice mixed with UF permeate was heated. This observation indicates the possible interaction of whey component such as whey proteins with fruit juice components including pectins and tannins and other possible coagulants. However the presence of protein (55% by weight) in the sediment obtained from one of the UHT processed fruit-juice prototypes developed at the University of Alberta, shows whey protein to be the main component responsible for the sedimentation.

2.3 Whey Proteins

About 20% of the total milk proteins is present in whey (Morr, 1979). Whey proteins, unlike casein, remain soluble at pH 4.6 typical for acid whey.

Whey proteins include different globular proteins such as β-lactoglobulin, α-lactalbumin, serum albumin and immunoglobulins (Gordon and Kalant, 1980). Among them, β-lactalbumin is the most important whey protein both qualitatively and quantitatively (DeWit, 1981). The different whey proteins have different properties as shown in the Table 2.4.

Table 2.4: Some important characteristics of whey proteins.

Protein type	weight contribution (g/L milk)	Molecular weight (daltons)	Isoelectric point (pH)
β-lactoblobulin	3.0	18,400	5.2
α-lactalbumin	1.2	14,200	5.1
Bovine serum albumin	0.3	69,000	4.8
Immunoglobulin	0.5	160,000	5.5-6.8

Adapted from DeWit (1981)

Whey proteins are quite sensitive to heat resulting in denaturation and subsequent precipitation in a pH range near 4.6 and are least sensitive at a pH range between 2.5-3.5 (DeWit, 1981). Unfolding of the globular proteins occurs as a result of the heat denaturation. The resulting random coil is more susceptible to protein-protein interactions (Morr, 1975). Besides heat sensitivity, whey proteins also show interactions with various chemical components such as polysaccharides, fats and tannins.

Among polysaccharides, carboxymethylcellulose and pectin have been reported to interact with whey proteins (Serov *et al.*, 1985; Hansen *et al.,* 1971; Hill and Zadow, 1974; Hill and Zadow, 1978; Hidalgo and Hansen, 1971; Hansen and Balachandran, 1983).

2.4 Protein-Polysaccharide Interactions

Interactions between polysaccharides and proteins resulting in the formation of various complexes have been found to play an important role in many biological systems such as in connective tissue of animals, different membranes found in eggs, the action of blood anticoagulants and antigen/antibody interactions (Ledward, 1978; Snoeren *et al.,* 1975). The interaction is also possible in vitro when proteins and polysaccharides are mixed under appropriate conditions.

Anionic polysaccharides such as carboxymethylcellulose (CMC), alginate and pectate may exhibit interactions with various proteins,

whereas there is little or no interaction between proteins and non ionic polysaccharides like guar gum and locust bean gum (Hansen *et al.*, 1969a; Ganz, 1974).

Among anionic polysaccharides, CMC is the most widely studied compound in the area of interaction with proteins (Imeson *et al.*, 1977; Ledward, 1978; Hansen *et al.*, 1971; Hill and Zadow, 1974; Hansen and Balachandran, 1983; Hidalgo and Hansen, 1971). Because of the ability to interact with proteins, CMC has been suggested as a means to isolate whey proteins at pH between 3 - 4, and to prevent precipitation of milk protein in fruit flavoured milk drinks in a pH range of 4-5 (Asano, 1966).

Very little fundamental work appears to have been done in the area of interaction of protein-polysaccharide systems containing pectates and alginates, both of which are widely used in food industry (Imeson *et al.*, 1977).

The anionic polysaccharides such as CMC, pectate and alginate have been found to interact with bovine serum albumen and myoglobin (Imeson *et al.*, 1977). Interaction of blood plasma proteins with sodium alginate and sodium pectate has also been reported (Imeson *et al.*, 1978).

CMC can interact with β-lactoglobulin and α-lactalbumin below their isoelectric point (Hidalgo and Hansen, 1971). The strongly sulfated polysaccharides such as carrageenan and cellulose sulphate can also interact with β-lactoglobulin in acidic pH (Hidalgo and Hansen, 1969b). Properties these complexes are similar to the

β-lactoglobulin-CMC complex, however the complex could not be readily dissociated as in the case of the complex formed with CMC.

An interaction of κ-carrageenan with κ-casein (which constitutes about 12% of all casein) has also been reported (Anderson, 1962). Because of the specific interaction with milk protein, carrageenan is widely used in dairy industry (Snoeren *et al.*, 1975).

2.4.1 Nature of interactions between proteins and polysaccharides

The interaction between a protein and a polysaccharide has been suggested as electrostatic in all the studies covering this area (Ledward, 1978; Ganz, 1974; Hidalgo and Hansen, 1969a). The interaction is dependent on pH and several other factors such as ionic strength, concentration and molecular weight of the reactants.

Dependence of the interaction on the charge carried by the macromolecules has been suggested from the study of the interaction of myoglobin and bovine serum albumin with pectate, alginates and CMC (Imeson *et al.*, 1977). The intensity of the interaction diminishes with increasing ionic strength and increases with change of pH from 7.0 to 5.0 due to increased number of positive charges on the protein molecules (Ledward, 1978).

According to Imeson *et al.* (1977), the electrostatic interaction between a protein and a polysaccharide can be used to

explain the fact why the interaction is stronger with myoglobin at pH 6.0 (at which it has a net positive charge) than bovine serum albumin, which has a net negative charge at that pH. Furthermore, Imeson *et al.,* (1977) give enthalpy values to provide tentative support for the electrostatic nature of the interactions.

The electrostatic interaction should involve carboxyl groups of polysaccharide and positively charged Ɛ-amino, α-amino, guanidium and imidazole groups. Thus the actual strength of the interaction will be dependent on the number and distribution of positively charged sites and overall net charges of the protein (Imeson *et al.*, 1977).

Heating has been reported to result in the formation of more stable complexes between proteins and polysaccharide molecules (Ledward, 1978). This could be due to increase in the "buried" basic groups of proteins as they are liberated during heat denaturation.

2.4.2 Polysaccharide-protein interactions in protein recovery

Anionic polysaccharides have been suggested for recovery of proteins from dilute effluents such as those from dairy and meat industries (Hansen *et al.*, 1971; Hill and Zadow, 1974; Imeson *et al.* 1978). This idea came after Gortner (1949) reported the interaction of negatively charged polymers and proteins resulting in the precipitation of the reactants (Ledward, 1978).

Protein from a polysaccharide-protein complex can be separated by increasing the pH or the ionic strength.

Smith *et al.* (1962), showed that a number of edible polysaccharides including alginic acid could be used to precipitate the protein from soya bean whey effluents.

Use of CMC in the recovery of whey proteins was quite extensively studied (Hansen *et al.*, 1971; Hill and Zadow, 1974; Hill and Zadow, 1978; Hidalgo and Hansen, 1971; Hansen and Balachandran, 1983). CMC can interact with both β-lactoglobulin and α- lactalbumin below their isoelectric point. An insoluble complex is formed due to interaction of β-lactalbumin with CMC and maximum precipitation occurs at pH 4.0. However, at that pH, α-lactalbumin/CMC complex is soluble (Hidalgo and Hansen, 1969b). This difference in the solubility could be used to fractionate whey proteins (Hansen *et al.*, 1971).

The interaction between CMC and whey proteins occurs only at lower ionic strength and no interaction is observed above the ionic strength of 0.2. Hence two fold dilution of whey is required for effective whey protein precipitation (Hill and Zadow, 1978).

Whey proteins can also be isolated using pectin as a complexing agent (Serov *et al.*, 1985). Protein yield in the complex was found to be dependent on the pH values and the relative pectin content in the system. Highest protein yield was found at pH 3.4

with ionic strength of 0.01. However no other confirmatory work has been carried out in this area.

CMC, sodium alginate and pectate have been used to recover blood plasma proteins (Imeson *et al.*, 1978). Alginate was found to be more effective as compared to pectate and CMC, in precipitating proteins at the low pH range of 3.5-4.0. Higher charge per unit residue for the alginate as compared to pectate and CMC could explain why the alginate is more effective (Ledward, 1978). Moreover, steric factor may also be one of the reasons. According to Imeson *et al.* (1978) the "kinks" in the pectin molecule introduced by L-rhamnopyranose units, may affect the number of freely accessible carboxyl groups available for participation in the protein pectate interaction. However pectate was found to be slightly more effective than alginate in destabilizing myoglobin at pH 6.0 (Imeson *et al.*, 1977). A different explanation is required for this kind of circumstances (Ledward, 1978).

2.5 Pectin

Pectin is a natural colloid present in higher plants including fruits and vegetables and is one of the five components of dietary fibres (Gregory, 1986; Biag and Cerda, 1983). It is present in cell walls of higher plants, associated with cellulose as an intercellular "cementing" material, appearing early during plant cell wall biogenesis (Biag and Cerda 1983). In plants, water soluble pectin is derived by hydrolysis of protopectin, which is insoluble in water

(Koster *et al.*, 1989). Being water soluble, pectin is present in most fruit juices. However the level and kind of pectin present differs in different juices.

2.5.1 Structure

Pectin is a polysaccharide composed primarily of a linear polymer of D-galactopyranosyluronic acid units joined in α-D (1-4) glycosidic linkages (Koster *et al.*, 1989) as shown in the following Figure 2.1.

Figure 2.1 has been removed due to the unavailability of copyright permission.

Fig. 2.1: Structure of pectin (After Koster *et al.*, 1989).

However, the regular structure of pectin is interrupted with insertion of 1-2 linked L-rhamnopyranosyl units, resulting in the

"kinking" of the linear polygalacturonic backbone (Thibault and Rinaudo, 1986), as shown in Fig. 2.2.

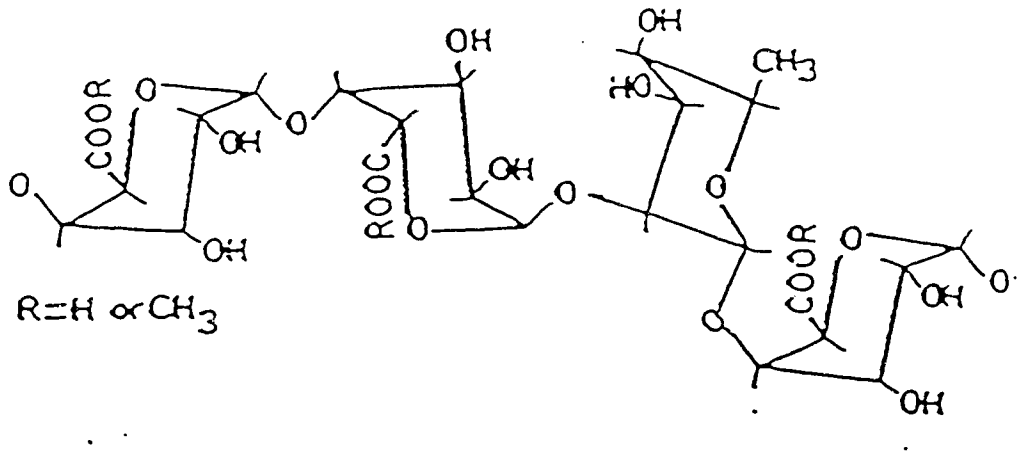

Fig. 2.2 Schematic illustration of the "Kink" in the pectin molecule (Adapted from Toft, 1982).

Whether L-rhamnopyranosyl units in the linear structure are evenly or unevenly distributed is a matter of argument (Bemiller 1986). According to some workers (Ress and Wight 1971), in citrus, apple and sunflower pectins, the rhamnopyranosyl units are more or less evenly distributed in the galacturonan chain occuring after about every 25 units .

The configuration of the rhamnopyranosyl linkage is not clearly known. However, calculations have shown that it should be

beta so as to provide the necessary degree of "kinking" in the structure (Bemiller, 1986).

Other neutral sugars such as galactose, arabinose, glucose and xylose are also present in the pectin as side chains (Thibault and Rinaudo, 1986). Total neutral sugar content differs with the source, extraction conditions and subsequent treatment. Presence of neutral sugars as side chains creates "smooth" and "hairy" regions within the pectin molecule (Brigand *et al.*, 1990).

In all natural pectin, some of the carboxyl groups are found in methyl ester form (Bemiller, 1986). The total number of esterified galacturonic acid units in the pectin molecule differs from source to source. Ratio of the esterified galacturonic acid units and total galacturonic acid units gives the degree of esterification in a pectin molecule (Towle and Christensen, 1973).

2.5.2 Types of pectin

Depending upon the degree of esterification (DE), pectins are divided into two broad groups. Pectin having DE higher than 50% is known as high methoxy pectin and pectin having DE lower than 50% is known as low methoxy pectin.

High methoxy pectins are further divided into three categories depending upon the degree of esterification, as shown in the following Table 2.5.

Table 2.5: Categories of high methoxy pectin with approximate degree of methoxylation and setting times.

Type	Degree of methoxylation	Setting time (Sec.)
Rapid set	72-75	20-70
Medium set	68-71	100-150
Slow set	62-66	180-250

Adapted from Crandall and Wicker (1985).

Natural pectins in most fruits are found in high methoxy forms (Christensen, 1982). However, high methoxy pectins can be converted to the low methoxy form by treating with different agents such as acid, alkali and enzymes (Kawabata *et al.*, 1981). Degree of esterification of the pectins found in various fruits differs from species to species. For example, pectin from apple normally has DE above 80% and citrus pectin can have DE up to 75% or more (May, 1990). Pectin present in fruit juices should be mostly of the high methoxy type, since pectin in fruits is found in high methoxy form. However, with time, high methoxy pectin (present in juices) could be converted to the low methoxy forms, particularly in citrus juices if

the enzyme pectin esterase is not properly inactivated (Baker and Bruemmer, 1972).

2.5.3 Industrial production

Even though pectin is present in most plant tissues, only a very limited number of plant species have been used as a raw material for the commercial pectin production. The most preferred materials include especially apple pomace and citrus fruit rinds (Brigand *et al.*, 1990). The reason for this could be the fact that world supply of citrus peel and apple pomace from juice industries far exceeds the possible demand for pectin production (Towle and Christensen, 1973). Banana peels, guava fruits and seed receptacle of sunflower are other potential sources for pectin production (Simpson *et al.*, 1984).

Percentage of pectin present in various fruits differs very strongly from species to species. Fine structure of the pectin is also dependent on the kind of fruits from which the pectin is derived (Koster *et al.*, 1989). Among the sources used for the commercial pectin production, lime peels are considered to be the best raw material (Gregory, 1986). Beside high yield, pectin obtained from lime peels is pure, lighter coloured and has high average molecular weight showing higher gel strength. But, according to Koster *et al.* (1989), apple pectin gives a better gelling performance and higher viscosity due to higher molecular weight as compared to citrus pectin. It might be possible that the quality of pectin depends on the

stage at which the fruits are picked and the cultivar of the specific fruit species used for pectin production. Among citrus pectins, lime shows the best gelling performance followed by orange, sweet orange and grapefruit (Koster *et al.,* 1989). Pectins obtained from certain plants such as sugar beets are of poor quality regarding their gelling power due to low molecular weight and presence of acetyl groups (Koster *et al.,* 1989).

Commercial production of pectin can be divided into three stages. The first step is the acid extraction of the pectin from plant species. Pectin extraction from various raw materials is carried out by treating the raw material with hot dilute mineral acid at pH 1.5-3.0 for different periods of time depending on the type of raw material used and type of pectin desired (May, 1990). For the production of high methoxy pectin, great care should be taken in controlling the time, temperature and acidity, as too harsh treatment would result in excessively de-esterified or hydrolysed pectin (Towle and Christensen, 1973).

The second step in the commercial production of pectin is the purification of the extracted pectin. Purification of the pectin from residues is done usually by centrifugation, filtration or by combination of both.

Finally, the purified pectin is isolated by precipitation using alcohol. The precipitate thus obtained is dried and ground to fine powder form.

2.5.4 Functional properties of pectin

Pectins are well known for stabilization and gel forming properties (Christensen 1982; Towle and Christensen, 1973; Gregory, 1986). However, the functions vary with the type of pectin used.

High methoxy pectins show the well known gelling property in presence of high sugar and low pH (Gregory, 1986). A satisfactory gel system is obtained with 58-75% of soluble solids, at a pH range between 2.8-3.5 (Towle and Christensen, 1973). Because of the requirement of high soluble sugar (about 60%), use of high methoxy pectin as a gelling agent is limited to conventional jam, jelly and confectionery production (Pedersen, 1980).

Low methoxy pectin can form a gel in presence of bivalent cations such as calcium (Thibault and Rinaudo, 1986). This gelling system is totally independent of soluble sugars. The gel formation results from crosslinking of the pectin chains by calcium in those sections which are free of ester groups. The 'egg box' mechanism shown in the Fig. 2.3, first proposed for alginates, has been suggested for this kind of gel formation (Morris *et al.*, 1982).

Low methoxy pectins are highly suitable for the production of gelled milk desserts, owing to the presence of calcium in the milk (Pedersen, 1980).

Figure 2.3 has been removed due to the unavailability of copyright permission.

Fig 2.3: Schematic representation of gelling mechanism of LM pectin by calcium ion crosslinking of the polymer chains. (After Morris *et al.*, 1982)

High methoxy pectins are also extensively used for stabilization of various dairy products including drinkable yoghurt and whey drinks (Foley and Mulcahy, 1989; Towler, 1984; Glahn, 1982; Iverson, 1984; Christensen,1982). This stabilization function is related to the ability of pectins to interact with dairy proteins.

2.5.5 Pectin as a stabilizer

Despite a report on the promotion of heat coagulation of bovine serum albumin and ovalbumin (Galston and Kaur, 1962), pectin has been considered to have protective effect against coagulation of protein in milk products (Foley and Mulcahy, 1989). Hence pectin is used as a stabilizer in various dairy products, such as drinkable yoghurt, to avoid precipitation and whey separation upon storage (Glahn, 1982).

During the manufacture of cultured dairy products, mechanical treatment such as homogenization could be used to break down the casein gel formed during fermentation of milk, resulting in suspension of casein particles in the products (Glahn, 1982). Heating of such cultured dairy beverages results in casein syneresis leading to the precipitation of casein from whey (Foley and Mulcahy, 1989). However high methoxy pectin can stabilize the milk protein, thus preventing the sedimentation in the cultured dairy drinks.

The stabilizing activity of the pectin results from the electrostatic interaction of pectin with milk protein resulting in the formation of a stable complex (Gregory, 1986). Hence stabilization by pectin is obtained only at pH below the isoelectric point of milk protein at which milk protein is positively charged. The stability is adversely affected at pH below 3.5, presumably due to suppression of dissociation of carboxyl groups present in pectin (Glahn, 1982).

During the stabilization of cultured dairy beverages, an increase in viscosity is noted by addition of pectin at low levels (up to 0.3% w/v). However as the level of stabilizer is increased to 0.4% or higher, the viscosity decreases (Towler, 1984; Glahn, 1982; Burton-Trapp, 1990).

The proposed mechanism for the stabilization and change in the viscosity by addition of pectin in the cultured dairy products is that pectin binds with casein particles having positive charges below their isoelectric point. The interaction of pectin with casein particles causes decrease in positive charges, lowering the tendency to repeal and increasing the tendency to adhere. As a result, an increase in viscosity is obtained. However with the increase in the level of pectin, the particles acquire a negative charge and repulsive force increases which prevents them from agglomerating (Gregory, 1986; Glahn 1982).

According to Christensen (1982) the stabilizing effect of pectin is not limited to casein particles. Pectin has been recommended as a stabilizer in acidified and fruit juice based whey drinks (Iverson, 1984; Christensen, 1982). This seems quite contradictory to the report on sedimentation of whey proteins by using pectin and more generally, in view of other reports on pectin-protein interactions (Serov *et al.,* 1985).

2.5.6 Pectin-protein interactions

Pectin has been reported to interact with various proteins. In a model system, pectin was found to interact with bovine albumin forming a complex resulting in change in viscosity (Takada and Nelson, 1983). Pectin has also been reported to interact with bovine serum albumin and ovalbumin effect ing the heat coagulation of these proteins. Heat coagulation was inhibited by pectin at pH between 5.3-5.7 and was promoted in pH lower than 5.3 (Galston and Kaur, 1962). Interaction of pectin with sunflower seed albumin resulted in the formation of an insoluble complex. The complex formation was caused by an electrostatic interaction and depended on pH values (Schwenke *et al.*, 1977).

Very little work has been found in literature on the interaction of pectin with whey proteins. However, there is a report on use of apple pectin as a means to isolate whey proteins from model systems (Serov *et al.*, 1985). This isolation method has been based on the insoluble complex formation between whey proteins and pectin due to electrostatic interaction between two oppositely charged macromolecules. Both high methoxy and low methoxy pectins were found to interact with whey proteins at pH between 2.8-3.6. Besides pH, the interaction between pectin and protein was also dependent on ionic strength. The complex was not formed at ionic strength (NaCl) exceeding 0.1, which was explained as due to screening of macroion charges.

Similar interactions between pectin and protein could be detrimental to the production of fruit juice containing whey beverages, because of the possibility of interaction of pectin present in fruit juices and the proteins present in the whey resulting in the formation of insoluble complexes. The pectin-whey protein complex thus formed might create the problem of sedimentation during heat processing or storage. Thus pectin seems to play an important role in the fruit juice-whey beverage technology.

2.5.7 Pectin in fruit juice-based whey beverages

Because of the various reports on the ability of anionic polysaccharides including pectin to interact with proteins, the pectin present in fruit juices might play a detrimental role in development of the turbidity in fruit juice-based whey beverages. Quite likely, the turbidity development in the laboratory prototype of a fruit juice-whey mixture studied at the University of Alberta (Patocka, personal communication) and the often observed sedimentation in fruit juice based whey drinks (Jelen, 1990), are the results of the pectin-protein interactions. However, the scant information regarding the role of pectin in sedimentation or turbidity development is in contrast to the proposed use of pectin as a stabilizer in these products. Thus a comparative study of the interactions of pectin with whey proteins and their role in the sedimentation of whey proteins in fruit-juice based beverages was

considered necessary as one of the important missing "building blocks" on which the development of a truly successful North American whey beverage may be based.

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Whey protein concentrate (WPC)

Alcan 840, the whey protein concentrate (WPC) used during the course of all experiments, was supplied by New Zealand Milk Products Inc. It contained 76.5% whey protein on dry weight basis and had 96% total solids, according to the technical data sheet provided by the producer.

3.1.2 Cottage cheese whey (CCW)

A batch (25 L) of CCW was supplied by Palm Dairies Ltd. of Edmonton every week throughout the experimental period. The CCW had a pH of 4.4 and a total solids content of 6.4%, with very little batch to batch variation.

3.1.3 Pectin

Low methoxy (LM) pectin was obtained from Sigma Chemical Company, St Louis Mo. 63178 U.S.A. Its degree of esterification was 8.9%. High methoxy (HM) pectin was obtained from both Pektin-Fabrik Hermann Herbstreith KG., Postfach 23, D-7540 Neuenburg/ Wurtt, Germany and Grindsted Products, Brabrand, Denmark.

The Herbstreith pectin was of the NS (rapid set) type with a degree of esterification 70-72%. Pectin from Grindsted had a degree of esterification of 70.7% (product # 044082).

3.1.4 Fruit juices

Except for cranberry juice, all fruit juices (Apple, grapefruit, lime, tomato) used in this work, were made in lab from fresh fruits purchased in the local market. The procedure given in Fig 3.1 was used to make the different fruit juices.

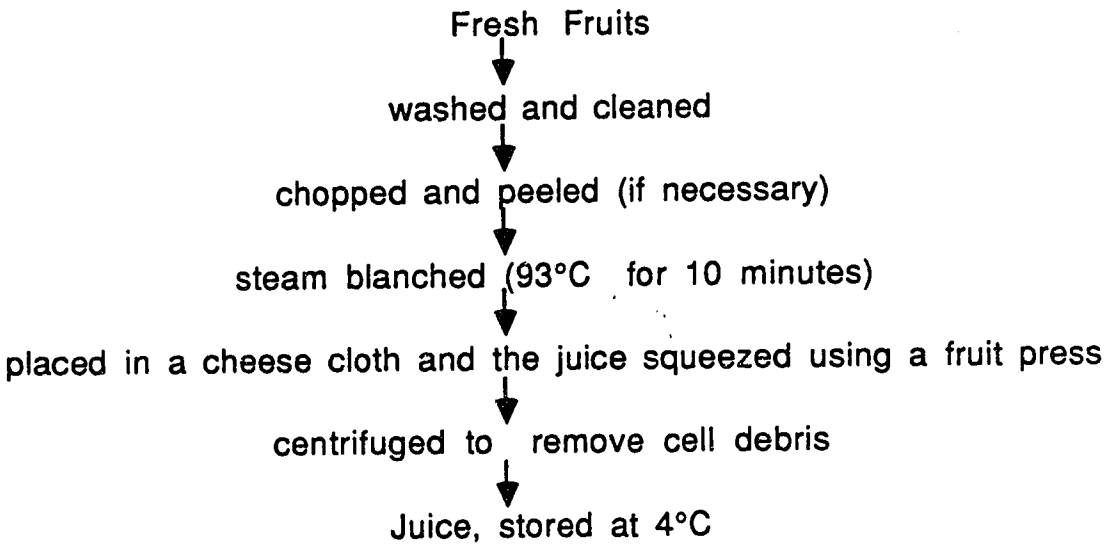

Fig 3.1: Procedure for making fruit juices

Cranberry juice was made from a frozen concentrate (Ocean Spray Cranberries Inc. St. Catharines, Ontario, Canada), purchased at a local market.

Greenhouse grown rhubarb was obtained from a local super-market. Juice was squeezed from the stems following the procedure given in Fig. 3.1.

3.1.5 Polygalacturonase

The enzyme polygalacturonase (pectinase) was purchased from Sigma Chemical Company, St. Louis, Mo 63178 U.S.A. The enzyme was in form of crude powder, isolated from Rhizopus spp. One unit (.002 g) of the enzyme was declared suitable to liberate 1.0 µmole of galacturonic acid per minute from poly-galacturonate chain at pH 4.0 and 25°C.

3.1.6 Chemicals

All chemicals required for protein and pectin analyses such as sodium tetraborate, m-hydroxydiphenyl, galacturonic acid, lactic acid, sulphuric acid etc. were purchased from Fisher Scientific Co. (Fair lawn, New Jersey 07410, USA).

3.2 Experimental Methods

Most of the experiments were carried out at three different pH levels; 3.4, 3.7 and 4.0. These pH levels were chosen because the whey proteins are highly susceptible to heat coagulation at pH 4.0 and are resistant to heat coagulation at pH 3.4.

3.2.1 Pectin-protein interactions in 1% WPC solutions

Solutions containing 1% WPC with 0.05%, 0.10%, 0.20%, and 0.30% of pectin were prepared by mixing the required amount of pectin in 1% whey protein concentrate solutions made by dissolving WPC powder in distilled water. In each case the pH was adjusted to three different values (3.4, 3.7 and 4.0) by addition of 80% lactic acid.

3.2.2 Whey protein-pectin interactions in cottage cheese whey systems

Whey with different percentage of pectin was made by dissolving the required amount of pectin directly in the whey as in case of 1% WPC solutions. Pectin was added gradually over extended time and was stirred with a magnetic stirrer for two hours so as to ensure the complete solubilization of the added pectin. Adjustment of pH was accomplished as mentioned in section 3.2.1.

3.2.3 Yield of sediment

Batches of 100 mL of 1% WPC solutions or CCW with different percentage of pectin (0.05%, 0.1%, 0.2%, and 0.3%), adjusted to the three different pH levels (3.4, 3.7 and 4.0), were left overnight at room temperature in separate beakers. The next day, the sediment developed in each beaker was harvested by centrifugation at a speed of 14,000 x g using JA 14 rotor in a Beckman centrifuge, model J2-21 (Beckman Instruments, Inc., Stanford Industrial Park, Palo Alto, California) for 30 minutes. This rather severe centrifugation was adopted to insure complete recovery of the fine visual sediments observed.

Supernatant was separated from the sediment and saved for further analysis. The sediment was washed with 100 mL of distilled water and was dried in hot air oven at 60°C for 15 hours or until constant weight was obtained. After drying, the sediment obtained from the WPC or CCW samples was weighed in an electric "Mettler AE 163" balance (Mettler Instrumente, AG, CH 8606, Greifensee, Switzerland).

3.2.4 Heating of cottage cheese whey with different concentrations of pectin

Effect of heat on the interaction of whey protein and pectin was observed by heating the whey with different levels of HM pectin.

Whey with various (0.05% - 0.3%) amount of pectin was heated in 100 mL aliquots at pH 3.4 or 3.7 or 4.0 in a hot water bath. Heating was done by holding the temp of whey at 90°C for 15 minutes. The time to reach 90°C (which was about 7 minutes) was not included in the 15 minute heating period. After heating the samples were cooled in an ice water bath.

The separation of supernatant and the determination of the sediment yield was completed as described in section 3.2.3

3.2.5 Whey protein-pectin interactions in fruit juice-cottage cheese whey mixtures.

Fruit juice-based prototype whey drinks were made by mixing various fruit juices separately with cottage cheese whey in a ratio of 1:9. A heat treatment at 90°C for 15 minutes in a water bath was applied to simulate the effect of heat processing. Since no visual sedimentation was observed, turbidity of the mixtures were determined before and after the heat treatment as described further in the section 3.3.3.

3.2.6 Enzymatic hydrolysis of pectin

Since the highest turbidity development was observed in a tomato juice and CCW mix (which also contained the highest amount of pectin), it was decided to repeat the experiment (as described in

the section 3.2.5), after breakdown of pectin present in the tomato juice using an enzyme. Polygalacturonase (0.01 g) was added to 100 mL of tomato juice and the pH was adjusted to 4.0, which was the optimum pH for the pectin hydrolyzing enzyme activity. The juice was left overnight at 25°C, a temperature suggested for the maximum enzyme activity.

3.2.7. Homogenization

As the pectin preparation is recommended as a stabilizer for various dairy drinks including whey beverages, HM pectin was added (0.3%) to a whey - apple juice prototype and the heat treatment of 90°C for 15 minutes was applied. This was done to observe the effect of pectin as a stabilizer in preventing the heat coagulation of whey proteins in whey drinks. The samples were homogenized before or after heating using a Westinghouse WK16 dairy type piston homogenizer (15M8BA Manton-Gaulin Mfg Co. Everett., Mass.) at 34,500 kPa.

3.3 Analytical Methods

3.3.1 Measurement of pH

In all samples the pH values were measured with help of a digital pH meter (model 601A/Digital Ionalyzer, Orion Research,

Boston, Mass.) equipped with an orion pH electrode (cat. no 91-04) also obtained from Orion Research.

3.3.2 Total Solids

The total solids contents of the different fruit juices and other samples were estimated by using a microwave oven (Cem Corp, model AVC-MP, Indian Trial, North Carolina). A sample aliquot was smeared on a pretared filter paper and was dried in the microwave oven for five minutes. Dry weight of the samples were displayed automatically by the microwave oven.

3.3.3 Turbidity measurements

A Spectronic 21 spectrophotometer (Bausch and Lomb Inc. Rochester, N.Y., U.S.A.) was used to measure the turbidity in each sample. The turbidity measurement was done at 900 nm. This wavelength is known to give sufficient sensitivity for turbidity changes in heated whey protein systems (Jelen and Buchheim, 1984). EQA, an equipment commonly used to measure turbidity of dairy products has a fixed wavelength of 900nm.

3.3.4 Protein content

Determination of protein content in supernatants and sediments

obtained with and without heating, was carried out by using the macro-Kjeldahl method (#16245, AOAC, 1980). The percentage of nitrogen was converted to the percentage of protein by multiplying the nitrogen content by a factor of 6.37, common for dairy proteins.

3.3.5 Pectin content

A colorimetric assay using m-hydroxydiphenyl was used for all the required pectin analyses. The method was a slight modification of the procedure given by Blumenkrantz and Asboe-Hansen (1973) as adapted and modified by Kintner and Van Buren (1982).

The modified procedure may be briefly described as follows:

Aliquots (1 mL) of standard solutions of galacturonic acid and samples dissolved in distilled water, containing between 5-75 μg uronic acid/mL were pipetted into separate test tubes, cooled in an ice water bath.

Sulphuric acid-tetraborate solution (6 mL) was added to each test tube in the ice water bath and the solutions were mixed with help of a vortex. After vortexing repeatedly, the test tubes were heated in a hot water bath at 100°C for five minutes and then cooled immediately in an ice water bath.

After the samples were cooled, 0.1 mL of 0.15% m-hydroxydiphenyl was added to each sample to develop color. A red color is formed in presence of uronic acid.

Since carbohydrates are also capable of producing reddish color, when heated with sulphuric acid tetraborate, a sample blank was run for each sample by replacing the m-hydroxydiphenyl reagent with the same amount of 0.5% sodium hydroxide (all other treatments were kept the same). The absorbance obtained for the sample blank was subtracted from the total absorbance to obtain the absorbance due to m-hydroxydiphenyl. Samples were vortexed after the addition of m-hydroxydiphenyl or 0.5 % sodium hydroxide. The samples were kept for about 20 minutes after which the absorbances were read at 520nm with Spectronic 20 spectrophotometer (Bausch and Lomb Inc., Rochester, N.Y., U.S.A.).

A reagent blank containing 1 mL of distilled water, 6 mL of sulphuric acid-tetraborate solution and 0.1mL of 0.5% sodium hydroxyde was used to calibrate the spectrophotometer.

A standard curve obtained with the different concentrations of galacturonic acid (as presented in Appendix 1), was used to convert the absorbance readings into pectin concentration for all the investigated samples.

3.3.6 Identification of the oxalic acid

The sediment obtained from the whey-rhubarb mixture (9:1 ratio) was separated from supernatant by centrifuging in Beckman model J-21 centrifuge at 14 000 x g for 20 minutes. The washed sediment was dried and then dissolved in concentrated sulphuric

acid or hydrochloric acid. The solution was then diluted with distilled water and was passed through a cation exchange resin (SPE column, catalog # 7095, J. T. Baker Chemical Co.).

The effluent collected after passing the sample through the cation exchange resin was analysed by HPLC by injecting the sample into "Rezex" organic acid column of 300-7.8 mm (Phenomenox, HPX-87H). A UV detector (LDC/Miltaroy, Spectromonitor D) set at 210nm was used. Eluent applied was 0.01 N sulphuric acid with a flow rate of 0.6mL/min. The HPLC analysis was done at room temperature.

3.4 Tomato Based Whey Beverages

3.4.1 Preparation of a prototype cottage cheese whey-tomato ("wheymato") drink

Tomato based whey drinks were prepared by mixing whey and commercially available canned tomato juice (H. J. Heinz Company of Canada Ltd., Leamington, Ont.) in 2:3, 1:1 and 3:2 ratio, with 2% sugar (sucrose) added. The drinks were adjusted to pH 3.7 and heated at 90°C for 15 minutes. After heating, the drinks were cooled in an ice water bath and stored in cold room at 4°C for 24 hours.

3.4.2 Sensory evaluation

All the sensory evaluation experiments were carried out in a sensory testing room having 10 individual tasting booths with an incandescent red light in each booth, at the Food Science Dept., U. of Alberta. All drinks were served cold (4°C) in polyethylene sample cups.

A preliminary sensory test panel was carried out to select the most liked tomato based whey drink prototype with three different whey tomato juice combinations. A total of 15 untrained panelists were included in the test panel.

Three drinks with different proportion of whey and tomato juice (identified by three digit random numbers) were served. Each panelist was asked to select the most liked formula by circling the code number assigned to the drink and to give comments as shown in the appendix 2

3.4.3 Comparisons of a tomato juice-based whey drink prototype with commercially available tomato drink

The most liked formula was selected for further sensory examination by comparing it with a commercially available tomato based drink "Clamato" (Cadbury Beverages Canada Inc., Mott's division, Mississauga, Ont., Canada).

First, a preliminary taste panel was conducted with a group of twenty panelists, to compare the colour and appearance, consistency and overall liking of clamato and the prototype drink.

The tomato-based whey prototype and clamato drink were served in separate polyethylene cups to each member of the taste panel. All the panelists were asked to assign a number for the colour and appearance, consistency and overall liking of the prototype and the clamato juice. A hedonic scale of 1 to 9 was used as shown in the Appendix 3.

A final taste panel was carried out to compare the "wheymato" with clamato juice in consistency, sweetness, acidity and overall liking as a breakfast drink or as a thirst quenching drink (Appendix 4). A group of 24 panelists (students and staff members of the University of Alberta) were included in the taste panel. In this sensory evaluation, two drinks were served separately, one drink at a time. The order of serving was randomized among the panelists.

3.5 Statistical Methods

All the data collected in the sensory evaluation as well as in other investigations if required, were analysed using appropriate statistical tests. Paired T test, analysis of variance (ANOVA) and Tukey's Test were the methods applied for the data analyses (Snedecor, 1956; Steel and Torrie, 1980).

4. RESULTS AND DISCUSSION

Protein-pectin interactions were studied using three different systems i.e. in model systems using WPC, in combinations of CCW with industrial pectin, and in mixtures of various fruit juices and CCW.

Different levels of pectin (0.05, 0.10, 0.20 and 0.30 % by weight) were added to 1 % WPC solutions or CCW at pH 3.4, 3.7 and 4.0. The sediment obtained in each case from equal amount of 1% WPC solution with various pectin concentration, was separated by centrifugation.

Various fruit juices containing different amount of pectin were mixed with CCW to observe the effect of the natural pectin present in the juices in production of whey beverage prototype.

4.1 Interactions of Pectins with Whey Proteins in Model Systems Using 1% WPC Solutions

Immediate visual sedimentation was observed when 1% WPC solutions containing different levels of HM or LM pectin were adjusted to the three different pH levels mentioned above.

4.1.1 HM pectin-protein interactions in 1% WPC solutions

Fig. 4.1 shows the amount of sediment obtained from 100 mL of 1% WPC solutions with different percentage of HM pectin added. At all pH levels used for the experiments, the amount of dry sediment increased with the increasing concentration of the pectin added i.e. the lowest amount of sediment was obtained in the solution with 0.05% pectin and highest amount was obtained from the solution containing 0.3% of pectin. Significant differences were found in the amount of the sediments obtained using the same levels (0.1%, or 0.2%, or 0.3 %) of pectin at pH 3.4 as compared to pH 3.7 and 4.0 at 5% level. The amount of sediment obtained at a particular pH without addition of pectin has been subtracted from the amount of the sediments obtained by addition of different levels of pectin at that particular pH. The amount of sediments obtained at pH 3.4 was significantly higher than those obtained at pH 3.7 and 4.0. However, no significant difference in amount of sediments obtained using same level of pectin at pH 3.7 and 4.0 was found except for the case in which 0.05% of pectin was used. Sediments obtained using 0.05% of pectin were significantly different at all pH levels studied. The highest amount of sediment was obtained at pH 3.4, followed by 3.7 and 4.0.

The protein content of the supernatant was found to decrease with the increasing level of pectin added. Hence the supernatant obtained from the solution with 0.05% of pectin showed highest amount of protein and the one obtained from the solution containing 0.3% of pectin had least amount of protein content at all pH levels as shown in Fig. 4.2.

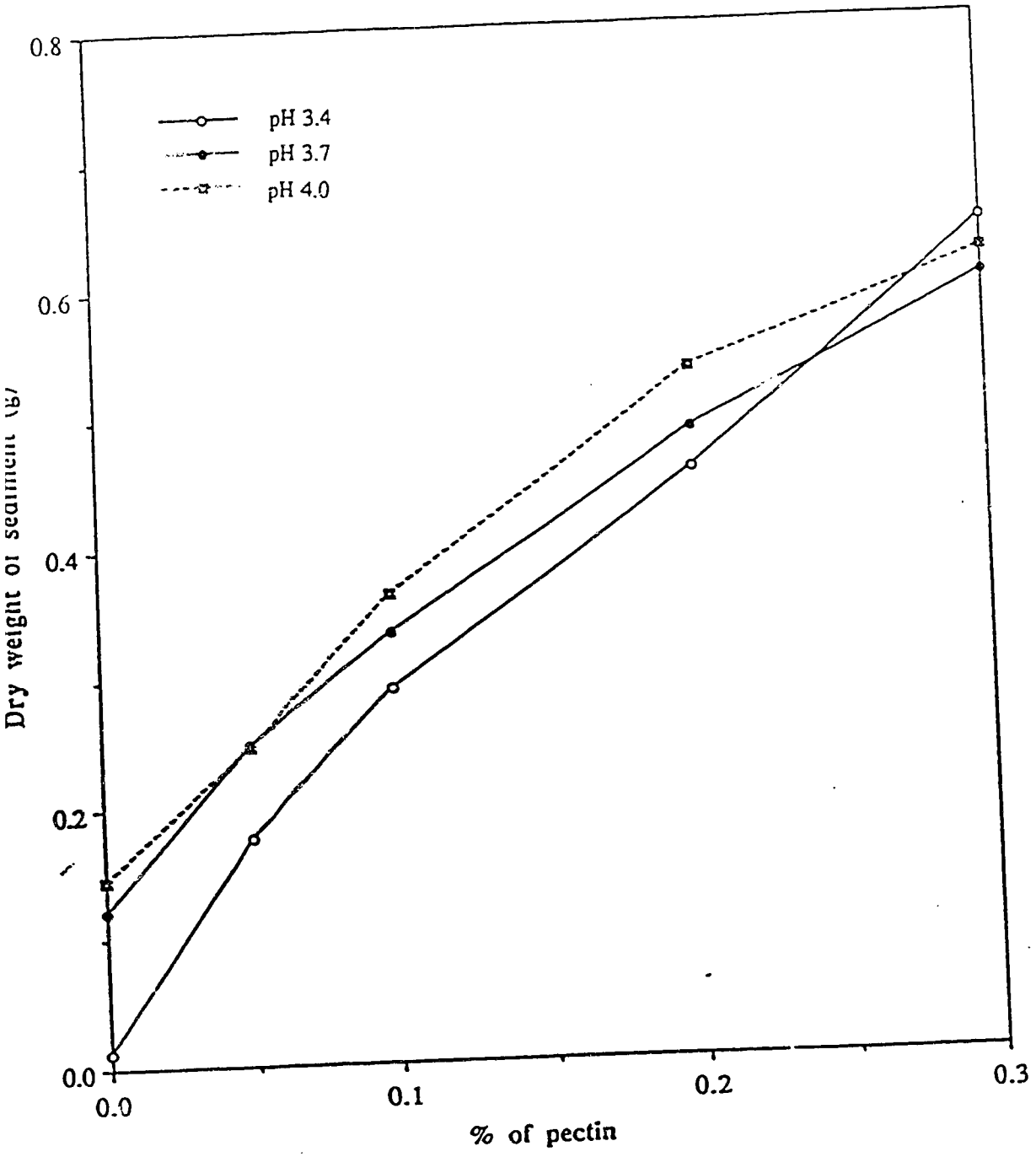

Fig. 4.1: Weight of the sediments vs % of HM pectin added to 1% WPC solutions.

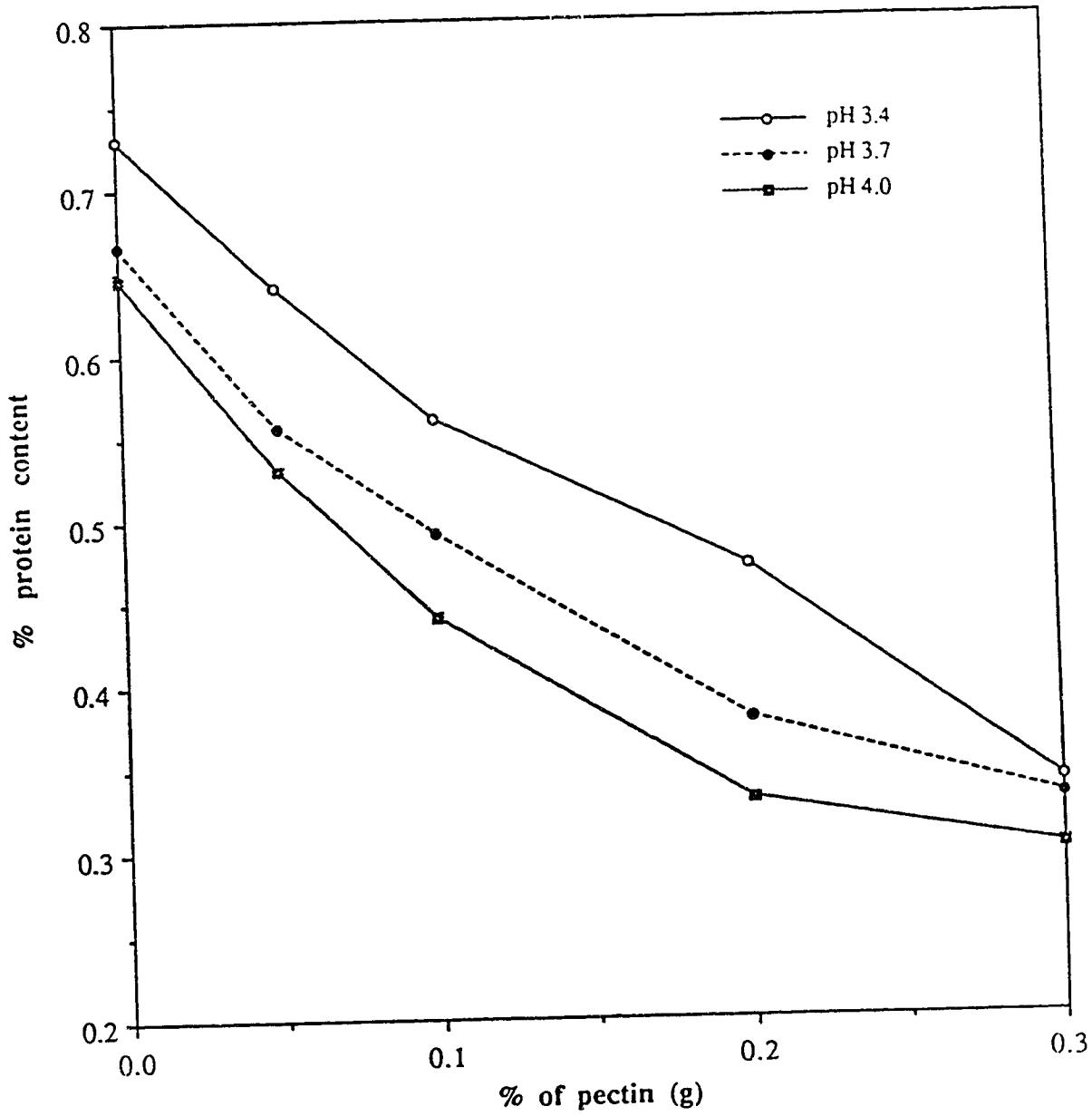

Fig. 4.2: Protein content of the supernatant v.s % HM pectin added to 1% WPC solutions.

Even though the decrease in the protein content of the supernatant with increasing level of pectin added can be used as an indirect measure of the presence of protein and pectin in the sediment, measurement of protein and pectin contents of the dry sediments was used in selected samples to determine the proportional composition of the sediments.

Figures 4.3 and 4.4 give the percentage of protein and pectin present in the sediments obtained from the whey protein solutions at pH 3.4 and 3.7. Presence of protein and pectin in all sediments indicated the ability of pectin to interact with whey proteins. The interaction appeared to have resulted in the formation of a complex between protein and HM pectin. The interaction between protein and pectin has been reported as the general phenomenon of complex formation between two oppositely charged macromolecular components (Serov *et al.*, 1985).

The sediments obtained in all cases were insoluble in water. Thus the interactions between pectin and whey proteins might cause the formation of insoluble complexes leading to the sedimentation in the pectin-whey protein systems. The visual sedimentation must have happened due to higher molecular weight and/or insoluble nature of the complex, or due to decrease in the repulsive force between the similarly charged molecules.

The interactions of pectin with whey protein molecules may change the hydrophilic properties of pectin which in turn could be the contributing factor to the insolubility of the complex. Similar

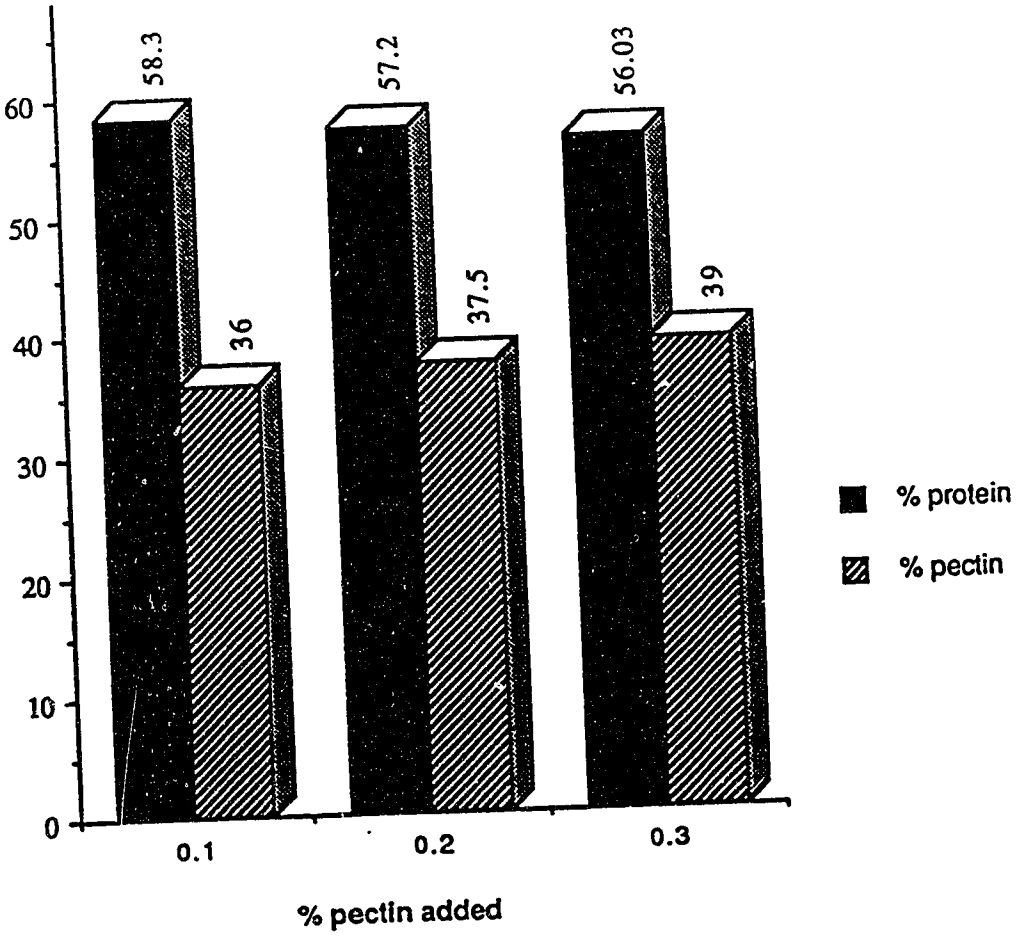

Fig. 4.3: Protein and pectin content of the sediments obtained from 1% WPC solutions at pH 3.4.

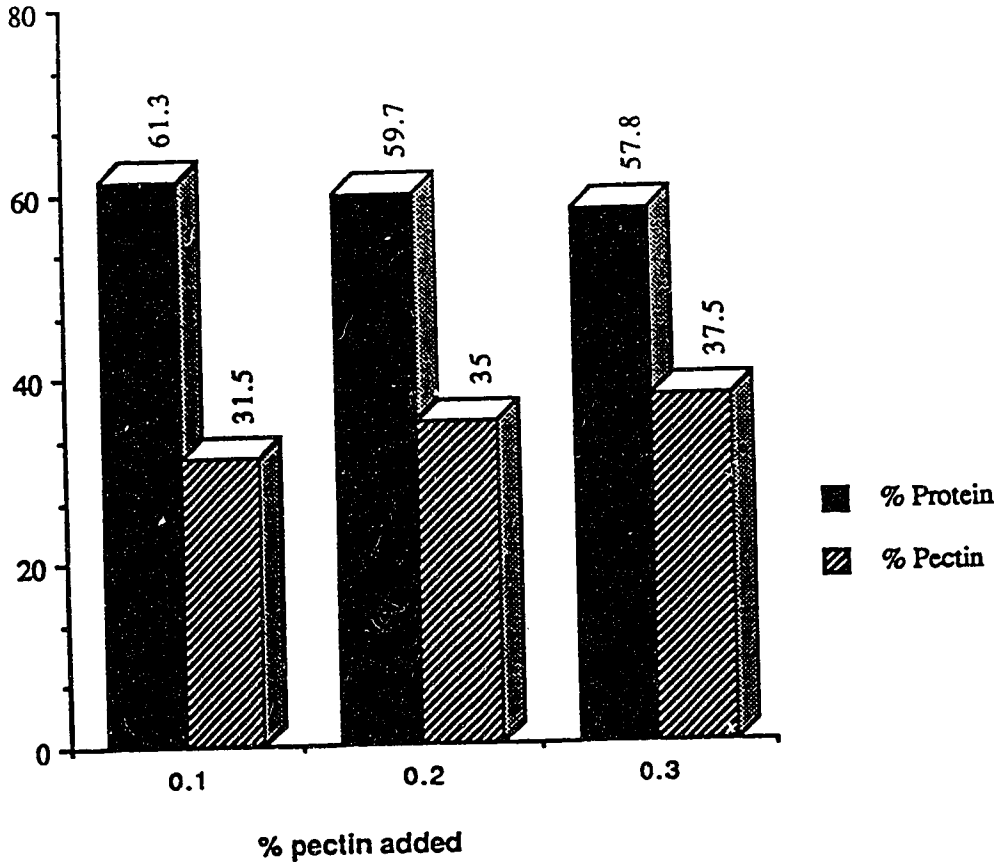

Fig. 4.4: Protein and pectin content of the sediments obtained from 1% WPC solutions at pH 3.7.

explanation has been given for the formation of insoluble complexes between β-lactalbumin and other ionic polysaccharides such as carboxymethylcellulose (Hidalgo and Hansen, 1969b).

The visual sedimentation did not occur after addition of the different levels of pectin at pH 5.2 (and higher) and the immediate sedimentation happened after adjusting the pH to 3.4, 3.7 and 4.0. This observation suggests that the interaction is pH dependent. Interactions between proteins and many other anionic polysaccharides also greatly depend on pH values (Imeson *et al.*, 1977; Ledward, 1978).

Dependence of the interaction of pectin with whey protein on pH indicates the electrostatic nature of the interaction. Many anionic polysaccharides such as CMC or alginates are known to show an electrostatic interaction with proteins (Ganz, 1974; Hidalgo and Hansen, 1969a; Imeson *et al.*, 1977; Ledward, 1978).

The highest intensity of interaction between pectin and protein was found at pH 3.4, as indicated by the significantly higher amounts of sediments obtained at this pH compared to pH 3.7 and 4.0. The possible reason for this could be the fact that electrostatic interaction between protein and pectin is dependent on the charges carried by the macromolecules. As the pH is decreased below the isoelectric region of proteins, the charges in the protein molecules increase. The increase in positive charges in the protein molecules should result in increased ability of proteins to interact with negatively charged pectin molecules. Hence the decrease of pH

should result in an increase in the intensity of the interactions between whey proteins and pectins. However, the pH should not be lower than the point at which dissociation of carboxyl groups in pectin molecule is supressed.

Highest amount of protein was found in the sediment obtained using 0.3% of pectin at pH 3.4. The highest protein yield was found to be 48.21% (of the protein present in 1% WPC solution) only, whereas Serov *et al.* (1985), reported isolation of whey proteins up to 85% (of the total protein present in the system) at pH 3.4. Further it should be pointed out that total concentrations of the macromolecular components in our experiment were 0.3% of pectin and 0.73% of protein whereas Serov *et al.*, (1985) have reported those concentration as being 0.39% of pectin and 0.26% of protein in a model protein-pectin system. One of the main differences between the experimental design of our experiments and those of Serov *et al.* (1985) is that in our experiment, the concentration of protein was kept constant and only the concentration of pectin was changed as we were concerned in observing the effect of different levels of pectin in sedimentation of whey proteins in the pectin-protein system. On the other hand, Serov *et al.* (1985), changed the concentrations of both pectin and protein in the pectin-whey protein system as they were interested in finding out the exact combination in which highest yield of whey protein (in the form of complexes with pectin) is obtained.

Even though the highest protein yield was found in the sediment at pH 3.4 with 0.3% of pectin added, the percentage of

protein present in the sediment was similar in all the sediments obtained by addition of 0.1%, 0.2%, or 0.3% of pectin as shown in figure 4.3. No significant difference was found when tested statistically. Similar ratio of pectin and protein was found in all the sediments obtained by addition of different levels of pectin at pH 3.4 and 3.7 (Fig. 4.3 and 4.4).

Addition of pectin at a level higher than 0.3% resulted in the development of cloudiness in the whey protein-pectin system as shown in Table 4.1. When such WPC solutions were centrifuged, the supernatant was quite cloudy and amount of sediment obtained was less compared to the one found in 1% WPC solution with 0.3% of pectin added.

This could be due to the fact that not all the pectin added above a certain level can interact with whey proteins. Hence the increased amount of pectin added might result in the increased viscosity of the system and whatever sediment was formed by the interactions between proteins and pectin, could not sediment freely as in low a viscosity system.

In a model system, Hidalgo and Hansen (1969b) showed that at lower concentrations of CMC, the interaction of β-lactalbumin with CMC resulted in the formation of a complex due to ionic binding between the positively charged protein and negatively charged CMC. However, the addition of CMC at higher concentration invariably resulted in solubilization of the precipitates. A similar phenomenon was also detected in whey systems (Hansen *et al.*, 1971). This

phenomenon could quite possibly be true for whey protein-pectin systems as a smaller amount of sediment was obtained at increased levels of pectin used (above 0.3%).

Table 4.1 Characteristics of the 1% WPC solutions with different levels of pectin

% of pectin	nature of the system	Supernatant
0.05	clear visual sedimentation.	Very clear
0.1	clear visual sedimentation.	very clear
0.2	clear visual sedimentation.	very clear
0.3	clear visual sedimentation.	very clear
0.35	cloudy, little sediment observed at the bottom.	cloudy
0.4	very cloudy, no sedimentation was observed.	cloudy
0.5	very cloudy, no sedimentation was observed.	cloudy

4.1.2 Interactions of LM pectin and whey proteins in 1% WPC solutions

Similar trends, as in the case of high methoxy pectin, were found for the amount of sediment obtained and protein content of supernatant, by addition of different levels of LM pectin (0.05 - 0.3%) in 1% WPC solutions. Figures 4.5 and 4.6 show the amount of sediment obtained and protein content of the supernatant obtained from 1% WPC solutions, respectively.

The increase in the amount of the sediment and a decrease in the protein content of supernatant with increased concentration of pectin added to 1% WPC solutions, indicates the power of LM pectin to interact with whey proteins.

Serov *et al.* (1975), found no difference in the amount of whey protein isolated by using high methoxy or low methoxy pectin as a complexing agent. However, in our experiments, in almost all cases, the amount of sediment obtained by addition of LM pectin in 1% WPC solutions was higher than that obtained by addition of same amount of HM pectin, at all pH levels studied. The reason for this could be the availability of more carboxyl groups in low methoxy pectin molecules (compared to high methoxy pectin), and consequently a higher electrical charge is available to interact with whey proteins.

HM and LM pectins showed exactly similar trends, indicating the ability of both HM and LM pectins to interact with whey proteins,

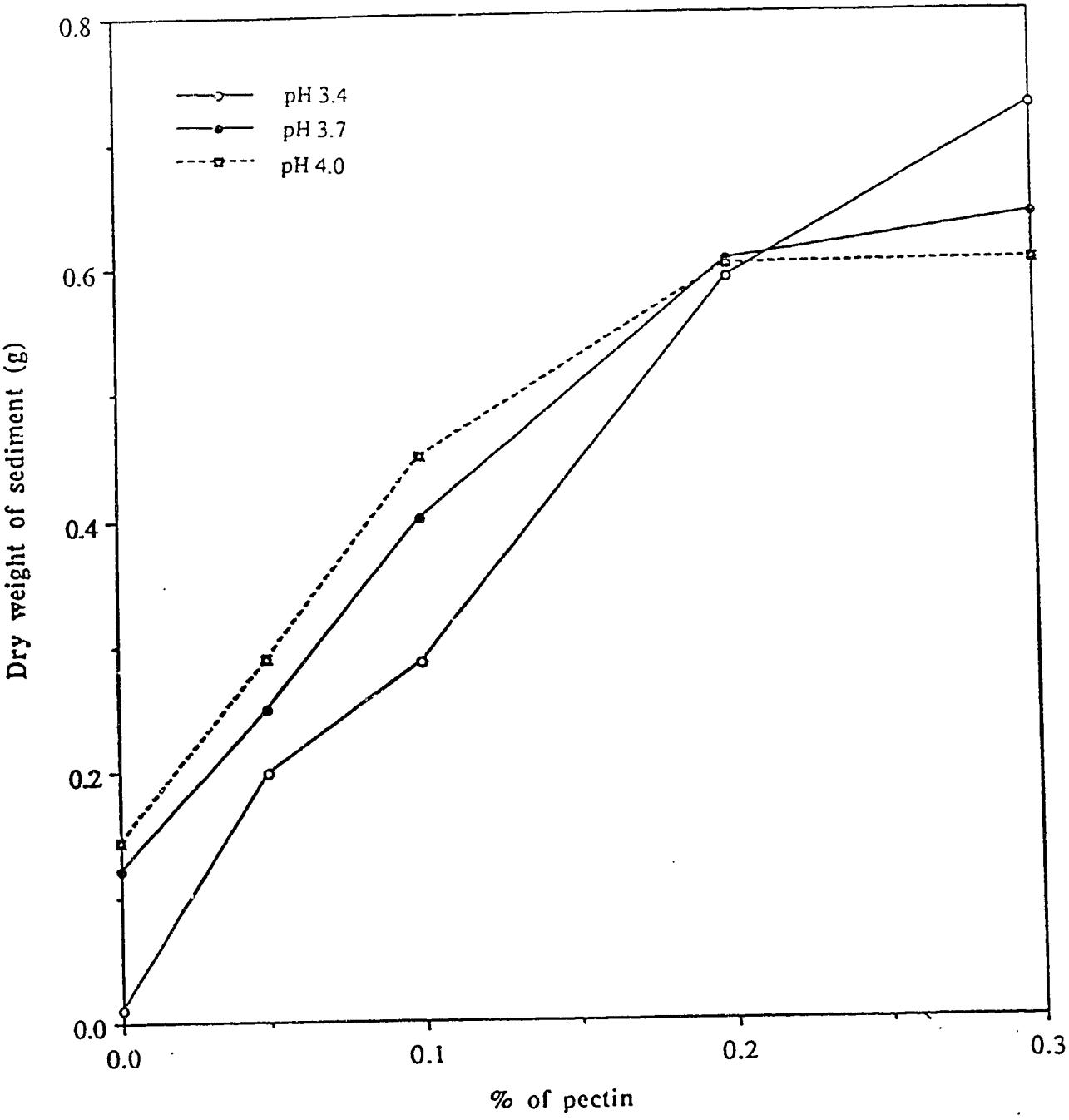

Fig. 4.5: Weight of the sediments vs % of LM pectin added to 1% WPC solutions.

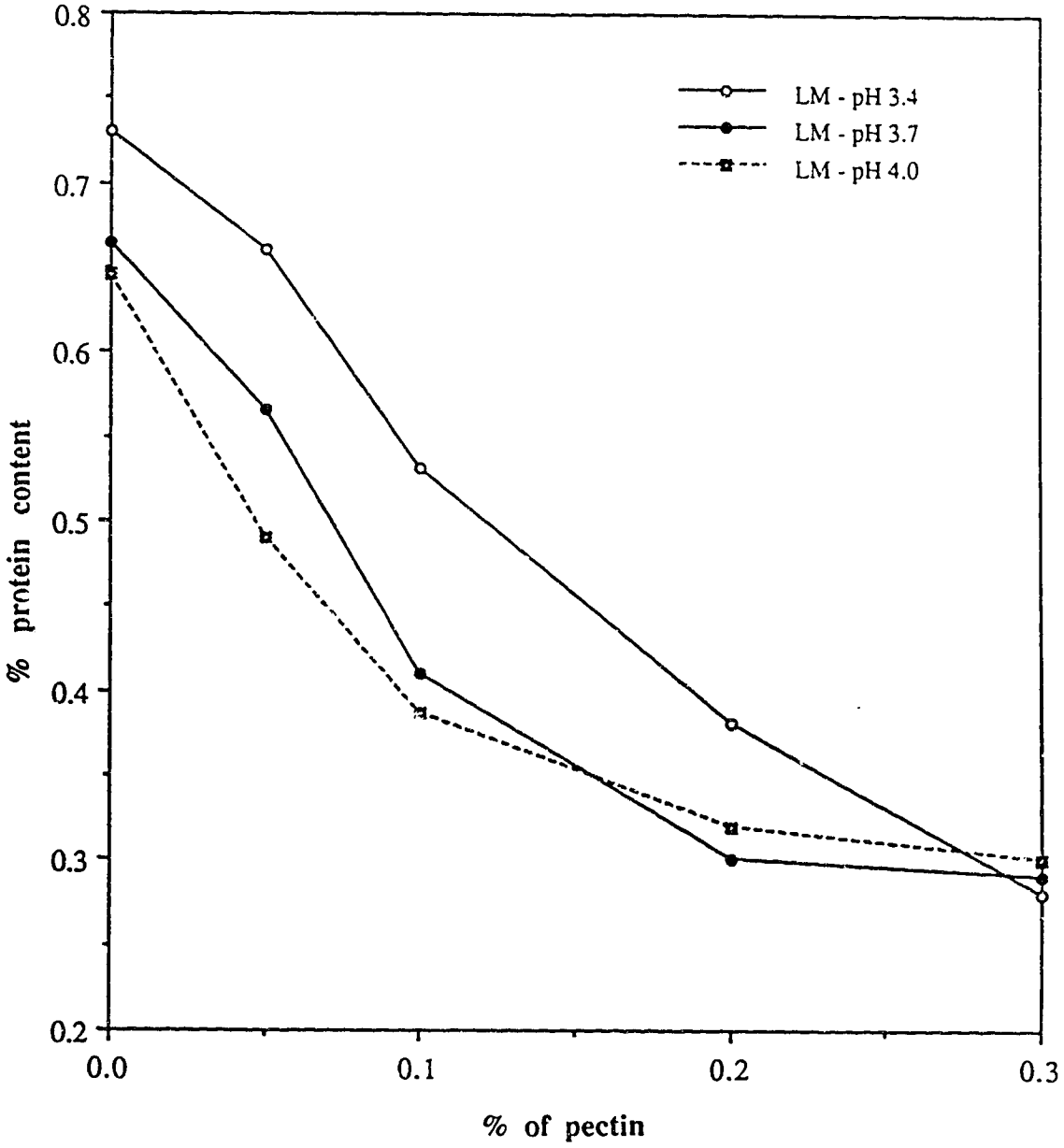

Fig. 4.6: Protein content of the supernatants vs % of LM pectin added to1% WPC solutions.

resulting in the visual sedimentation at all the pH ranges chosen for the experiments.

Since both high and low methoxy pectin were found to induce sedimentation of whey proteins in a similar manner, only high methoxy pectin was used for further experiments. The main reason for choosing high methoxy pectin was that pectin present in fruits is mostly found in the high methoxy form in natural conditions.

4.2 Interactions of HM Pectin with Whey Proteins in Cottage Cheese Whey Systems

4.2.1 Unheated systems

High methoxy pectin was added to 100 mL of cottage cheese whey in concentrations ranging from 0.05-0.3% and pH was adjusted to 3.4, 3.7 and 4.0 as in previous cases.

Sedimentation was observed in each case. However the sedimentation was not as immediate as in previous experiments with 1% WPC solutions. The reason for this might be the effect of greater ionic strength of CCW compared to WPC solution, as ionic strength could affect the interactions between whey proteins and pectins.

High salt concentration has been reported to interfere with the complex formation between proteins and polyelectrolytes (Hidalgo

and Hansen, 1971). Serov *et al.* (1985) reported that the intensity of interactions between pectin and whey proteins decreases with increasing ionic strength. Similar cases have been found with the interactions of whey proteins with CMC i.e. the precipitation of protein by CMC decreased as the salt concentration was increased (Hidalgo and Hansen, 1969a).

Formation of an insoluble complex due to interaction between whey protein and pectin has been reported to occur only at low ionic strength (Serov *et al.*, 1985). However, in our experiment the insoluble complex formation was observed in undiluted CCW also. Since the ionic strength of whey has been reported to be approximately 0.2 (Hill and Zadow, 1974), our result does not agree with that of Serov *et al.* (1985). They reported the insoluble complex formation between pectin and protein did not occur at ionic strength exceeding 0.1. Besides the interactions between proteins and pectin, the insoluble complex formation between whey proteins and CMC has also been reported to occur only in whey diluted with an equal volume of water so as to reduce the ionic strength (Hill and Zadow, 1974); no complex was formed above ionic strength 0.2 (Hidalgo and Hansen, 1969a).

In our experiments, the amount of sediment obtained, by addition of different levels of pectin in CCW, increased with increasing amount of pectin used as in the case of 1% WPC solutions (Fig. 4.7, 4.8 and 4.9). However the sediment obtained was significantly lower as compared to the sediments obtained from 1% WPC solution by addition of same amount of pectin at all pH levels

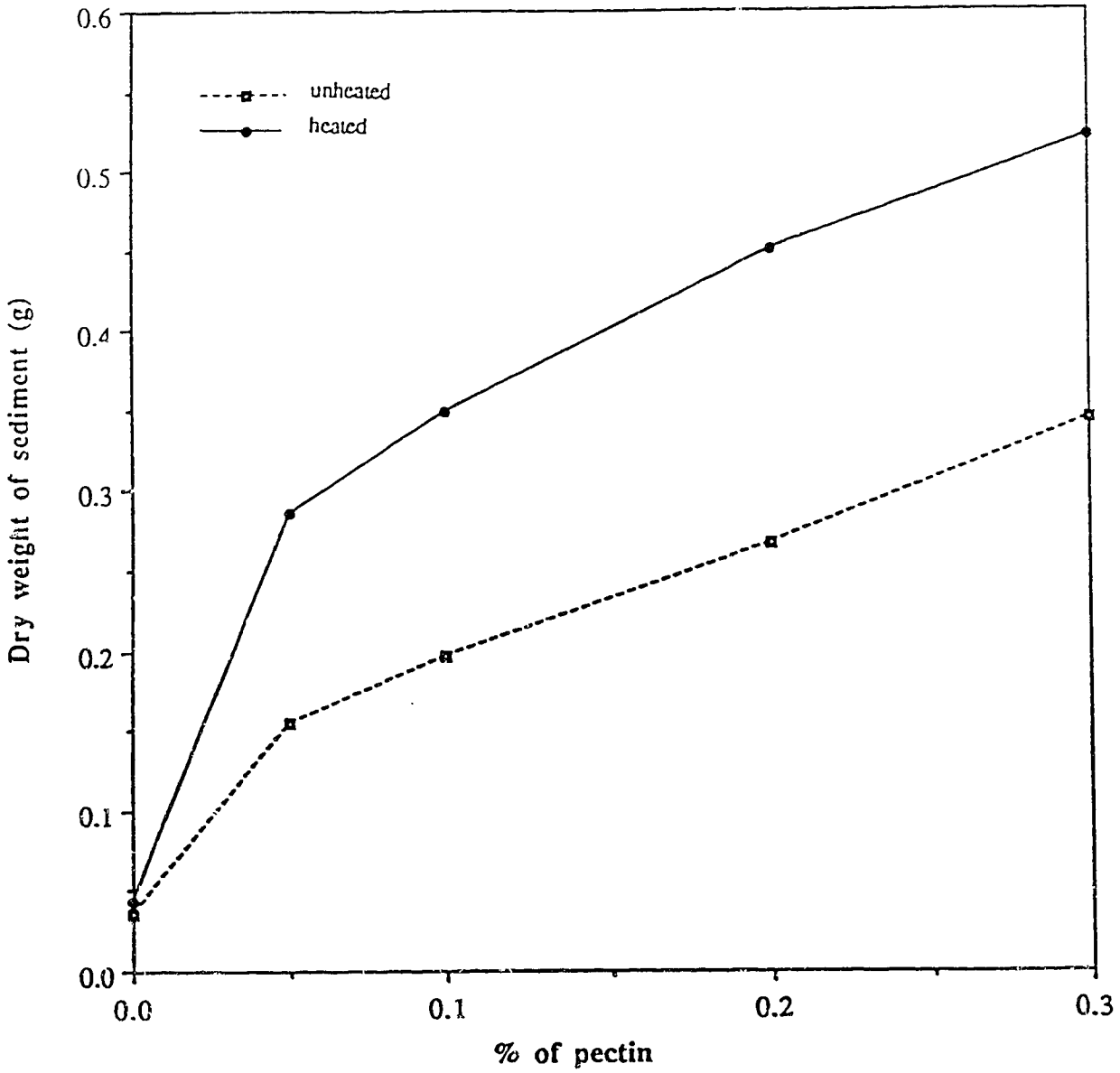

Fig. 4.7: Weight of the sediments vs % of HM pectin added to cottage cheese whey at pH 3.4.

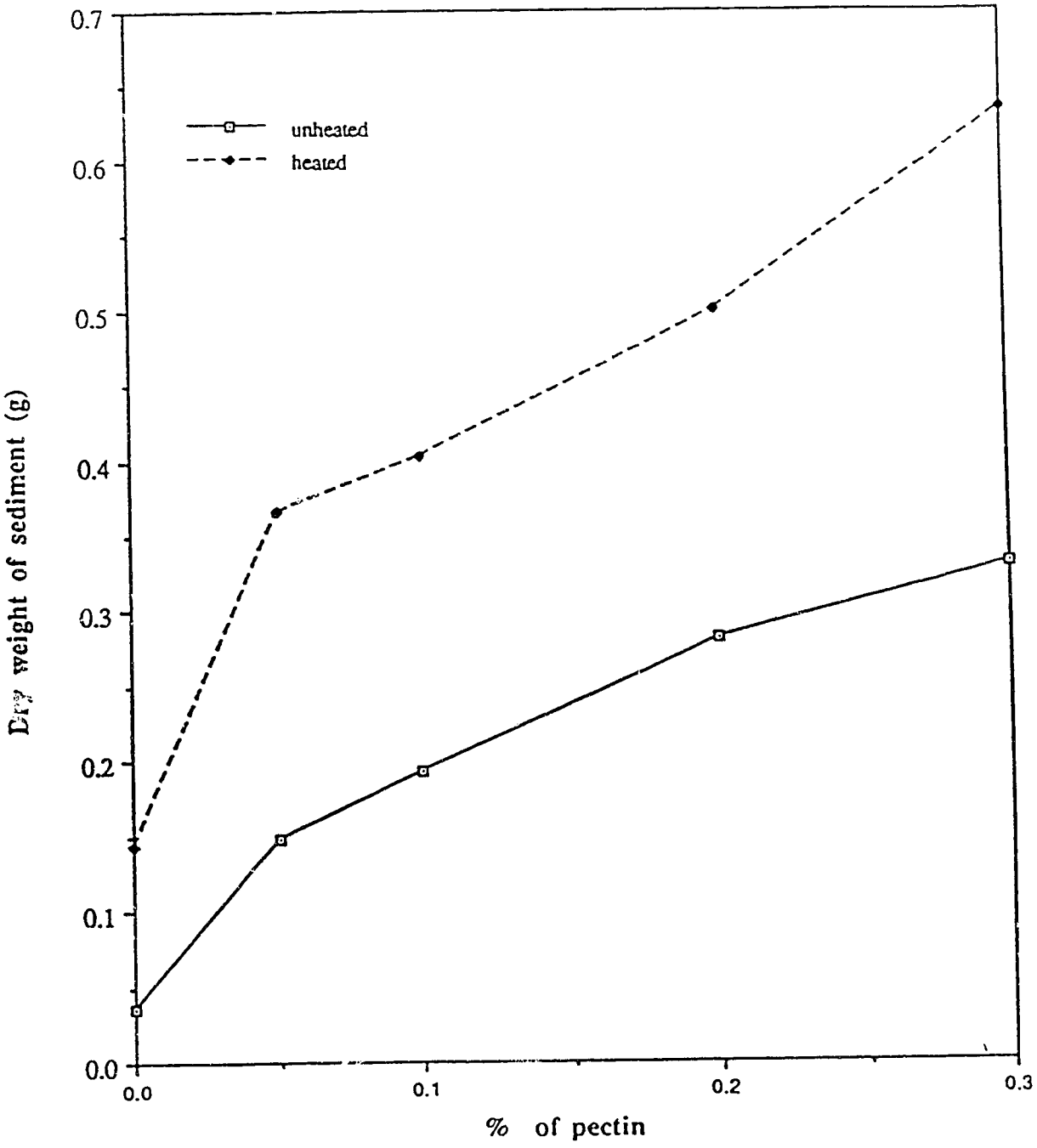

Fig. 4.8: Weight of the sediments vs % of HM pectin added to cottage cheese whey at pH 3.7.

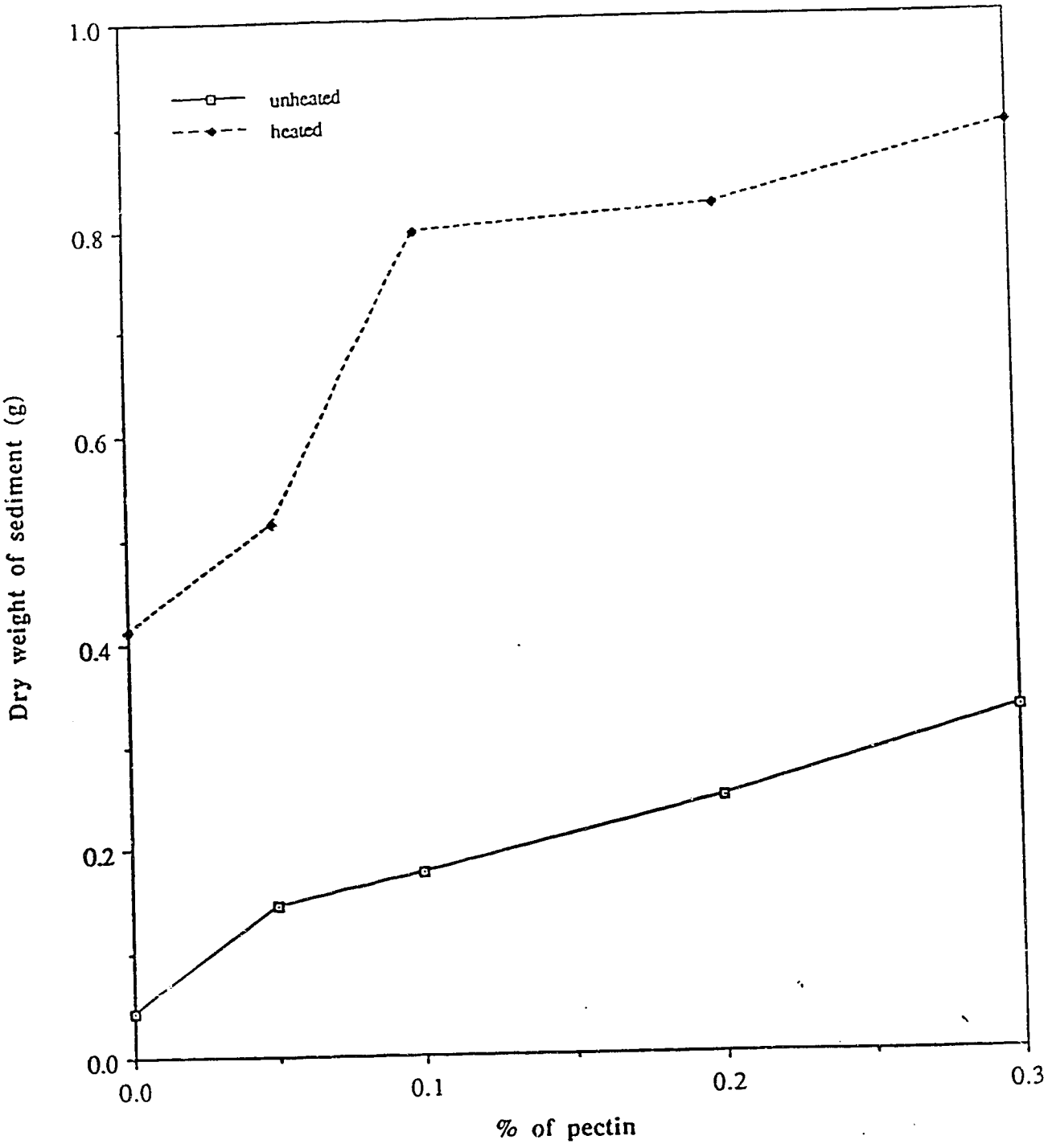

Fig. 4.9: Weight of the sediments vs % of HM pectin added to cottage cheese whey at pH 4.0.

studied. This could be due to the lower ionic strength of 1% WPC solution as compared to that of the CCW, since the intensity of interactions between pectin and proteins has been reported to decrease with increasing ionic strength.

Statistical test showed no difference between the amount of sediment obtained by addition of the same amount of pectin, at pH 3.4 , 3.7 and 4.0.

Figures 4.10 and 4.11 show percentage of protein and pectin present in the sediment obtained at pH 3.4 and 3.7 respectively. The sediments obtained from CCW at pH 3.4 and 3.7 showed relatively higher pectin content in the insoluble complex than the sediment obtained from 1% WPC solutions. This may be due to the presence of higher amount of calcium in CCW (compared to 1% WPC solution). Serov *et al.* (1985) have pointed out that the presence of calcium salts in water-pectin-protein systems would result in an increased relative pectin content in the composition of the insoluble complex.

4.2.2 Heat treatment of the pectin-cottage cheese whey mixture

The effect of heat on the interactions between pectin and whey proteins was observed by heating CCW with different concentrations of pectin (0.05, 0.1, 0.2 and 0.3%) at pH 3.4, 3.7 and 4.0 at 90°C for

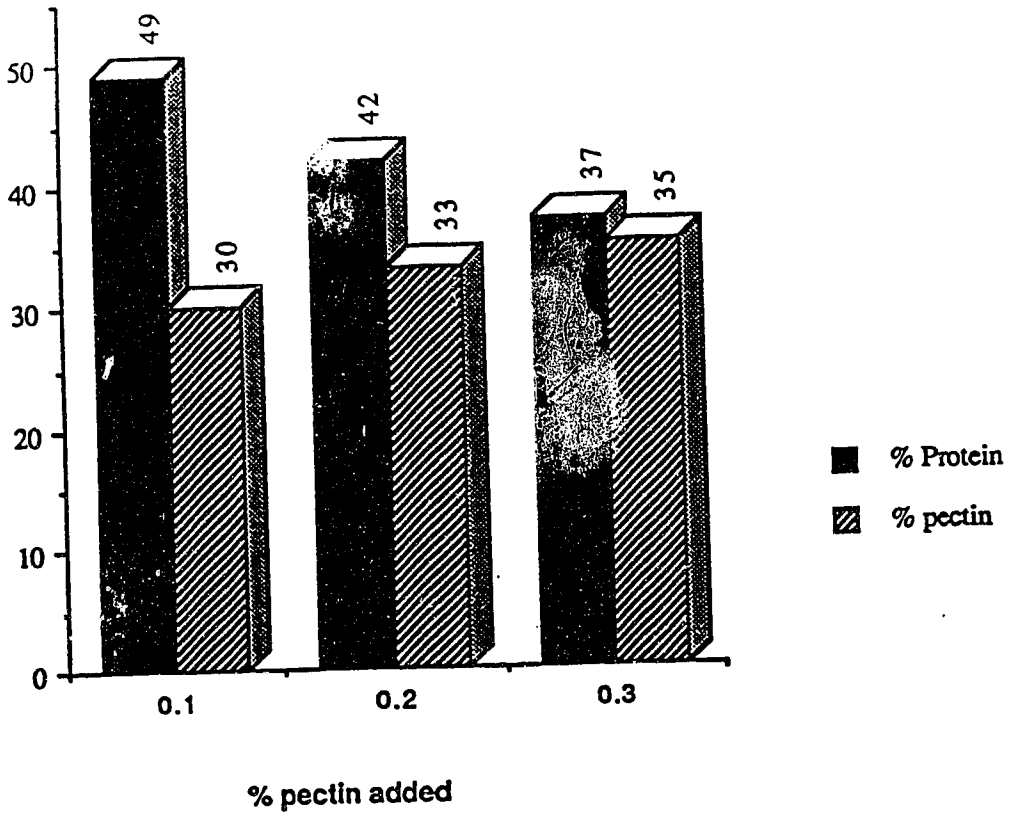

Fig. 4.10: Protein and pectin content of the sediment obtained from cottage cheese whey at pH 3.4.

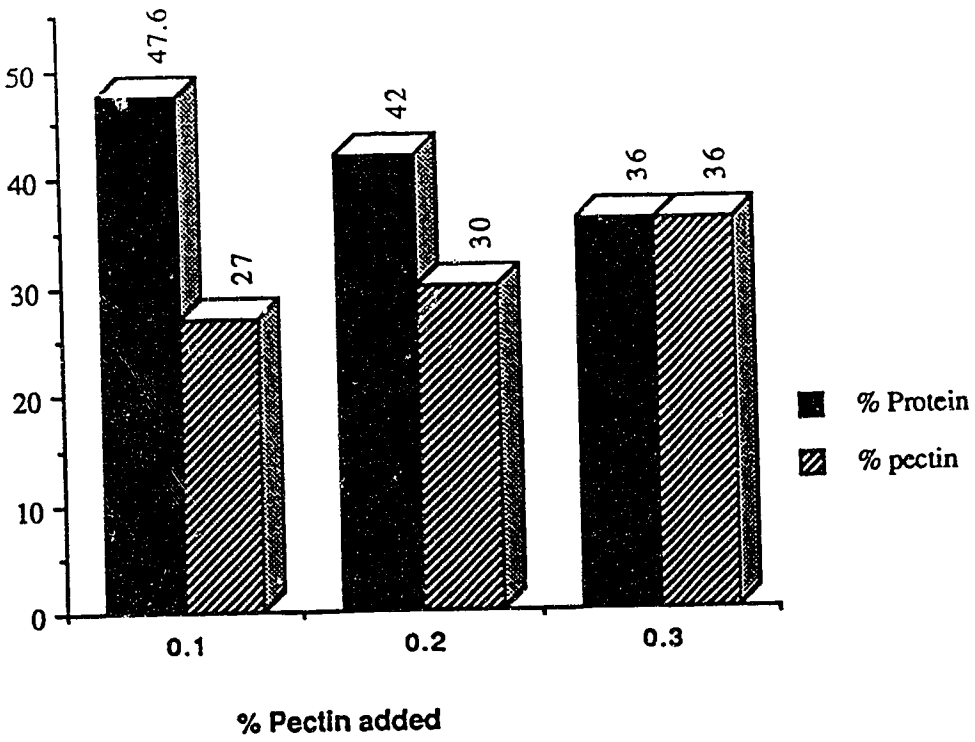

Fig. 4.11: Protein and pectin content of the sediment obtained from cottage cheese whey at pH 3.7.

15 minutes. The pH of the pectin-CCW system did not change as a result of heating.

The heating caused a significant increase in the amount of sediments obtained at all pH levels studied, which is obviously seen in Figures 4.7, 4.8 and 4.9. Pectin has been reported to promote heat coagulation of proteins in lower pH ranges i.e. at pH values lower than 5.0 (Galston and Kaur, 1962). Heating increases the strength of interactions between protein and anionic polysaccharides, presumably due to exposure of more basic groups present in the protein molecules as a result of denaturation (Imeson *et al.*, 1977). Moreover, the flexibility of the denatured random coil of the protein molecules aids to the configurational adjustment to increase the intensity of the interactions resulting in the formation of a more stable complex than those formed from the native proteins (Ledward, 1978).

The sediments obtained from the heated and unheated whey were different in appearance. A weak jelly like sediment was obtained from unheated whey and more or less curd-like sediment was obtained from heated whey. Takada and Nelson (1983) reported the formation of the more stable irreversible complex between pectin and protein as a result of heating. This could have been the case in our experiments also.

In contrast to our observations (in 1% WPC and unheated whey), the amount of sediment obtained by addition of 0.1%, 0.2% or 0.3% of pectin at pH 4.0 was higher than those obtained at pH 3.4 by addition

of the same amount of pectin. This could be due to the fact that whey proteins are highly susceptible to heat denaturation at pH 4.0 and above.

The dry sediments from the heated whey at pH 3.4 and 3.7 with different levels of pectin showed presence of protein and pectin in an approximate ratio of 2:1 (figure 4.12 and 4.13) similar to the ones obtained from 1% WPC solutions. The protein contents of the sediments were significantly higher than those of the sediments obtained from unheated CCW. At this point a question arises - was the higher percentage of protein present in the complex due to heat precipitation of whey proteins?. But it is obvious in Fig 4 7 that there was no heat precipitation of whey proteins at pH 3.4 in absence of pectin and the amount of sediments increased significantly with the heat treatment. This shows that more proteins must have interacted with the pectin added as a result of heating. The protein and pectin content of the sediment obtained after heating at pH 4.0 was not estimated, since at that pH whey proteins precipitate as a result of heat denaturation even in absence of pectin.

4.3 Interactions of Pectin and Whey Proteins in Mixtures of Fruit Juice and Cottage Cheese Whey

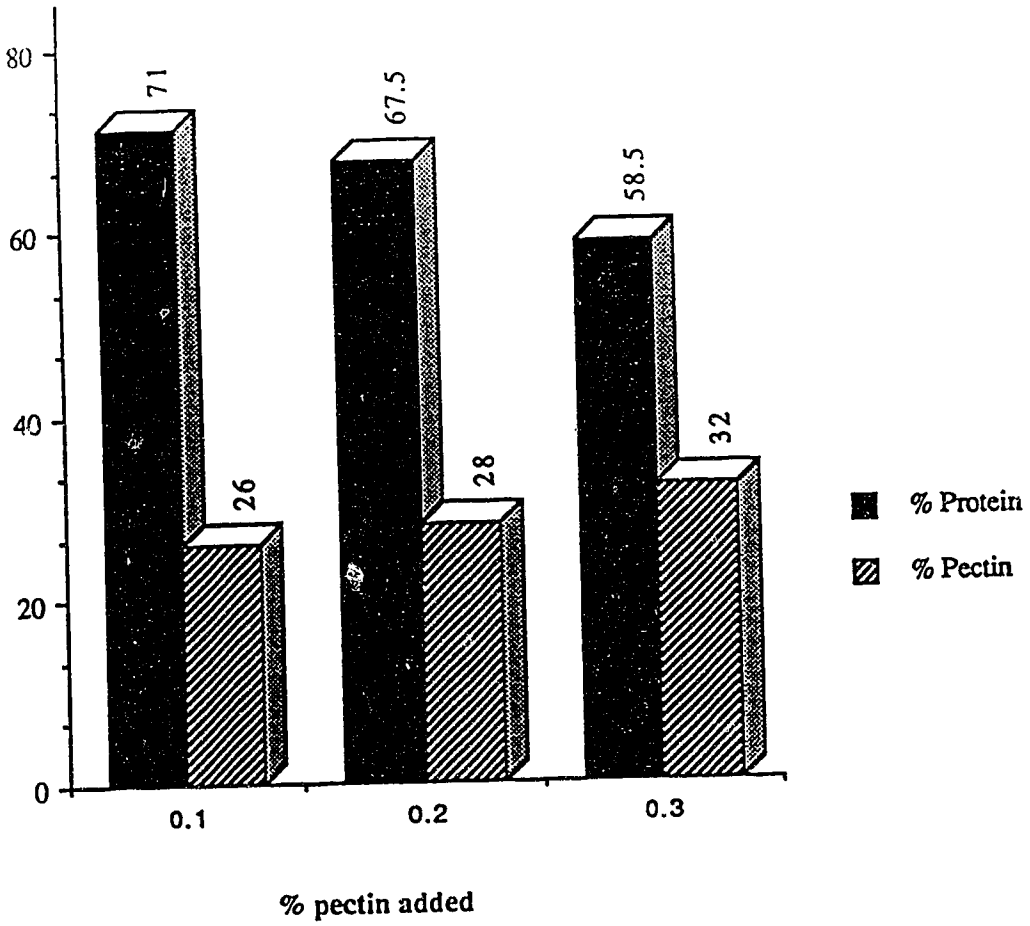

Fig. 4.12: Protein and pectin content of the sediment obtained from heated cottage cheese whey at pH 3.4.

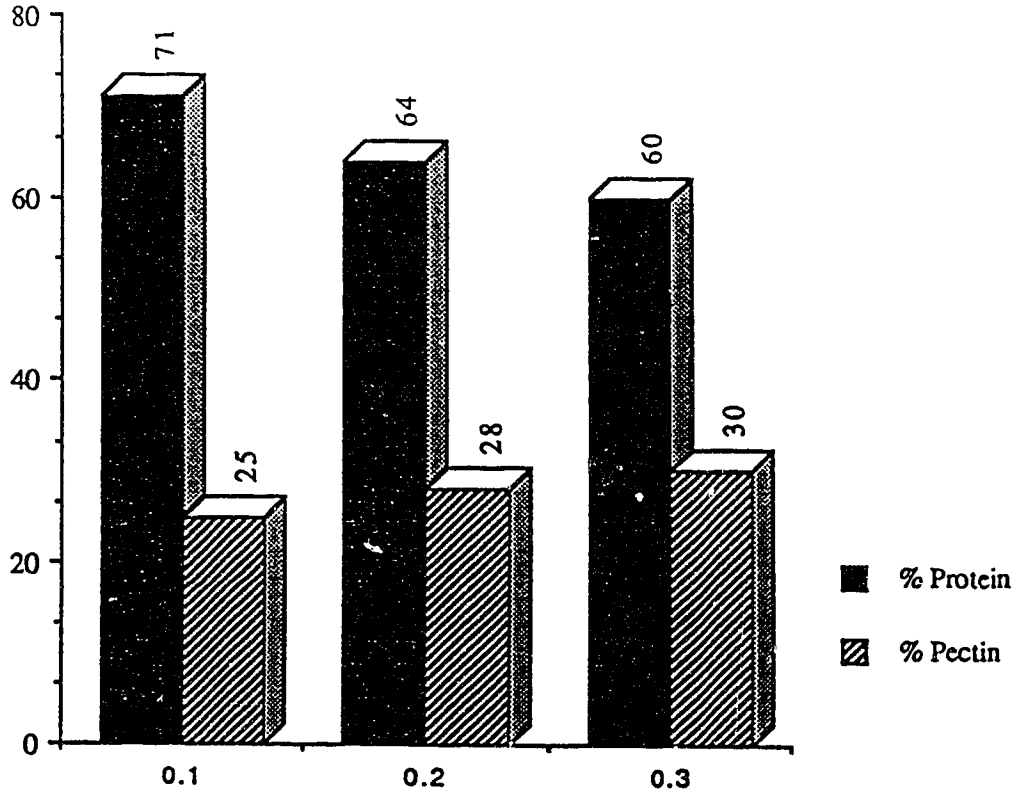

Fig. 4.13: Protein and pectin content of the sediments obtained from heated cottage cheese whey at pH 3.7

4.3.1 Comparison of various fruit juices

Different fruit juices such as apple, cranberry, grapefruit, lime and tomato were mixed separately with centrifuged CCW in 1:9 ratio and were heated at 90°C for 15 minutes at pH 3.4. Turbidity might develop in whey after heating due to heat susceptibility of the whey proteins at pH value higher than 3.7. However due to the stability of whey proteins under acidic conditions, heating of whey at 3.4 does not result in any turbidity development. This was the reason why heating was done only at pH 3.4 (thus avoiding the turbidity developed due to heating of the whey which was subsequently mixed with the fruit juices).

Heating resulted in the development of turbidity in fruit juice-whey mixtures except in case of cranberry-whey mix. (Fig. 4.14). Turbidity did not develop when fruit juices or whey were heated separately. This suggests the possible development of turbidity as a result of the interactions between the chemical components present in the whey and fruit juices.

The intensity of the turbidity development varied with the kind of juices used. In general, the turbidity values are low as only very small amounts of pectin were present in the 1:9 mixtures. Highest turbidity development was found in a tomato juice-whey mixture. However, no visual sedimentation was observed. But with the increase in the ratio of tomato juice and whey to 1:1, visual sedimentation was observed. The washed sediment obtained from the tomato juice-whey mixture showed the presence of protein pectin in

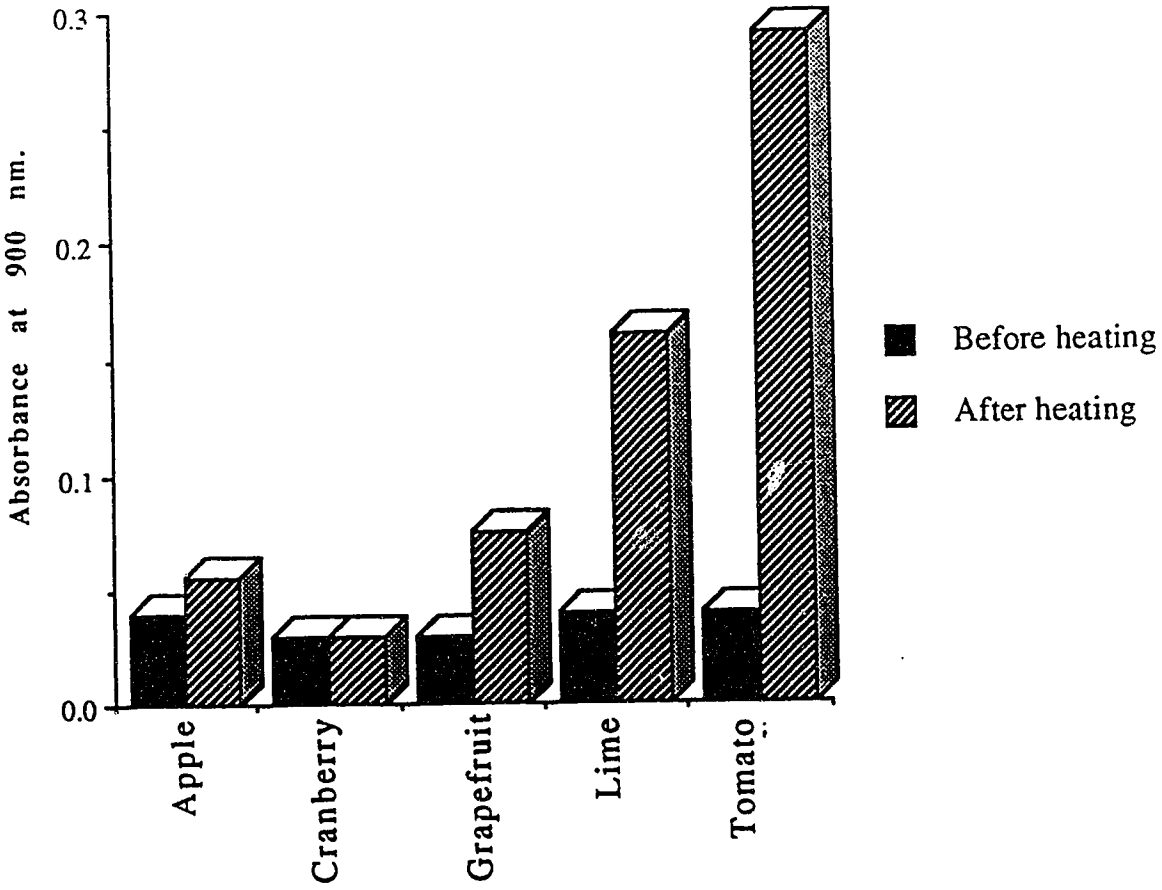

Fig. 4.14 : Turbidity of whey-Fruit juice mix. (9:1) at pH 3.4

an approximate ratio of 3:1 (Fig. 4.15). The tomato juice used in above experiments, was a clear serum of reddish yellow colour, obtained after the centrifugation to remove the cell debris. Thus the juice made at our laboratory looked quite different from the commercially available thick and red tomato juice.

The presence of protein and pectin in the sediment obtained from the tomato juice-CCW indicates the sediment to be the result of the interactions of the pectin present in tomato juice and whey proteins from the whey.

Difference in the development of the turbidity among various fruit juice-CCW mixtures, could be due to different amount and quality of the pectins present in different juices. However the apple juice which showed the least turbidity development, contained pectin in an amount (Table 4.2) that was not very much different from the amount present in tomato juice, which showed the highest turbidity development. But it also possible that there were differences in the quality of the pectin (such as molecular weight, methoxy content etc.) present in the apple juice and the tomato juice. Molecular weight of pectin has a great effect in the interactions with oppositely charged molecules. The higher the molecular weight higher would be the interactions with other molecules. Molecular weight and methoxy content of pectin vary greatly in different fruit juices depending upon the genus, species and cultivar of the fruit from which the juice is extracted (Milkeladze and Chogovadge, 1971). The quality of the pectin in juices also depends on the juice extraction conditions.

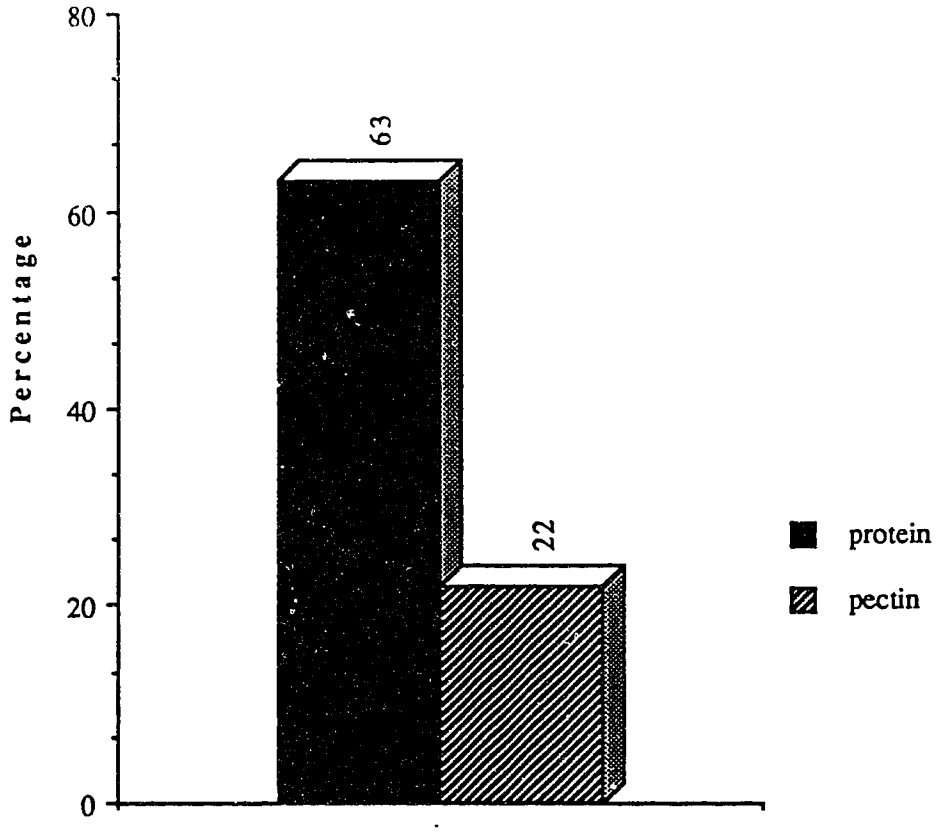

Fig. 4.15: Protein and Pectin content of the sediment obtained from heated tomato juice-whey mix. (1:1)

Table 4.2: Some relevant characteristics of the juices used.

Juice	pH	Total solids (%)	Pectin content(%)
Apple	4.0	12.6	0.50
Cranberry	2.5	18.1	0.10
Grape-Fruit	3.4	11.0	0.50
Lime	2.9	8.5	0.55
Rhubarb	3.2	4.8	0.12
Tomato	4.5	4.3	0.60

Cranberry was the only juice which did not show any turbidity development after heating at 90°C for 15 minutes. Here it should be noted that the cranberry juice was made from frozen concentrate bought commercially and showed the least amount of the pectin present compared to other fruit juices. Moreover, the commercially available juices such as cranberry and apple are usually clarified using pectic enzyme preparations (both polygalacturonase and pectin esterase), which results in the hydrolysis of the pectin present in the juices (Luh, 1971).

The incidental tasting of the prototypes during the experiment showed that all juices used in our experiments are compatible with whey in production of whey-based beverages.

4.3.2 Enzymatic hydrolysis of the pectin present in the tomato juice

The presence of pectin and protein in the sediment obtained from the tomato juice-whey mixture (1:1) indicated the pectin to be one of the components responsible for the turbidity development. Hence it was decided to observe the turbidity development in the mixture of CCW and tomato juice that has been treated enzymatically to break down the present pectin.

Prior to mixing with whey, polygalacturonase (the enzyme that breaks down the pectin molecules) was added to tomato juice and was left standing overnight. The juice was then mixed with whey in 1:9 ratio and the heating was carried out at 90°C for 15 minutes.

Significant reduction in the turbidity development was observed when enzymatically treated juice was used as compared to the turbidity developed by mixing regular tomato juice with whey in the same ratio and after the same heat treatment (Fig. 4.16). This strongly suggests the primary role of pectin in turbidity development of fruit juice-based whey drinks. Hence to avoid the turbidity or the sedimentation developed in the fruit juice-based whey drinks due to interactions between whey proteins and pectin present fruit juice, enzymatic breakdown of the pectin present in fruit juices can be used.

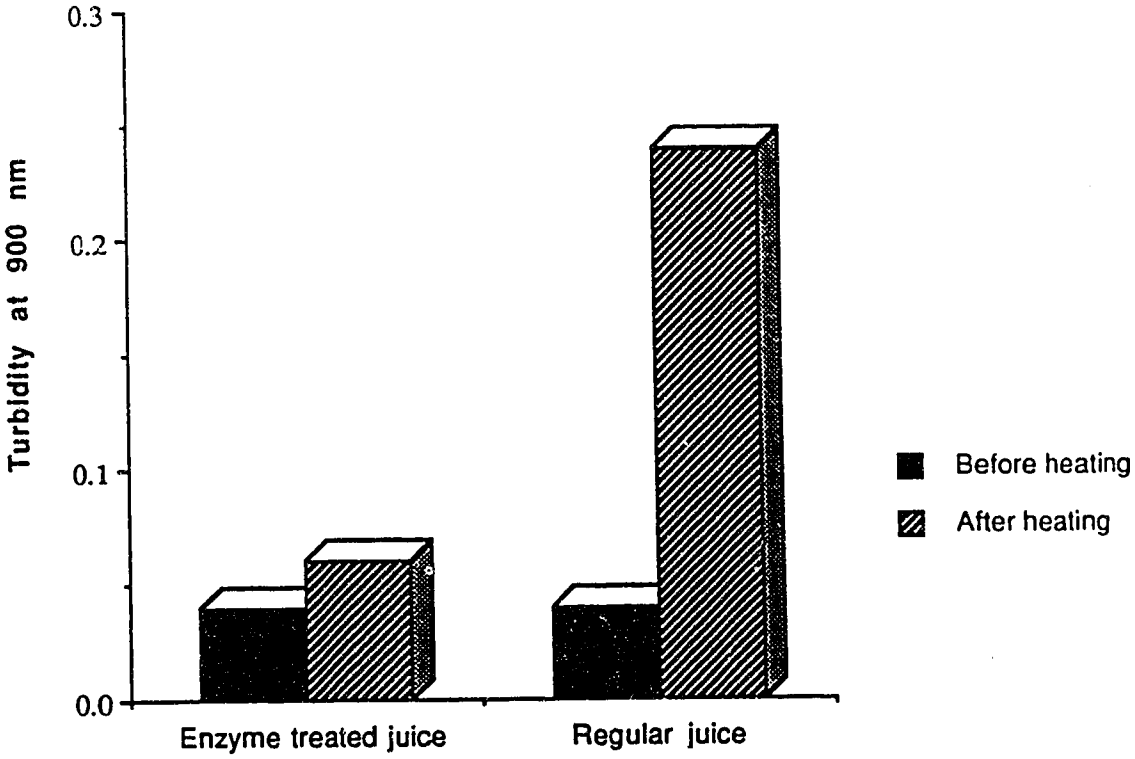

Fig. 4.16: Turbidity of the cottage cheese whey-tomato mix. (9:1) at pH 3.4.

4.3.3 Mixing of cottage cheese whey with rhubarb juice

Rhubarb juice was one of the selected juices to mix with whey in the production of juice based whey-drink prototype. But when rhubarb juice was mixed with whey in 1:9 ratio, immediate sedimentation was observed even without heating or adjustment of pH levels. This sediment appeared totally different from the sediment obtained due to interactions of pectin and protein.

The sediment was collected by centrifugation. The washed sediment was dried overnight in an oven. The dry sediment thus obtained looked like chalk powder and was suspected to be calcium oxalate because of the presence of oxalic acid and calcium in the rhubarb juice-whey mixture. Identification of the sediment was carried out with the use of HPLC. The chromatogram obtained from HPLC showed presence of oxalic acid.

The formation of the sediment of calcium oxalate in rhubarb juice and whey indicates the interaction between oxalic acid present in the juice and calcium present in whey. Thus this observation indicates that not all fruit or vegetable juices could be used to formulate juice based whey drinks.

It should be mentioned here that same type of sedimentation was observed when rhubarb juice was mixed with UF permeate obtained from CCW which contains almost the same amount of calcium but no or very little (0.1%) of whey proteins as compared to regular CCW.

4.4 Pectin as a Stabilizer in Whey-Based Drinks

High methoxy pectin has been recommended as a stabilizer in whey drinks. However the sedimentation of whey protein due to addition of pectin in model systems at pH range between 3.4 - 4.0, a pH range suitable for whey beverages, raises a question whether pectin really acts as stabilizer in prevention of sedimentation or induces sedimentation due to interactions with whey proteins.

Thus to see the effect of addition of pectin in whey-fruit juice mix, 0.3% of high methoxy pectin, (the dose recommended by Grindsted Co. for the stabilization of long-life fruit juice-based whey drinks) was added to apple juice-whey mixture and was heated at 90°C for 15 minutes at pH 3.6. Visual sedimentation was observed in the pectin added apple juice whey mix whereas no sedimentation was noted in the mixture without pectin added. Homogenization of the pectin added CCW-apple juice mix prevented visual sedimentation upon heating. However, there was intense development of turbidity in the mixture. This could be due to the mechanical disintegration or breakdown of the complex formed between pectin and protein. Hence the broken particles could not sediment but formed a suspension in the system. The development of the turbidity in the homogenized apple juice-CCW mixture with pectin added was significantly higher than the turbidity developed in the mixture without pectin added (Fig. 4.17).

The visual sedimentation in non-homogenized fruit juice-CCW mix and increased turbidity development due to addition of pectin

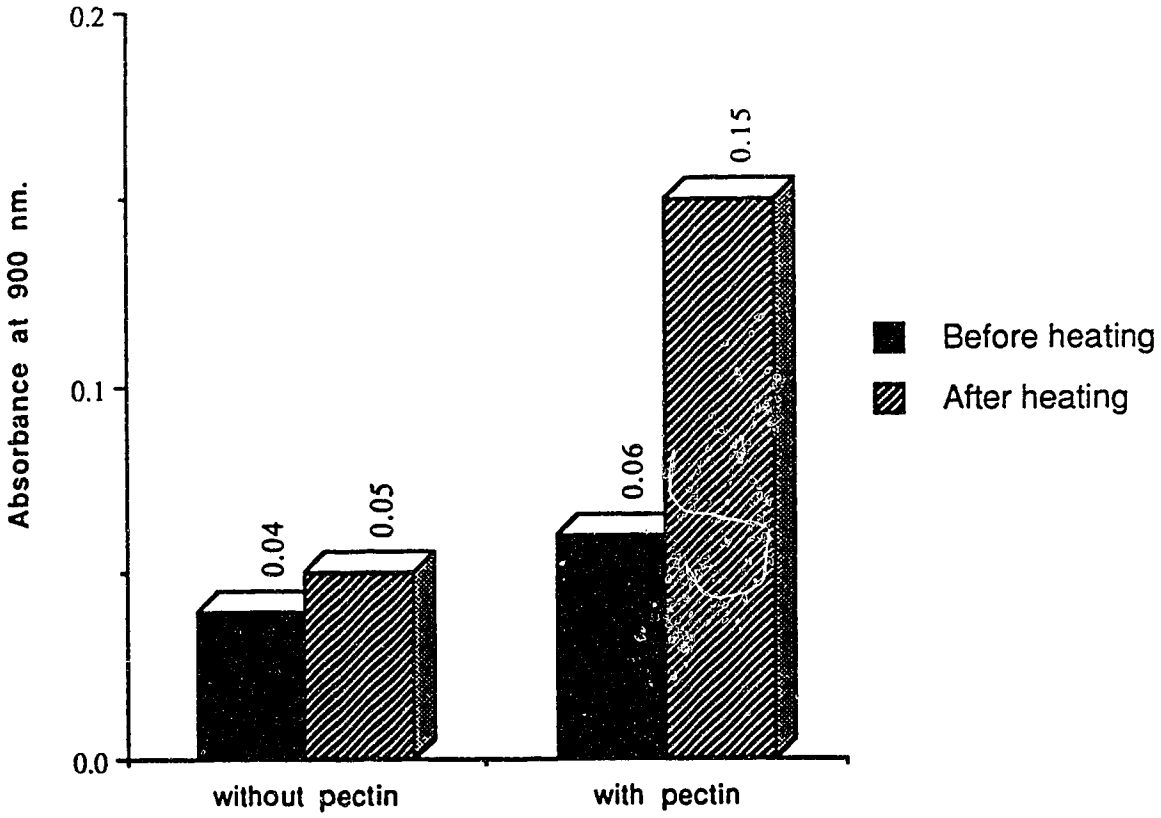

Fig. 4.17: Turbidity change in homogenized cottage cheese whey-apple juice mix.(9:1) with and without pectin added.

(which indicates the loss of solubility) in the homogenized fruit juice-CCW mix, raises a question whether use of pectin in fruit juice whey drinks is detrimental or beneficial in maintaining the quality of the product.

From our experiment it can be concluded that addition of pectin up to 0.3% in a clear fruit juice-whey mixture certainly seems inappropriate, as it results only in higher turbidity development upon heating. However, at higher level of pectin (above 0.3%), probably the added pectin may be beneficial in prevention of the sedimentation of whey proteins, because of the increased force of repulsion due to higher number of similar charges.

The explanation for this may be that at lower levels of pectin added, most of the pectin would interact with the whey proteins forming the complexes, hence reducing the repulsion between the positively charged proteins and resulting in sedimentation. However with the increased level of pectin added, there will be an increase in viscosity as well as increase in the pectin molecules carrying negative charges, which would increase the repulsive forces in the system resulting in the reduced sedimentation (Burton-Trapp, 1990).

4.5 Tomato Based Whey Drinks

Even though the highest turbidity development was found when the whey-tomato juice mix was heated, incidental tasting of the products during the experiments indicated that flavour of tomato

was highly compatible with CCW. In comparison to the other juices used, the whey tomato mix appeared to be the most promising prototype for possible commercialization. Further work was carried out with the tomato juice-based CCW drink prototype to ascertain its market potential.

Many workers have tried developing fruit juice-based whey drinks, using many different fruits such as orange, grapefruit, passion fruit, mango, pineapple, kiwi etc. (Holsinger *et al.*, 1974). However, very little work has been done in the development of tomato-based whey drinks.

In our experiments, a prototype of tomato based whey drink was made by mixing whey with commercially bought tomato juice in 9:1 ratio. Visual sedimentation of the suspended particles present in the juice occured after mixing. This was obviously due to the decrease in viscosity as a result of addition of whey in nine parts. In order to minimize the problem, whey was mixed with tomato juice in a higher ratio. Three different ratios i.e 40:60, 50.50 and 60:40 were tried. All the prototype products thus developed were quite viscous and thick and had very deep red colour, without any visual sedimentation of the suspended particles. Those prototype drinks were heated at 90°C for 15 minutes. The turbidity that may have developed during heating was totally masked by the intense red colour of the drinks as these did not look much different than regular tomato juice. In addition, because of the many suspended particles present in tomato juice, even the insoluble particles developed (if any) did not look atypical compared to normal tomato

juice as they must have blended with the other suspended particles present in the juice. In the soup developed by Webb (1937) by mixing tomato juice and whey, the presence of minute particles of coagulated whey proteins were found to be unobjectionable in the presence of tomato pulp.

As all three prototype drinks did not show any visual sedimentation even after heating, a preliminary taste panel was conducted to choose the most preferred one. The prototype with whey and tomato juice in 50:50 was found to be the most popular, whereas the one with whey and tomato in 60:40 was reported to be too thin and the one with 40:60 to be too thick by majority of the taste panelists.

Based on the preference of the taste panelists, the prototype containing tomato juice and whey in a 1:1 ratio with 2% sugar added (pH 3.7) was tested for the acceptance comparing with another commercially available tomato-based drink, clamato.

A taste panel consisting of 20 panelists was carried out to compare the different characteristics of the prototype with that of clamato as shown in appendix 3. Rating of the products was done in a hedonic scale of 1 to 9. No significant difference was found in the attributes of color and appearance, consistency and overall liking of the products (Table 4.3)

Table 4.3: Comparison of sensory acceptability of the prototype ("wheymato") with clamato using a 9 point hedonic scale.

Characteristics	Average panel score	
	Prototype	Clamato
Color and appearance	6.2	6.5
Consistency	6.5	6.05
Overall liking	6.0	6.2

Data in the same horizontal rows are not significantly different at 5% level.

A second taste panel consisting of 23 panelists was held to characterise the prototype ("wheymato") in comparison to clamato for specific quality attributes regarding taste, flavour and overall liking. The format of the score sheet was different from the previous taste panel (Appendix 4). No significant differences were found in consistency, taste, flavour and overall liking of the two products as presented in the Table 4.4.

Table 4.4: Comparison of acceptance of "wheymato" with clamato (second taste panel).

	Average panel score	
	Wheymato	Clamato
Taste	3.8	4.1
Flavour	3.9	3.6
Consistency	3.7	3.7
overall liking		
1. As breakfast drink	5.08	4.96
2. As thirst quencher	5.27	5.25

Data in the same horizontal rows are not significantly different at 5% level.

None of the panelist were able to detect any taste of whey in the wheymato. Most of the panelist thought wheymato to be very much suitable as a thirst quenching drink whereas a few thought that it was too acidic

A few panelists who had previously tried whey-based drinks thought wheymato to be the first whey based drink they have ever liked, when they came to know the presence of whey in the drink they were served.

The data obtained from the sensory evaluation of the prototype tomato-based whey drink showed that it may be a good commercial product. Such product would have three main advantages, as the price would be lower because of the presence of 50% whey (which is a waste product); It would aid in utilization of whey contributing to the reduction of environmental pollution created by disposal of whey, and the products would have higher nutritional value than the regular tomato juice because of the presence of whey proteins.

Thus it can be concluded that fruit juices (such as tomato) can be mixed with whey to produce marketable whey beverages. However there are some limitations in the production of fruit juice-based whey beverages if the clear type drink is desired. One of the limitations noticed from our experiments was the interactions of the pectin present in the juice (used to mix with whey) and the whey proteins present in the whey, resulting in the sedimentation or the turbidity development of the product. But the problem of this pectin-whey protein can be overcome easily by using clarified juices to mix with whey in the production of whey-based beverages. This is because the clarified juices contain hydrolyzed pectin which can not interact with the whey proteins.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary of the Research Findings and Conclusions

This study of the interactions of pectin with whey proteins using 1% WPC solutions showed that both high methoxy and low methoxy pectins are capable of interacting with whey proteins in similar manner, resulting in the visual sedimentation at all levels of pH (3.4, 3.7 and 4.0) studied. Since the visual sedimentation was dependent on the pH values, electrostatic interactions between the two oppositely charged macromolecules resulting in the formation of complexes, were assumed to be responsible for the sedimentation.

High methoxy pectin (HM) was also able to interact with CCW proteins inducing the visual sedimentation at all three pH levels, when the HM pectin was added to CCW. Heating resulted in the increased sedimentation in the CCW-pectin system indicating an increase in the intensity of the interactions between pectin and proteins. Amount of the protein present in the dry sediment obtained from heated samples was significantly higher than the amount of protein present in the sediment obtained from unheated samples. Release of more basic groups in the protein molecules as a result of heat treatment could be the reason for the increased intensity of the interactions between protein and pectins.

Development of turbidity in fruit juice-CCW mixtures was observed after heat treatment. Sediment obtained from CCW-tomato

juice showed the presence of both pectin and protein. Moreover, significant reduction in the turbidity development after heating was observed as compared to the turbidity developed in tomato-CCW mixture, when tomato juice treated with the enzyme polygalacturonase was used to mix with the CCW. This indicates that the protein-pectin interaction could be the reason for turbidity development or sedimentation in fruit juice based whey beverages after heat processing. This often reported problem interferes in maintaining quality of the fruit juice based whey beverages.

Besides the sedimentation due to interaction between pectin and whey proteins, sedimentation may also result in fruit juice based whey drinks from the interaction of other components present in juices and whey. For example, juice containing oxalic acid may interact with calcium present in the whey resulting in the sedimentation of the product due to formation of calcium oxalate. This was the observed case in our experiment when rhubarb juice was mixed with CCW to produce a juice-based prototype whey drink.

Addition of pectin up to 0.3% as a stabilizer in fruit juice-whey prototype drink had an adverse effect, as the added pectin caused sedimentation in unhomogenized products and resulted in intense turbidity development after homogenization. Thus the recommendation of addition of pectin (0.2-0.3%) in the fruit juice based beverages to avoid sedimentation during storage seems controversial. In order to get the stabilizing effect, the amount of the added pectin should be higher than 0.3%. The assumed reason is that at an increased levels of pectin used, there would be enough

negative charges in the pectin molecules to create a repulsive force between the molecules which would result in inhibitition of the sedimentation in the pectin-protein systems.

Tomato juice was found to be highly compatible with whey flavour compared to other juices used in our experiments. However, problem of sedimentation or turbidity development was observed in a tomato based whey drink prototype (clear type). The turbidity development could be controlled by hydrolysing the pectin present in the tomato juice using enzyme polygalacturonase. The turbidity development was not noticeable when commercially available tomato juice was mixed with whey to produce a tomato juice-based whey drink prototype. The reason was the intense red colour and the increased viscosity of the juice used masking the turbidity that might have developed as a result of the protein-pectin interaction.

5.2 Recommendations for future research

The presence of pectin and protein in all the sediments, obtained by addition of pectin in 1% WPC solutions or CCW indicated the ability of pectin to interact with whey proteins. However the fractionation of the proteins from the sediments was not carried out. It is not known clearly whether any of the individual whey proteins are more reactive with pectin. Separation of proteins from the sediments obtained from 1% WPC, unheated CCW and heated CCW (by electrophoresis or other method) would be desirable to determine exactly which protein is more susceptible to the

interaction under the conditions investigated in our study. Comparison of the protein present in the supernatant from the pectin added samples with control samples should also provide the answer.

The amount of sediment obtained from 1% WPC solutions (unheated) was significantly higher than the sediment obtained from CCW (unheated) by addition of same amount of pectin at all pH levels studied. This result was thought to be caused by the difference in Ionic strength of the two systems. Hence further confirmatory work in the area of effect of ionic strength in the pectin-whey proteins interactions seems necessary.

Even though the protein-pectin interaction was assumed to be mainly due to electrostatic interaction on the basis of dependence of the interaction on pH values, other forces could also be a part of the interaction. Thus more fundamental research on elucidation of the exact mechanism of the interaction should be carried out.

In our study, it was observed that the turbidity development and the unpleasant flavour of whey was totally masked in the prototype made by mixing whey and commercially available tomato juice in 1:1 ratio. The reason for it was the color and presence of suspended cell particles in the tomato juice. However, in the prototype, some of the proteins present in the whey must have interacted with pectin resulting in the formation of protein-pectin complexes. Hence, from a nutritional point of view, there might have been loss of some of the available whey proteins of high quality. However, the complex might be dissociated in the highly acidic

condition of the stomach when consumed by humans. This would warrant further research in the conditions and pH required for the dissociation of the protein-pectin complex.

Many whey-based beverages have been reported to suffer commercially from sedimentation. The whey protein-pectin interaction was found to be the reason for the turbidity or sediment development in tomato juice-whey prototype in our experiments. Same reason could be true for the development of turbidity or sedimentation in other fruit juice and whey mixtures. Hence further research in the development of whey drinks by mixing fruit juices (that have been treated enzymatically to hydrolyze the pectin present) with whey might be carried out. Our understanding in this area should be helpful in production of high quality fruit juice-based whey beverages without any turbidity or sediment formation.

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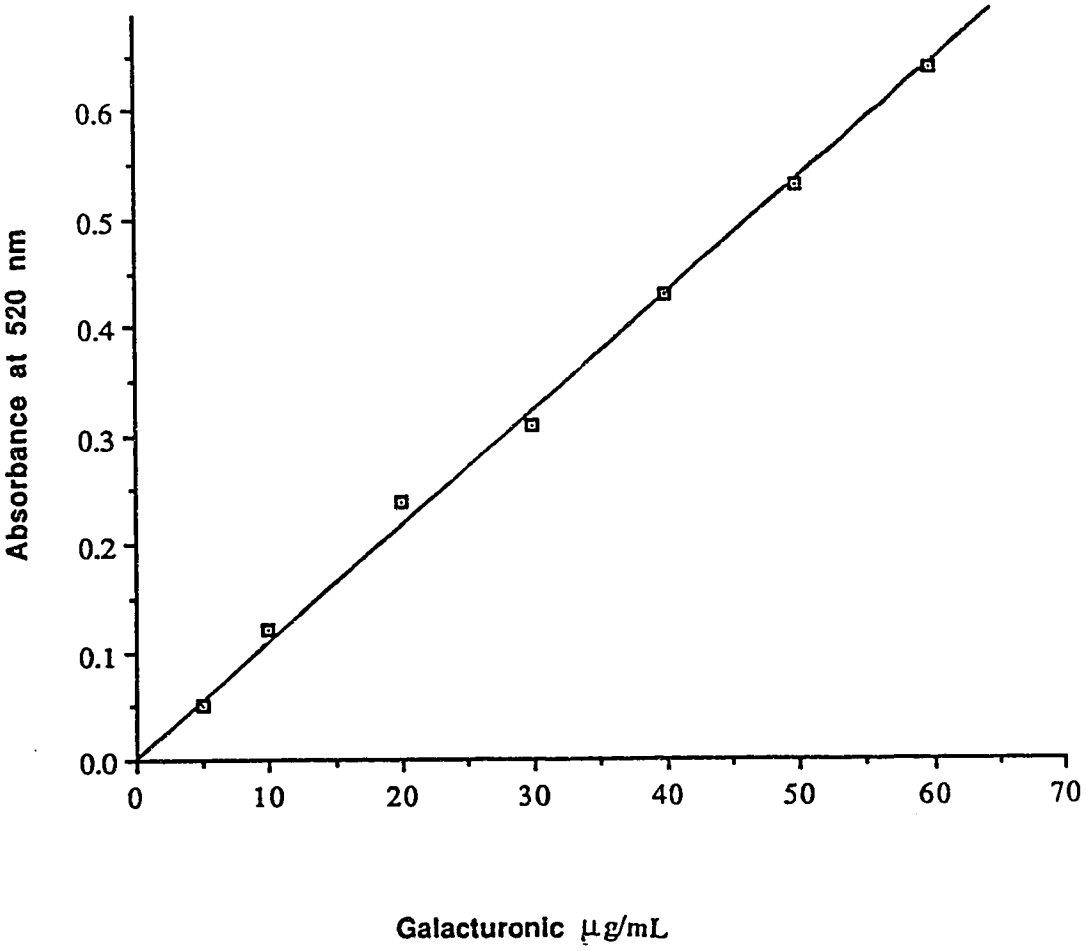

Appendix 1: Standard curve for galacturonic acid.

Appendix 2: Preference test for selection of [illegible]-based whey prototype drink.

Name..... Date.....

You are given three refreshing summer drinks

A) Please indicate the one that you prefer the most by circling the number

456 564 645

B) Please give reason for the preference

_____

_____

_____

Appendix 3: Questionnaire for the comparison of the prototype with clamato using hedonic scale.

You are given tomato based refreshing drinks. Please indicate the degree of liking of each product by circling the # between 1 to 9 in each appropriate hedonic scale. As you are the only one who knows what you like, please help us by giving an honest expression of your personal feeling about each product provided.

A) Liking of color and appearence

	Product#356	Product#547
Like extremely	9	9
Like very much	8	8
Like moderately	7	7
Like slightly	6	6
Neither like nor dislike	5	5
Dislike slightly	4	4
Dislike moderately	3	3
Dislike very much	2	2
Dislike extremely	1	1

Comments.....

.....

B) Consistency of the products

	Product#356	Product#547
Like extremely	9	9
Like very much	8	8
Like moderately	7	7
Like slightly	6	6
Neither like nor dislike	5	5
Dislike slightly	4	4
Dislike moderately	3	3
Dislike very much	2	2
Dislike extremely	1	1

Comments.....

.....

C) Overall liking of the products

	Product#356	Product#547
Like extremely	9	9
Like very much	8	8
Like moderately	7	7
Like slightly	6	6
Neither like nor dislike	5	5
Dislike slightly	4	4
Dislike moderately	3	3
Dislike very much	2	2
Dislike extremely	1	1

Comments.....

.....

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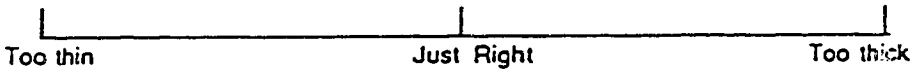
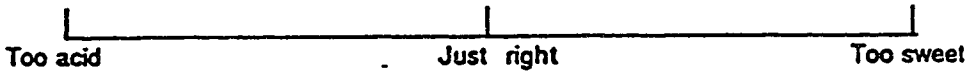
Appendix 4: Questionnaire for the comparison of the prototype with clamato (second taste panel).

'ou are given a refreshing tomato based drink. Please indicate the degree of liking by marking he line at appropriate spot in the provided scale

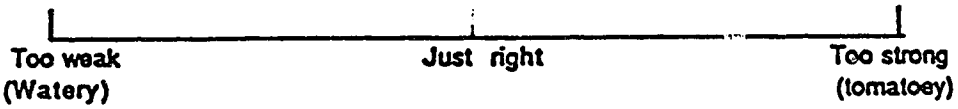
\) Consistency



) Taste

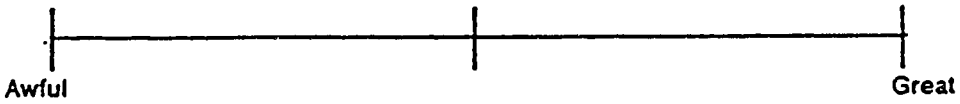


:) Flavour

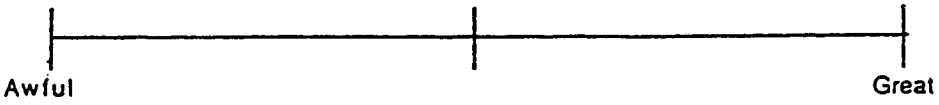


) Overall liking

1. As thirst quenching drink



11. As breakfast drink



omments.....
.....

roduct # 169

Thankyou.