

DEVELOPMENT OF CLOUD STABLE WHEY-FORTIFIED BANANA BEVERAGES

by

ERNEST KOUADIO KOFFI

(Under the Direction of Louise Wicker)

ABSTRACT

Banana puree is a complex mixture of proteins, carbohydrates and phenolics. The addition of whey protein to produce fortified banana beverages may be a better way to utilize wasted bananas in banana producing countries where malnutrition prevails. This work characterized the components of the banana pulp and their chemical interactions and selected sensory and physical measurements to get a better understanding of the formulated whey-banana beverages.

High methoxyl pectin ($DE > 80$) and low molecular weight proteins were present in banana, mango and orange pulps. Aspartic acid in protein pulps was predominant while methionine and tyrosine contents were very low. Phenolic compounds were highest in banana pulp (138 ± 2 mg gallic acid equivalent/L = mg GA/L) compared to 102 ± 8 mg GA/L for orange. Phenolic compounds were about 10 fold less (15 ± 2 mg GA/L) in mango. The interaction between whey proteins (10%, w/w) and pectin (0.4%, w/w) was evaluated in acidified banana beverage model systems using rheology and particle size measurements. Pectin-protein interaction influenced the mechanical properties whereas sucrose influenced the particle size of the whey-banana beverage. Except for the first week, the particle size was constant for 60-day

storage at 4, 20, 30 and 40 °C. While the products remained light colored ($L^* \sim 71$) for 60 days, redness (a^*), yellowness (b^*) and saturation (c^*) increased at elevated temperatures. The hue angle (H^*) was less at elevated temperatures however. The product was a sour, sweet, smooth beverage with distinctive banana flavor and minimum off-flavor. Sourness and acidity were critical quality factors for consumer acceptability. Sedimentation was maximum at 40C while serum separation was minimum at 4C.

Sedimentation was greater for non-UHT than UHT treated whey-banana beverages and increased with increased storage temperature for 17 days of storage. The flow behavior and consistency indexes were comparable for both UHT and non-UHT treated samples. The flow behavior decreased with increasing temperature as the consistency increased. The particle size measurements (D_{43} , D_{32}) were greater for the non-UHT product and were independent of time and temperature. Higher color values were observed for the UHT beverage.

INDEX WORDS: Banana, pectin, cloud, neutral sugars, phenolics, whey proteins, polysaccharide-protein complexes, flow behavior, consistency index, particle size

DEVELOPMENT OF CLOUD STABLE WHEY-FORTIFIED BANANA BEVERAGES

by

ERNEST KOUADIO KOFFI

B.S., The University of Abidjan, Ivory Coast, 1986

M.S., The University of Florida, 1990

A Dissertation Submitted to the Graduate Faculty of

The University of Georgia in Partial Fulfillment

of the

Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2003

@ 2003

Ernest Kouadio Koffi

All rights Reserved

DEVELOPMENT OF CLOUD STABLE WHEY-FORTIFIED BANANA BEVERAGES

by

ERNEST KOUADIO KOFFI

Major Professor:

Louise Wicker

Committee:

Robert Shewfelt

Casimir Akoh

William Kerr

Aaron Brody

Sandra Kays

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
December 2003

DEDICATION

This dissertation is dedicated to my parents and my wife who have always supported me.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the following people:

My major professor, Dr. Louise Wicker for being a very inspiring example in my graduate student life. She has always encouraged and advised me to plan my work and manage my time better. Thanks to my committee members, Dr. Robert Shewfelt, Dr. Casimir Akoh, Dr William Kerr, Dr. Aaron Brody and Dr. Sandra Kays for their guidance during this research.

Dr Stanley Kays for all his help with the neutral sugar analysis, Dr Robert Phillips for the amino acids analysis, Dr. Joseph Frank for his help with the microbiological analysis and Dr Romeo Toledo for the packaging material.

I am thankful to The Fulbright, The graduate school and Dr. Donald McLellan for their support during my graduate studies. I am also thankful to Dr. Jack Reeves for his help with the statistical analysis and Dr. Rakesh Singh for his advice.

My lab mates Panida Banjongsinsiri, Kim Yoo-Kyung, Maureen Bishop, Jennifer Ackerley and Janice Hunter for their help all these years.

David Peck, Revis Chmielewski and my entire sensory panel for their participation and input and Rajdeep Singh for his friendship.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	v
CHAPTER	
1. INTRODUCTION	1
2. LITERATURE REVIEW	7
3. CHARACTERIZATION OF CLOUD COMPONENTS FROM SELECTED TROPICAL FRUITS	70
4. INTERACTIONS OF WHEY PROTEINS WITH HIGH METHOXYL PECTINS IN ACIDIFIED BANANA BEVERAGES.....	97
5. STORAGE STABILITY AND SENSORY ANALYSIS OF UHT PROCESSED WHEY-BANANA BEVERAGES.....	114
6. CHANGES IN THE PROPERTIES OF UHT WHEY-BANANA BEVERAGE DURING STORAGE.....	144
7. SUMMARY AND CONCLUSIONS	172
8. APPENDIX.....	175

CHAPTER 1

Introduction

Bananas

Banana is one of the most important food crops in the world (FAO, 1998). World production of table bananas is greater than that of either grapes or apples, and is exceeded only by that of citrus (FAO, 1998). The banana tree belongs to the family Musaceae, and the varieties frequently consumed in the United States are from the species *Musa cavendishii* and *Musa sapientum*.

There is a large body of literature available on the history, physiology, morphology, production and use (Von Loesecke, 1950; Stover and Simmonds, 1987); diseases (Wardlaw, 1950; Stover and Simmonds, 1987); technology, chemistry, post-harvest physiology and biochemistry (Von Loesecke, 1950; Palmer, 1971; Freiberg, 1969; Stover and Simmonds, 1987) of bananas. Ivory Coast is among the leading banana producing countries in Africa. However, there are large post-harvest losses of bananas in rural areas of the Ivory Coast. The problem is that over 40,000 Mt of the more than 200,000 Mt (FAO, 1998) of bananas produced in the Ivory Coast are lost annually because of poor storage, poor transportation, or overproduction at certain times of the year. Bananas are always harvested green, but by the time they reach the port, the ripening process has already started (green skin turning yellow). Although transportation is not a problem for the majority of large firms located close to highways, for the small farmers who produce 40% of the bananas, transportation is never certain, and often the harvested fruit reaches the port at a yellow skin stage. Therefore, for small farmers who produce banana in countries like

the Ivory Coast, other uses must be found for the surplus fruit. Because bananas have a high sugar content (200g/kg for the fully ripe fruit) (Wills et al., 1984) and a desirable fruity flavor, one alternative could be to mash or puree banana for use in bakery products, dairy products, and baby food. But a better alternative could be production of banana juice.

Whey

Whey, an aqueous solution containing lactose, proteins, fat, and minerals is concentrated to yield whey protein concentrate (WPC), with desirable functional properties. Whey contains approximately 6% total solids of which 70% is lactose and 11% is whey protein (Zall, 1984). The increased use of whey proteins in recent years is due to a large number of functional components identified (Morr, 1982). Whey protein has a protein efficiency ratio (PER) of 3.2 compared to 2.6 for casein (McIntosh et al, 1998). A PER of 2.5 is considered good for a protein. Whey possesses more sulfur-containing amino acids than casein and a surplus of the essential amino acids, which are often limited in plant proteins. The high-sulfur amino acid is important because of its ability to enhance immune function and antioxidant status. Because the banana pulp has low protein content (Brady, 1970; Wills et al., 1984), adding milk proteins, especially whey proteins, to this pulp will increase their nutritional value. The non-denatured whey proteins are soluble at an acid pH, a characteristic that facilitates their incorporation into beverages (Swaisgood, 1996).

One way to stabilize the banana juices may be the use of valve homogenization, commercial pectins, or adjustment of the pH of the product to a range of 3.4-3.8 for stability of the whey proteins to heat coagulation (Jelen and Buchheim, 1984). By acting as dispersant, pectins in milk drinks minimizes sedimentation problems.

Acid whey in the powdered or liquid form, is the type of whey commonly used in the formulation and processing of fruit beverages because it blends well with most fruit flavors including lemon, orange, and grapefruit) (Shekilango, 1996).

Whey-banana beverages

Shekilango et al. (1997) found that a blend of 3 parts (w/w) acid whey to 2 parts (w/w) banana was the most acceptable formula in terms of flavor, texture and viscosity. However, the shake separated immediately when left on standing. The authors suggested that interaction between constituents, such as the banana pectins and tannins were responsible for the sedimentation.

Homogenization can be used to alleviate sedimentation by reducing particle size (Jinescu, 1974). Under high pressure, the flocks of whey protein-pectin aggregates are ruptured thereby releasing entrapped fluid and decreasing the volume of the sediment. Homogenization can also reduce viscosity. Crandall and Davis (1988) reported that homogenization reduced the viscosity of an orange juice concentrate. Factors that will affect apparent viscosity of a whey-banana product include structure, volume, size, shape, number and interactions of particles.

Suitable stabilizers including pectins, propylene glycol alginate (PGA) and carboxymethylcellulose (CMC) have been added to drinks to prevent coagulation during pasteurization (Glahn, 1982). Pectin solutions exhibit the non-Newtonian pseudoplastic behavior typical of most polysaccharides. The viscosity of a pectin solution depend on the molecular weight of the pectin, its degree of esterification, concentration, pH and presence of counter-ions in the medium. The ability of pectins to bind to proteins and protect them from aggregating is very important in formulated beverages during heat pasteurization. Upon heating, β -lactoglobulin aggregates at temperature above 80 °C over a wide range of pH values (de Wit and

Klareenbek, 1984). However, it is well known that this aggregation occurs more readily at alkaline pH.

Whey proteins have the potential to improve the nutritional value of tropical fruit juices through fortification. However, previous works indicate that there is still great potential for improving the acceptability of the fortified beverages. A great deal is known about the composition of citrus juices. As mentioned earlier, the structure of the pectin and protein interactions is influenced by pH, ionic strength, and thermal treatment. Therefore, it can be assumed that cloud stabilization is also greatly affected by the same factors. Cloud stabilization in citrus juices is believed to be primarily due to protein-pectin interaction. Thus, it is reasonable to think that by manipulating the properties of pectins, we may improve the stabilizing effect of these molecules. While many researchers believe that cloud stabilization is restricted to protein-pectin interaction, others feel that other components of the fruit juice have stabilizing effect. As mentioned earlier, pectin structure and functionality can be tailored to specific applications. In addition, milk-processing technology has engineered whey protein concentrates with improved functionality for various foods.

Objectives of the research

The main goal of this study is to develop a cloud stable whey-banana beverage from whey protein concentrate.

The specific objectives are:

- 1-To determine the chemical composition of banana, orange and mango pulps.
- 2-To determine the degree of esterification (DE) of pectins present in banana, orange and mango pulps.

3-To investigate the effect of storage time on the sedimentation, sensory and color of whey-banana beverage prototypes.

4-To investigate the composition of the sediment harvested from the whey-banana beverages.

5-To evaluate the effects of commercial pectins added as stabilizers in the whey-banana beverages.

6-To characterize whey-banana beverage by identifying and quantifying sensory characteristics using sensory descriptive analysis.

7-To evaluate the market potential of a whey-banana beverage product using a three-point acceptability scale.

References

Brady, C.J., Palmer, J.K., O'Connell, P.B.H., and Smillie, R.M. An increase in protein synthesis during ripening of the banana fruit. *Phytochemistry* **1970**, 9, 1037-1044.

Crandall, P.G. and Davis, K.C. Viscosity reduction and reformation of structure in orange concentrate as affected by homogenization within commercial taste evaporators. *Journal of Food Science* **1988**, 56, 1360-1364.

De Wit, J. N.; and Klareenbek, G. Effect of various heat treatments on the structure and solubility of whey proteins. *Journal of Dairy Science* **1984**, 67, 11, 2701-2710.

FAO Production yearbook. Food and agriculture organization of the United Nations. Yearbook Production. **1998**.

Freiberg, S.R. Encyclopedia of food and food science. John Wiley and Sons, Inc., New York, **1969**.

Glahn, P.E. Hydrocolloid stabilization of protein suspensions at low pH. *Progress in Food and Nutrition Science* **1982**, 6, 171-177.

Jelen, P. and Buchheim, W. Stability of whey protein upon heating in acidic conditions. *Milchwissenschaft* **1984**, 39, 215-218.

Jinescu, V.V. The rheology of suspensions. *International Chemical Engineering* **1974**, 14, 3, 397-401.

McIntosh, G.H., Royle, P.J., Le Leu, R.K., Regester, G.O., Johnson, M.A., Grinsted, R.L.,

Kenward, R.S. and Smithers, G.W. Whey proteins as functional food ingredients. *International Dairy Journal* **1998**, 8, 425-434.

Morr, C.V. Functional properties of milk proteins and their use as food ingredients. In P.F. Fox (ed.), *Developments in Dairy Chemistry* 1, p 375. London, UK: Applied Science **1982**.

Palmer, J.K. **1971**. The banana. In: the biochemistry of fruits and their products. Hulme, A.C. ed. Vol 2, p. 65.

Shekilango, S.A. Technical, nutritional, and sensory investigations of whey-banana beverages. Masters thesis. University of Alberta. **1996**.

Shekilango, S. A.; Jelen, P and Bagdan, G.C. Production of whey-banana beverages from acid whey and overripe bananas. *Milchwissenschaft* **1997**, 52, 209-218.

Stover, R.H. and Simmonds, N.W. Bananas. 3rd ed. John Wiley & Sons Inc., New York, NY, **1987**.

Swaigood, H.E. Characteristics of milk. In Food Chemistry 3rd ed. Fennema, O.R., Ed.; Marcel Dekker: New York,Basel, **1996**.

Von Loesecke, H.W. Banana, 2nd ed. Interscience Publishers, New York, **1950**.

Wardlaw, C.W. Banana diseases, 2nd ed. Longmans Group Limited, London, **1950**.

Wills, R.B.H., Lim, J.S.K., and Greenfield, H. Changes in chemical composition of “Cavendish” banana *Musa acuminata* during ripening. *Journal of Food Biochemistry* **1984**, 8, 69-74.

Zall, R.R. Trends in whey fractionation and utilization, a global perspective. *Journal of Dairy Science* **1984**, 11, 2621-2629.

CHAPTER 2

Literature Review

Banana composition

In banana, compositional changes following harvest are important since banana is a climacteric fruit. Dramatic changes in banana peel color and pulp texture occur during the rise in respiration during climacteric. In commercial trade, the ripening is initiated after transporting the green banana to locations where the fruits are treated with about 1000 ppm ethylene. Ripening temperature, humidity, and other conditions can be varied to suit fruit variety and trade conditions (Palmer, 1971; Golding et al., 1999).

Color changes in banana during ripening are based on the peel color rather than the pulp color. Color of banana peel has been classified by Anon (1972) in banana ripening guide for commercial use in which 7 stages of peel color were reproduced and translated to a numerical scale where 1+2= green, 3= yellow with green tip, 4= more yellow than green, 5= yellow with green tip, 6= full yellow, 7= full yellow with brown spots. Green banana peel contains 50-100 ug/g of chlorophyll, 5-7 ug/g of xanthophyll, and 1.5-3.5 ug/g of carotene. During ripening, all the chlorophyll is lost and total yellow pigment remains approximately constant (Von Loesecke, 1950; Stover, 1987).

The proximate composition of 'Cavendish' banana pulp during ethylene induced ripening has been studied by Wills et al. (1984). Table 1 shows the composition of banana at different color stages. The major changes in the pulp during ripening of the bananas is in the carbohydrates. About 21 g/100 g of the pulp of the unripe fruit is starch, which decreases

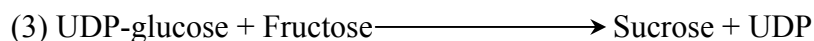
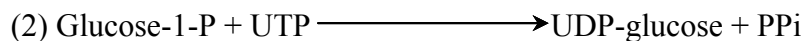
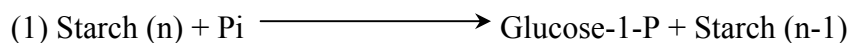
Table 1. Proximate composition of banana pulp during ripening (g/100 g) (Wills et al., 1984)

Color Stage	Fruit State	Water	Protein	Fat	Sugars					Dietary Fiber
					Fructose	Glucose	Sucrose	Total	Starch	
1+2	green	71.9	1.9	0.1	0.1	0.2	1.0	1.3	21.2	3.2
3	green turning yellow	73.2	1.7	0.1	1.0	1.6	6.4	9.0	12.0	2.7
4	more yellow than green	73.6	1.7	0.1	1.4	1.9	7.8	11.1	10.2	2.8
5	yellow with green tip	74.0	1.7	0.1	2.0	2.9	9.5	14.4	5.8	2.5
6	fully yellow	75.1	1.7	0.1	2.4	3.6	11.2	17.2	1.9	2.5
7	fully yellow with brown spots	76.1	1.6	0.1	3.2	4.2	8.8	16.2	0.8	1.9

continuously during ripening to reach 0.8 g/100 g in the fully ripe fruit (stage 7= completely yellow with brown spots). Sugars are at low level in the unripe fruit (1.3 g/100 g), but increase as a result of starch hydrolysis during ripening. Sucrose is always the major sugar present; the major increase in sucrose occurs early in the ripening process (stage 1= green to stage 3= green turning yellow) and reaches a maximum (about 11 g/100 g) in firm ripe fruit (stage 6= fully yellow). Fructose is always present at slightly lower levels than glucose. Both fructose and glucose increase continuously during ripening and reach their maximum level in fully ripe fruit (about 3 g/100 g and 4 g/100 g, respectively). Total carbohydrates decreases by 5% during ripening, presumably because sugars are utilized in respiration (Palmer, 1971; Wills et al., 1984). The water content of the banana pulp increases during ripening from 72 g/100 g to 76 g/100 g. According to Wills et al. (1984), this increase is the result of water accumulation as an end product of respiration. The movement of water from peel to pulp is another reason for the increase of water during the ripening process of bananas (Von Loesecke, 1950). There is a decrease in dietary fiber early in the ripening process (stage 1 to 3) and again when the fruits are fully ripe (stage 7). This change is from 3.2 to 2.7 g/100 g as a result of hydrolysis of hemicelluloses and breakdown of pectic substances (Von Loesecke, 1950).

Many researchers, such as Areas and Lajolo (1981) and Terra et al. (1983), observed that the accumulation of sucrose in banana fruit preceded the increase of glucose and fructose during starch degradation. Several researchers (Kayisu and Hood 1981; Lii et al., 1982) have studied banana pulp starch. They reported that the starch contained around 80% amylopectin. The gelatinization temperature range was 74-83 °C. This suggests that debranching enzymes must be present to break α -1,6 glucosidic linkages. Several enzymes, including alpha and beta amylases, glucosidases, phosphorylases and phosphatases, are involved in sugar metabolism and starch

hydrolysis (Garcia and Lajolo, 1988). Terra et al. (1983) suggested that the transformation of starch to sucrose was one of the possible mechanisms involved in the ripening of bananas. They proposed the following reaction mechanisms leading to the formation of sucrose, the major sugar in banana.



Reaction (1) is catalyzed by phosphorylase enzyme.

Reaction (2) is catalyzed by UDP-glucose pyrophosphorylase.

Reaction (3) is catalyzed by sucrose-synthetase.

Mao and Kinsella (1981) found that amylase activity was present in both banana pulp and peel. They also reported that the enzyme activity was higher at both distal and proximal ends than in the middle section. Further investigation by the same authors showed that amylase activity increased most significantly in both pulp (9.3 to 17.0 units) and peel (4.2 to 9.8) from maturity stage 3 (green fruit turning yellow) to stage 4 (more yellow than green). They found the highest amylase activity in banana pulp (19.9 units) in fruits at stage 5 (yellow skin with green tip remaining). The banana amylase had pH optimum 6.3, and retained significant activity until temperature exceeded 62 °C, when activity dropped sharply. Amylase activity was destroyed after five minutes of steam blanching at 100 °C. Later research by Mao and Kinsella (1981) demonstrated that over 80% of the observed amylase activity was attributed to alpha-amylase. The activity of beta-amylase was ten folds lower. The amylase preparation from banana hydrolyzed soluble potato starch and banana starch at similar rates.

The interconversion of pectic substance is presumed to be involved in the characteristic softening which occurs during fruit ripening. In the pulp of bananas, insoluble protopectin decreases from about 0.5 to 0.3% of fruit weight and soluble pectin shows a corresponding increase during ripening (Von Loesecke, 1950). Prabha and Bhagyalakshmi (1998) found that total hemicellulose content lowered considerably from 2.4% to 0.9% during ripening, whereas pectin decreases from 1.1% to 0.8%. Hultin and Levine (1965) observed a very large increase in pectinesterase activity in the pulp of the ripening banana fruit. Their finding was confirmed by De Swardt and Maxie (1967), who noticed an apparent increase in pectinesterase in the ripening fruit. However, Palmer (1971) reported constant pectinesterase activity in ripening banana. He suggested that phenolic compounds were inhibiting the enzyme. This assertion was verified when he determined pectinesterase activity in the presence of polyvinylpyrrolidone (a chemical commonly used to bind phenolics). Polygalacturonase may be involved in softening of banana pulp, but little information is available.

Banana pulp contains 2-3% cellulose and this decreases only slightly during ripening (Kayisu and Hood, 1981; Prabha and Bhagyalakshmi, 1998). In the green fruit, the hemicelluloses make up 8-10% of the fresh banana pulp, decreasing to about 1% in the ripe fruit (Palmer, 1971; Barnell, 1943). Despite this decrease, the hemicellulose content of ripe banana pulp is higher than that of most fruits and vegetables (Southgate, 1976; Vansoest and Robertson, 1977), whereas cellulose and lignin contents are lower.

The main organic acids in banana pulp are malic, citric, and oxalic (Shimokawa et al., 1972; Wyman and Palmer, 1964). In the “Gros Michel” variety, malic acid increased during ripening, whereas oxalic acid decreased. Palmer (1971) found a wide variety of other acids in trace amounts. Other changes during ripening include a fall in pH from 6.0 in the green fruit

(stage 1) to 4.6 in the fully ripe fruit (stage 7) due to organic acids synthesis via the Krebs cycle (Lustre et al., 1976).

About 0.7% of the fresh banana pulp is protein and there is an increase in synthesis during ripening (Brady et al., 1970). Banana pulp contains high concentrations (mg/100 g dry weight) of glutamic acid (265 mg/100 g), aspartic acid (328 mg/100 g), leucine (127 mg/100g), histidine (325 mg/100 g), and valine (204 mg/100 g) (Askar, 1973). There is a marked increase in the content of valine and leucine during the ripening of bananas. They are possible precursors for certain volatile constituents which contribute to banana flavor (Palmer, 1971). Further work by Tressl and Drawert (1973) showed that radio labeled leucine and valine were converted into their corresponding methyl-branched esters, alcohols, and acids. Ripe banana fruit contains at least 200 volatile components (Wick et al., 1969; Tressl and Drawer, 1973). No significant quantities of volatiles appear until about 24 hours after the climacteric (Palmer, 1971), which is why only ripe bananas have typical banana flavor. The complex banana flavor has been studied extensively (Issenberg and Wick, 1963; Wick et al., 1969; Teranishi et al., 1971; McCarthy et al., 1964; Myers et al., 1969; Salmon et al., 1996; Boudhrioua et al., 2003). Characteristic “banana-like” flavor is caused by the amyl esters of acetic, propionic, and butyric acids. The distinctive “fruity” and “estery” notes are attributed to butyl acetate, butyl butyrate, hexyl acetate, and amyl acetate. The “Valery” variety of Cavendish banana was considered to have fuller and more interesting flavor than other varieties primarily because Valery bananas contained higher concentrations of “fruity” and “estery” constituents. “Green”, “woody”, or “musty” notes are attributed to methyl acetate, pentanone, butyl alcohol, amyl alcohol, and hexyl alcohol (McCarthy et al., 1963).

Banana processing

Stover and Simmonds (1987) have published a long list of processed banana products. By far, the most important product is banana puree. Most puree consist of aseptically canned ripe pulp with no preservatives or sugar added (Crowther, 1979). However, the high water content of banana puree makes packaging and freight very costly. The next most important canned product after puree is sliced bananas in a heavy acidified syrup (30 Brix, pH 4.4). This product is used mainly in bakery products, desserts, fruit cocktails, fruit salads and toppings. For dried banana products, banana figs and flour are by far the most important. Banana figs are made by drying entire ripe fingers to a moisture content of 33% (Dupaigne, 1967). United Brands developed the first natural banana essence extracted from ripe fruit. It is a clear, colorless liquid with excellent aroma and no additives or preservatives. It can be used in products for which banana puree is used.

Banana juice studies have been under way for about two decades in order to produce a commercial product (Anon, 1981). Previous research on banana juice by Dupaigne and Dalnic (1965) indicates that pressing banana pulp without any preliminary treatment does not produce a juice. These authors found that by extracting the pulp after treatment with pectinase, a cloudy, characteristic banana flavored juice was obtained with a low viscosity. Mumyanganizi and Coppens (1974), simplified the process by extracting the juice from the pulp after treating with lime (CaOH), leaving for 15 minutes, then neutralizing the lime with 6N sulfuric acid. The lime eliminates the pectin in the pulp, making extraction of the juice easier using either presses or centrifuges. The process was said to produce a better juice than previously reported with yields up to 88%. It was suggested that the juice could be used as a starting point for the production of local beers in developing countries where banana beers have been traditionally produced for

centuries. Further work by Dupaigne (1974), using enzyme mixtures (polygalacturonase and pectinesterase) to break the pectin in the banana pulp, showed that a good juice could also be obtained provided that the bananas used were ripe and could be processed rapidly. Casimir and Jayaraman (1971) also describe a process for producing an acceptable drink, involving acidification of the pulp to pH 4.2 to 4.3, rapid heating in the absence of oxygen, separation of fiber, diluting, centrifuging and sweetening, filling, retorting and cooling. The first two steps were carried out in one operation using a Pfaudler vacuum tumble blancher and the third step in a screening De Laval centrifuge or a Brown screw press. The authors suggested that the drink could be blended with other tropical fruit juices or carbonated to produce a refreshing drink. Sreekantiah et al. (1971) found that settling the juice overnight at 5 °C could separate most of the suspended particles in banana juice. Dupaigne and Dalnic (1965) recommended the use of fully ripe bananas for juice extraction to ensure complete flavor development and maximum sugar content. Viquez et al. (1981) studied the effects of six commercial pectinolytic enzymes on the viscosity reduction and clarification of juice from ripe banana pulp. They reported clear juice yields of between 55 and 60% (based on pulp weight) with pulp incubated at 45 °C for one hour using 0.01% w/w of the pectinolytic enzyme (Ultrazym 100) and subsequent centrifugation at 2900 maximal relative centrifugal force for 20 minutes. Kyamuhangire et al. (2002) reported a mechanical banana juice extraction that improved the juice yield to 75% when water was added to the spent pulp. Little information is available concerning storage stability of banana drinks. Canned banana drink in tinfoil cans has maintained quality for 18 months at ambient temperatures (Casimir, 1971). Artificially colored drinks have also maintained quality, provided the coloring material used is compatible with the container. Further research by Iwamoto et al.

(1970) showed abnormal de-tinning in canned banana drink due to the presence of naturally occurring nitrate in the bananas.

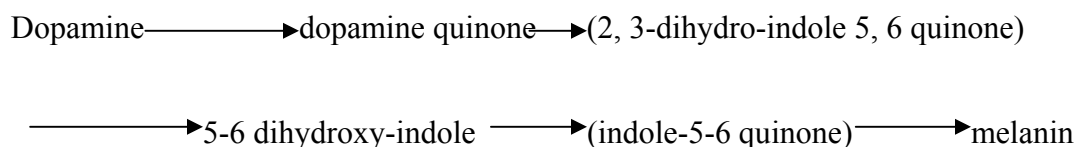
Browning reactions in banana

Enzymatic browning occurs in banana when the tissues are damaged mechanically or exposed to abnormal conditions. It is initiated by an enzyme (or multiple enzymes) causing oxidation of dopamine (3, 4-dihydroxyphenyl-ethylamine) to o-quinones (Griffiths, 1959). In mammalian systems, dopamine is known to be synthesized from the decarboxylation of DOPA, which is formed from tyrosine by a tyrosine hydroxylase. In the banana tissue, however, tyramine is oxidized directly to dopamine and dopa is not an intermediate in dopamine biosynthesis (Deacon and Marsh, 1971; Kanazawa and Sakakibara, 2000). The enzymic system which converts tyramine to dopamine was isolated by the same authors.

Polyphenoloxidase is also known as phenolase or phenoloxidase. The injured banana tissue rapidly darkens on exposure to air due to the conversion of the phenolic compounds to brown melanins, which is dependent upon molecular oxygen. Most phenolases catalyze two different reactions, the hydroxylation of monohydroxyphenols into dihydroxyphenols, and the oxidation of dihydroxyphenols into quinones. To enable these reactions to proceed, both a copper prosthetic group and oxygen must be present. The complex polyphenoloxidase in banana has yielded nine iso-enzymes by electrophoresis (Montgomery and Sgarbieri, 1975). Studies on the substrate specificity of these enzymes showed that L-tyrosine was oxidized at a slower rate than dopamine. Other good substrates for banana polyphenoloxidase were catechol, 4-methyl catechol, and pyrogallol. All these compounds are diphenols, which suggest that the banana PPO were more specific for o-diphenols. Furthermore, all diphenol substrates cited above either lack or have a short side chain on the ring in the 4 position. This could be the reason why there

was a difference in the activity between dopamine and DOPA, which has a carboxyl group.

Palmer (1963) developed methods for preparation of banana phenolase and determined some of its properties in comparison with phenolase from other sources. He proposed a reaction mechanism leading to the formation of melanins in banana.



Spectrochemical evidence was obtained for the presence of the bracketed compounds only. The remaining intermediates were assumed by analogy with DOPA(3-hydroxytyrosine) oxidation, and not all the probable intermediates were shown in the mechanism (Palmer, 1963).

Maillard reactions between amines, amino acids, and proteins with sugar, aldehyde, or ketones are the primary basis of non-enzymatic browning during heating or prolonged storage of foods (Ellis, 1959). Five major steps are involved in the browning: formation of a glycosamine; rearrangement of the glycosamine to a ketosamine or aldosome; formation of a diketosamine or a diamino-sugar; degradation of the amino-sugar to amino or non-amino intermediates; and condensation of the amine with intermediates formed in previous steps. These reactions are followed by polymerization with formation of brown pigments (Reynolds, 1965; Song and Chichester, 1967). Draudt and Huang (1966), working on freeze-dried bananas, detected many carbonylamine browning intermediates using paper chromatographic techniques. Among them some yielded γ -aminobutyric acid and valine, and 5-hydroxymethylfurfural as the carbonyl part after hydrolysis with 1N HCl. Their chromatographic behaviors, color reactions, and hydrolysis products were identical with those of authentic reference compounds prepared from D-glucose

and valine. Therefore, these authors identified the compounds as fructose- γ -aminobutyric acid with fructose-valine, respectively.

Caramelization involves the thermal degradation and polymerization of sugars in the absence of amino acids or proteins. When sugars are heated above their melting points, pyrolysis occurs and sugars darken to brown caramels. The chemical composition of caramel is extremely complex and little understood (Bryce and Greenwood, 1963; Sugisawa and Edo, 1964; Anet, 1964; Wolform et al., 1948, and Holtermann, 1966). Mao (1974) attributed the browner color of the dehydrated ripe banana puree by drum drying to caramelization of sugars present at higher concentration in the pulp of the riper fruit.

Ascorbic acid oxidation is another type of non-enzymatic browning in foods. Banana contains varying amounts of vitamin C (~15 mg/100 g). Ascorbic acid may be involved in at least two ways (Curl and Talburt, 1961). One way is by forming dehydroascorbic acid which reacts with amino acids to give strongly colored compounds. The other way in which ascorbic acid could contribute to browning is by its conversion to 2-furaldehyde, which readily darkens, especially in the presence of amino acids.

Many methods for inhibition of phenolase are known (Ponting, 1960). Most methods for controlling enzymatic browning are based upon destroying the active enzymes, inhibiting the primary oxidation or removal of oxygen. However, problems with off-flavor, off-odor, potential toxicity, and economics often limit the methods or substances, which can be employed in food systems. Chemical methods used to inhibit the enzymatic browning can be grouped into dipping and chemical modification of the substrate. Bisulfite and sulfur dioxide are powerful inhibitors of phenolase (Sayavedra and Montgomery, 1986; Koffi et al., 1991). Muneta (1966) suggested that the inhibition of oxidative hydroxylation is the primary mechanism by which bisulfite

inhibits enzymatic browning. Sulfite also prevents browning by combining with the enzymatically produced ortho-quinones and stopping condensation to melanin (Embs and Markakis, 1965). However, there are problems with bisulfite or sulfur dioxide, such as off-flavor, off-odor, toxicity at high levels, destruction of vitamin B₁, (Koeing et al., 1983). However, due to the effectiveness and low cost, bisulfite is used by the food industry to prevent or reduce many of their enzymatic browning problems (Feinberg et al., 1967).

Sodium chloride, ascorbic acid, and citric acid have all been used as chemical inhibitors for phenolase (Hope, 1961; Joslyn, 1951), but not as effectively as bisulfite or sulfur dioxide. Methylation of the phenolase substrates was also proposed to make fruits and vegetables resistant to browning (Finkle, 1964). Studies by Galeazzi and Sgarbieri (1981), using several chemical inhibitors of phenolase, showed that the most effective on the banana polyphenoloxidase was ascorbic acid, followed by cysteine, and then sodium bisulfite.

Physical methods are also used to inhibit the enzymatic browning. Ultrafiltration of banana juice through a 10,000 MWCO (molecular weight cutoff membrane) removed all polyphenoloxidase activity and reduced juice browning (Sims et al., 1994). Steam blanching is very effective in inactivating the phenolase in bananas (Mao, 1974; Koffi et al. 1991). The minimum heat treatments for complete inactivation of phenolase of bananas needs to be established in order to avoid excessive heat treatment, which could destroy texture, flavor, etc. according to the United Fruit Company (1972), polyphenoloxidase activity drops sharply after heating the banana pulp at 80-85 °C for one minute. Microwave energy was also reported to effectively inactivate phenolases (Collins and McCarty, 1969).

Milk proteins

Bovine milk contains about 30-36 g/L of total protein. About 80% of the proteins correspond to the caseins, whereas the other 20% constitute the whey proteins (Eigel et al., 1984). Agglomeration of the casein micelles during cheese manufacture leaves a serum containing the whey proteins.

Caseins

The caseins are composed mainly of calcium sensitive caseins (α_1 , α_2 , and β caseins) with multiple anionic clusters and κ -casein as determined by electrophoresis (DeMan, 1990). The proportions of these proteins in the casein micelle are 10%, 38%, 36% and 13% (α_2 , α_1 , β and κ) (Davies and Law, 1980). The C-terminal macropeptide of κ -casein is believed to protrude from the surface of the casein micelle in the hairy micelle model. A negative hydrophilic outer layer formed on the surface of the casein micelle prevents casein molecules aggregation and ensures steric stabilization (Holt and Horne, 1996). Calcium phosphate complexes and suppresses the positive charges of the phosphoserine clusters to favor hydrophobic stabilization of the micelle structure (Horne, 1998; Walstra, 1999). Isoelectric precipitation and destabilization of caseins micelle occur at pH 4.6 with release of the calcium phosphate in the serum (Dalglish and Law, 1989).

Whey composition

Acid whey and sweet whey are the two types of whey. They differ in pH with sweet whey being pH 5-7 and acid whey pH 4-5. Acid whey contains more calcium and phosphate owing to its method of processing. Wong et al. (1978) found that the calcium content of acid whey was three times greater than that of sweet whey while the zinc content was twenty times greater in acid whey. Mavropoulou and Kosikowski (1973a) indicated that the lecithin content

of sweet whey was higher than that of the acid whey, however acid whey was more stable when stored at room temperature. Whey is not a good source of iron. Glass and Hedrick (1976) found that acid whey contained higher levels of iron (about 1.3 mg/ 100 g) as compared to sweet whey (about 0.9 mg/ 100g). Demott (1975) showed that acid whey did not tolerate heating as well as sweet whey did.

Bovine milk contains about 0.65% whey protein, with a high organic content (about 5.5 to 6.5% w/w). Because of its high organic content, this milk by-product has long been recognized as a serious pollutant. However, its well balanced ratio of essential amino acids makes it a satisfactory source of protein for food fortification (Morr, 1982). To alleviate protein deficiency in many areas of the world, it seems desirable to use this protein source. Whey proteins have a biological value superior to most other proteins (Harper, 2000). Many of the same components found in mother's milk are present in whey proteins. This reason is key to the incorporation of whey proteins in infant formula. When breast-feeding is not possible, whey proteins are the next best option.

Whey proteins are complete proteins that supply the body with all the essential amino acids for active teens and healthy adults. The abundance of branched amino acids in whey proteins is important to athletes as they are metabolized directly into muscle tissue unlike other amino acids that are metabolized into the liver (German et al., 2001). Individuals following strenuous activity or malnourished patients need more proteins to help the body repair and rebuild muscle tissue. whey proteins can help meet that need. Another important benefit of whey proteins is their ability to help prevent bone loss in elderly individuals. A diet including the recommended amount of whey protein can help keep bones healthy and strong as the body ages (McIntosh et al.,1998).

A compromised immune system can benefit from a diet rich in whey proteins. A powerful anti-oxidant, glutathione, has the ability to enhance the body's immune system. Whey proteins are rich in sulfur-containing amino acids (e.g., cysteine, methionine) which are rate-limiting for the biosynthesis of glutathione, an antioxidant, anticarcinogen, and immune stimulating sulfur-containing tripeptide (German et al., 2001). HIV patients often have reduced levels of glutathione and these individuals can get a "boost" to their immune system by consuming whey protein rich diet. Recent studies have shown that whey proteins inhibit the growth of breast cancer tumors.

Whey processing

Whey is a by-product of cheese manufacture (Morr and Ha, 1993). Whey contains about half the solids of the original milk, mainly lactose with some valuable proteins and with much of the original mineral content of the milk. Worldwide, the amount of lactose present in whey is about 12 billion pounds, which is equivalent to 5% of the world production of sucrose (Clark, 1987). Whey is industrially processed to whey protein concentrate (WPC, 50-80% protein) or whey protein isolate (WPI, $\geq 90\%$ protein) by precipitation of the lipid fraction (mainly phospholipoproteins) followed by microfiltration and ultrafiltration.

Separation of the main individual whey proteins, β -lactoglobulin (β -lg) and α -lactalbumin (α -lac) is based on the fact that the latter aggregate more extensively than the former above 55 °C for 30 min in the pH range 4-4.5, with only minor changes in β -lg (Pearce, 1987). Denaturation of α -lac is reversible and the protein is restored to its original form on cooling and adjusting the pH to 6.5. Thus it is possible to separate α -lac aggregates from the β -lg remaining in solution (Maubois, 1991). Removal of phospholipoproteins is important because their amphiphilic properties allow them to adsorb strongly on membrane materials and cause membrane fouling

(Lee and Merson, 1976). A pilot-scale isolation of β -lg used a combination of fractionation processes of Fox et al. (1967) and Caessens et al. (1997). In the first step, hydrochloric acid (2M) precipitated the caseins from skimmed milk at pH 4.6 to yield acid whey free of caseinomacropeptide. Then the whey was added to an equal volume of 6% (w/v) trichloroacetic acid, and centrifuged at 4000g for 15 min at 4C. Ultrafiltration followed by diafiltration using alumina-based ceramic membranes (15 kDa cut-off) produced a retentate of β -lg. Vacuum oven drying was used to obtain β -lg powder. Other methods including the salting-out procedure of Mate and Krochta (1994), the selective thermal precipitation method (Maubois et al., 1987) and the peptic hydrolysis methods by Konrad et al. (2000) have been used for isolating β -lg.

Whey proteins

The major proteins in whey are β -lactoglobulin (β -lg), α -lactalbumin (α -La), bovine serum albumin (BSA), and immunoglobulin (Ig). Whey contains low concentration of other proteins such as lactoferrin, and enzymes such as lysozyme, lipase and xanthine oxidase. The most abundant whey proteins are β -lg which represents about 50% of the total whey proteins in bovine milk. β -lg plays a key role in any processing operation (Swaigood, 1982). At the pH of the milk (pH 6.7) β -lg is a dimer (36.7 kDa) due to electrostatic interactions between amino acids Asp¹³⁰ and Glu¹³⁴ of one monomer with the corresponding lysyl residues of the other monomer. At pH values below 3.5 and above 7.5 the protein forms monomers. In the pH 3.5-5.2 an octamer is formed (147 kDa). Most of the tertiary structure of β -lg is maintained by a thiol group (residue 119 or 121) and two disulfide bonds (66-160 and 106-119 or 121) (Swaigood, 1985).

Alpha-lactalbumin is the second most important whey protein and represents about 20% of the serum protein in bovine milk. This protein is a co-enzyme, which plays a role in the final

stage of lactose synthesis (Sienkiwicz and Riedel, 1990). A total of 123 amino acids are present in the primary structure of this protein which has a molecular weight of 14,000 Daltons and four disulfide bonds. Bound calcium stabilizes the molecule against irreversible thermal denaturation (Hiraoka and Sugai, 1984). Removal of the calcium leads to the reduction of transition temperature and thermal denaturation and aggregation (Kronman et al., 1981).

BSA represents about 10% of the whey proteins. This large monomer protein (66,267 Daltons) contains 17 disulfide bonds and a thiol group. BSA and the immunoglobulins originate from blood.

The immunoglobulins represent about 10% of the serum proteins. The naturally occurring enzymes are acid phosphatases, xanthine oxidase, catalase, lysozyme plasmin, ribonuclease, amylase, superoxide dismutase, lactoperoxidase, sulphhydryl oxidase and δ -glutamyl transferase (Farkye and Imafidon, 1995; Pearce, 1989). All the functions of these enzymes have not been explained; however some are associated with flavor modification in milk.

Interactions of whey proteins

Whey proteins can interact with or without heating, with various non-dairy components including pectins, tannins and polyols (Rawel et al., 2001). Low methoxyl pectins interact with whey proteins yielding sedimentation. Devkota (1991) found that the presence of both whey protein and pectin in a 2:1 ratio resulted in the production of sediment in a dispersion containing a mixture of whey and pectin.

Incompatibility was found for mixtures of native whey protein-nonionic hydrocolloids such as maltodextrins, dextrans and methylcellulose at pH 5 to 7 (Syrbe et al., 1995). However, ionic hydrocolloid-whey proteins solutions including high-methoxyl pectin, sodium carboxymethylcellulose or sodium alginate performed differently at pH 6 to 7. Unless whey

proteins were denatured, no polymer segregation occurs in these mixtures. If denatured, phase separation as well as gel formation occurs concurrently. Two types of gels are observed with both anionic and nonionic hydrocolloids (Syrbe et al., 1998). Heat induced gelation of β -lactoglobulin-low-methoxyl pectin solutions is influenced by calcium ions on both sides of the pI (Ndi et al., 1996). At pH 3.5 self-association of low-methoxyl pectin in presence of high levels of calcium was promoted, which prevented formation of insoluble complexes with positively charged β -lactoglobulin. However, at pH 6.5 repulsive forces were reduced between protein and polysaccharide, leading to more homogeneous gel structures.

Whey-fruit beverages

Some success has been achieved in developing beverages fortified with whey. The beverages produced are nutritious (acid whey contains about 120 mg Ca/100ml), refreshing but less acidic than fruit juices, and have a good potential for profit margins (Holsinger et al., 1974). Rivella^R is one of the most successful beverages developed in Switzerland in 1952 (Anon, 1960). It is a sparkling, crystal clear infusion of herbs in deproteinized whey, promoted as therapeutic tonic. Other beverages that have received attention include Bodrost^R (Len'kov, 1969), an alcoholic beer-like beverage made in Russia from pasteurized, clarified whey with the addition of sugar and raisins. Tai^R from Brazil is a soft drink fortified with a whey protein concentrate to contain 1.5% protein (Anon, 1973). Other whey beverages (from deproteinized whey) sold in Europe includes, Big M^R, Frusighurt^R (Germany) and Taksi^R (Holland) (Lang and Lang, 1969). Heat precipitation of the whey proteins by steam injection is commonly used during deproteinization (Sienkiwicz and Riedel, 1990).

Research in the United States has been directed toward utilizing whole whey (to minimize costs) in the form of nutritious whey beverages, both carbonated and non-carbonated

(Holsinger et al., 1974). The citrus flavored beverages (particularly orange), have received the highest acceptability. The orange-flavored drink contained 33% untreated cottage cheese whey. Citric acid was added to pH 3.6 to overcome the buffering capacity of the whey (Nelson et al., 1971). No more than 33% of unmodified whey could be used without some objections being raised about the unpleasant insipid taste of whey. The same author noticed that the choice of the flavor was very important. Passion fruit juice at 2-3% has contributed considerably to the acceptability of whey beverages. Synthetic flavors including raspberry have been used successfully. When using fruits, it is recommended that natural fruits be well ripened. Various fruits including peach puree (20% level), strawberry (10%) and red raspberry (10%) have been used successfully at these levels. Hannigan (1978) developed what he called citrus milk. The beverage was obtained by mixing (85-92%) of 14 Brix orange juice (or 54-57% of 6-14 Brix grapefruit juice), soluble protein (WPC) to obtain 1-6% protein (3-4% best). Soluble stabilizers in the range of 0.1-1% were added to improve texture, viscosity and to prevent sedimentation during storage. A number of sweeteners are added as well as various extracts (vanilla, citrus essences etc). The author recommends that the viscosity should be at least about 40 cps at 80 °F. Lactofruit^R, a whey drink developed in Switzerland was made by hydrolyzing deproteinized-whey to 50% with lactase to increase sweetness and avoid lactose intolerance. The beverage contained 25g/L galactose, 4.5 g/L minerals, 2 g nitrogenous substances, B vitamins and vitamin C.

Jelen (1992) reported that the lack of high marketability for whey beverages, when compared to other beverages, was due to poor flavor blends. Whey contains high amounts of lactose and salts which makes it a difficult material to utilize. Various flavor notes including

diacetyl, acid, saltiness, astringency, bitterness and sweetness have been found (McGugan et al., 1979).

Phenolics

Phenolics represent a large group of chemical compounds that have at best one aromatic ring bearing one or more hydroxyl groups together with a number of other substituents. Fifteen major groupings have been identified in the plant kingdom (Harborne and Simmonds, 1964). However, the common phenolic constituents of plants could be divided broadly into two main groups:

Phenolic acids and coumarins (C_6-C_1 and C_6-C_3 structures).

Two families of phenolic acids are distributed in plants. A range of substituted benzoic (C_6-C_1) acid derivatives and those derived from cinnamic acid (C_6-C_3). Both types of phenolic acids usually occur in conjugated or esterified form. Gallic acid (found in green tea) (Rawel et al., 2001) represents the simpler type of benzoic acid derivative. Chlorogenic acid present in sunflower seeds and coffee beans is an ester of caffeic acid (cinnamic acid) and quinic acid.

Flavanoid compounds, include anthocyanidins ($C_6-C_3-C_6$ structures)

This group includes the largest and most diverse range of plant phenolics (Walker, 1975, Robards et al., 1999). Flavanoids are built upon a $C_6-C_3-C_6$ flavone skeleton in which the three-carbon bridge between the phenyl groups is commonly cyclized with oxygen (Robards et al., 1999). Flavanoids include the red and blue anthocyanin of flowers, the yellow flavones, flavonols, flavanols and the isoflavones. Most flavanoids occur as glycosides in which the $C_6-C_3-C_6$ aglycone part of the molecule is esterified with a number of different sugars. The flavanols are unique in that they do not occur as glycosides, but show reactivity through polymerization into "condensed tannins".

Polymeric phenols could be further divided into two broad groups: tannins and lignin. Tannins have been classified by Freudenberg (1920) into two groups based on structural types: hydrolyzable tannins and the condensed tannins (or procyanidins). The former is readily hydrolyzed by acids, bases or certain enzymes. Tannins are responsible for a dry, puckery, astringent sensation in the mouth. The ability of tannins to bind proteins is of considerable importance in fruit ripening, and manufacture of tea, cocoa and wine. Important taste attributes of astringency and bitterness are contributed by tannins. Bitterness is the sensation perceived at the back of the tongue whereas astringency is the “dry-mouth” feeling produced by the interaction of tannins with the proteins of the mouth (Singleton and Esau, 1969).

Lignins are complex, three-dimensional polymers of phenyl-propanoid (C_6-C_3) units which encrust and penetrate the cellulose cell walls of higher plants, thus contributing to their mechanical strength and rigidity. Alkaline hydrolysis of lignin releases a variety of benzoic and cinnamic acid derivatives together with other unrelated compounds.

Protein-phenolic interactions

Tannin-protein interaction causes haze in beverages (White, 1957; Siebert 1999) and astringency in foods (Rawel et al., 2001). Multiple bond formation between the hydroxyl group of tannins and the carboxyl groups of proteins and peptides have been reported (Van Sumere et al., 1975). Hydrophobic reactions have also been found as important in the formation of tannin-protein complexes (Hagerman and Butler, 1980; Oh and Hoff, 1987). The hydrophobic nature of tannins was demonstrated by their effective adsorption on an uncharged polystyrene resin (Oh et al., 1980).

Tannins precipitate proteins at pH values up to the isoelectric point of the individual protein (Oh et al., 1985). The tannin-protein complex is one of the main reasons for turbidity in

beverages. The concentration of salt and the pH of a solution tend to affect turbidity values. At pH 3, salt has an effect on the turbidity values, while at pH 4, higher turbidity occurs irrespective of the salt concentration. At pH 5, the salt concentration decreases the turbidity values of the solution (Oh and Hoff, 1987). Binding of condensed tannins occurs at any pH < 8. When protein conformation is more open the protein tends to bind the tannins more strongly (Hagerman and Butler, 1978). The extent of turbidity reflects the extent of tannin-protein formation (Oh and Hoff, 1987).

Increasing protein concentration influences the turbidity because of the increase in soluble tannin-protein complexes. BSA reacts with tannins to form a soluble compound depending on the relative amount of tannins and proteins as well as on pH and salt concentration (Calderon et al., 1968). Interaction between BSA and tannin is optimum at pH 4 (Oh and Hoff, 1987). Siebert (1999) reported that the protein/ polyphenol ratio had a strong influence on the amount of haze formed; the largest amount was obtained when the numbers of polyphenol binding ends and protein binding sites were nearly equal.

Carbohydrates

The interactions between polysaccharides and proteins are essential for satisfactory food texture and shelf life improvement. Water binding by hydrocolloids helps prevent ice crystal growth in ice cream caused by temperature fluctuations upon storage as well as a slow melt down behavior. On the other hand hydrocolloids prevent whey separation, settling of dispersed particles or flocculation and creaming of emulsion droplets (Hansen, 1994). Whey proteins mixed with hydrocolloids in dairy products form polyelectrolyte solutions composed of various chemically different monomer units in water (Syrbe et al., 1998).

Polymer interactions usually result in complex formation. Three situations can be found (Syrbe et al., 1998). Incompatibility between polymers of different nature leads to segregation of the biopolymers into two distinct, immiscible aqueous phases each of them loaded with only one biopolymer. In this case, the interaction between different biopolymers is more repulsive than the average interaction between like polymers. Second, complex coacervation occurs when two distinct aqueous phases form with one phase is loaded with both polymers. This is the case of solutions of oppositely charged polyelectrolytes. Miscibility occurs when contact between like polymers or different biopolymers is similar and, the mixture remains homogeneous. Conditions responsible for complexation or phase separation depend on the physico-chemical characteristics of proteins and polysaccharides and the environment (pH, ions type, ionic strength etc.) (Tolstoguzov, 1991).

Pectin

Pectins are structural polysaccharides. They occur mainly in primary cell walls and middle lamella of higher plants, where they are largely involved in the integrity and coherence of plant tissues. Despite their low levels (1% w/w), they have a major effect on the consistency of homogenized fruits and vegetables and on the cloudy appearance of juices. Chemically, pectins are heteropolysaccharides with molecular weight ranging from 30,000 to 300,000 Daltons. Pectins are complex polysaccharides consisting of mainly galacturonic acid, rhamnose, arabinose, glucose, xylose and galactose. The neutral sugars represent about 5-10% of the galacturonic acid by weight (Turquois et al., 1999). Depending on the plant source, other sugars can be detected as minor components (de Vries et al., 1981; Ridley et al., 2001). The galacturonan backbone containing 1,4-linked α -D-galactosyluronic acid makes up the smooth region of the pectin molecule. The linear polymer of (1-4)-linked α -D galacturonic acid is

interspersed with (1-2)-linked-rhamnopyranosyl units in the hairy regions (de Vries, 1981; Mukhiddinov et al., 2000). The rhamnose inserts in the galacturonan chain give rise to the rhamnose kink. The neutral sugar side chains are attached to the main chain via both rhamnose and galacturonic acid units. The neutral sugars are significant with regard to a variety of functional properties such including solubility, gelling, freeze-thaw stability and rheological properties (Hwang and Kokini, 1992). Side chains prevent intermolecular association of pectin molecules. Thus, water molecules can penetrate more readily the intermolecular spaces caused by steric hindrance to enhance solubility. Swelling in branched polysaccharides is promoted for the same reasons. Side branches however inhibit the formation of junction zones required for gelling. The limited intermolecular association at low temperature of branched molecules makes them more freeze-thaw stable.

Pectins also contain non-sugar substituents including methanol, acetic acid, phenolic acids, and amide groups in some commercial samples (Pilnik, 1990; May, 1990). Ferulic acids are attached to arabinose and galactose in the side chains in pectin fractions isolated from spinach cell and sugar beet pulp (Fry, 1979; Fry, 1982; Rombouts and Thibault, 1986). Carboxyl groups are partially esterified by methanol and sometimes acetylated. Acetylation occurs at carbon C2 and C3 of the galacturonide monomers. If more than 50% of the carboxyl groups are methylated the pectins are called high-methoxyl pectins (HMP); if fewer than 50% are methylated low methoxyl pectins (LMP) are obtained. The LMP are further subdivided into low methoxyl amidated (LMA), and low methoxy conventional (LMC). Recently, calcium-sensitive pectins (CSP) have been introduced. CSP are high-methoxy pectins in which de-esterified blocks allow them to gel in the presence of calcium without the addition of sucrose (Joye and Luzio 2000; Hotchkiss et al., 2002).

The pH of greatest stability of pectin solutions is at pH 3-4. At pH values below and above 4, deesterification and depolymerization occur, with the rate of depolymerization being lower than the rate of deesterification. Methoxyl and acetyl groups are removed and the neutral sugar side chains hydrolyzed (May, 1990).

Milk proteins are charged negatively in neutral milk (pH 6.5-6.7). These proteins are mutually repellent, preventing sedimentation. The proteins have their lowest charges and weakest hydration at the isoelectric point (pH 4.6). When acidified milk products are heated, proteins will contract due to water loss. Beverages made with the proteins have a sandy structure and are subject to sedimentation. The agglomeration of proteins in sour milk products and the sandy structure that results from heating can be prevented by adding high methoxyl pectins (Parker et al., 1994). The stabilization of proteins is a result of the protective colloid effect of pectins. The negatively charged pectins accumulate around the protein particles which are positively charged at a pH of 4.0. The protein particles in turn receive a uniformly distributed negative charge. This negative charge leads to electrostatic repulsion of the particles which continue to remain suspended. The acidified milk drink is stable. The optimal acidified milk stabilization depends on the type of pectin used as well as on the recipe and on the manufacturing parameters of the drink to be stabilized.

The pH value of acidified milk drinks is critical for optimal stabilization because it affects the dissociative behavior of pectins and their reaction with calcium. The drink's pH-value should be between 3.9 and 4.1 (Dickinson, 1998). The concentration of the proteins as well as the size of the protein particles influences the pectin dosage. The higher the protein content, the higher the dosage of pectin needed for stabilizing milk drinks (Xia and Dubin, 1994; Parker et al., 1994).

Homogenization is crucial for stabilization. The pectin dissolved in acidified milk drinks will be uniformly distributed on the surface of the protein particles by applying a high shear force and can thus lead to a protective colloid effect (Parker et al., 1994). Heat treatment of the drinks is necessary to ensure that they are microbiologically stable. According to the desired product shelf-life, different heat treatment methods are used (pasteurization, UHT-heating). To counteract the loss of stability caused by heating influences, a higher pectin dosage is necessary. The amount of added pectin required also depends on the intensity of thermal treatment involved (Glahn, 1982). Higher calcium content in the acidified milk drink also necessitates a higher pectin dosage. The stability of an acidified milk drink is evaluated according to viscosity, amount of sediment according to defined centrifugation and microscopic observation of the protein particles. If the pectin dosage is too small, the protein particles adhere to each other which results in high viscosity. An increase in the concentration of pectin results in increasingly better stabilization, the protein particles are less able to agglomerate and optimal stabilization is achieved when the viscosity is at its minimum level (Parker et al., 1994). At this point, the protein particles can repel due to aforementioned charge effects and thus move freely resulting in a lower viscosity. This optimal stabilization dosage (OSD) gain depends on the pectin type, the acidified milk recipe and the manufacturing parameters. With an increased pectin dosage, the thickening qualities as well as the stabilizing abilities of pectin come into force, increasing the viscosity of the drink. Whether or not this effect is desired depends on the specific consumer requirements.

In dairy beverages, interaction between the charged groups of pectins and those of proteins is necessary for the stabilization effect (Kravtchenko et al., 1992; Pereyra et al., 1997; Syrbe et al., 1998). Furthermore, it has been found that, not only the degree of esterification

(DE) but also, the molecular weight distribution and molecular weight averages are linked to the stabilization effect of pectin (Deckers and Olieman., 1986). If stabilization of milk beverages is required, homogenization in presence of pectin is to be considered. The usual level of pectin under these circumstances is less than 0.5% (Syrbe et al., 1998).

Carrageenan

Carrageenan are sulfated polymers extracted from red seaweeds. This compounds are composed of linear chains of D-galactopyranosyl units linked alternately β -1,3 and α -1,4 glycosidic bonds, with most sugar units having one or two sulfate groups esterified to a hydroxyl at carbon atoms C-2 or C-6 (Stanley, 1990). The principal structures are termed kappa (κ), iota (ι), and lambda (λ). Carrageenan products dissolve in water to form highly viscous solutions. The viscosity is quite stable over a wide range of pH because the sulfate half-ester groups are always ionized, even under strongly acidic conditions, giving the molecules a negative charge (Mleko et al., 1997). Segments of molecules of kappa- and iota-type carrageenans exist as double helices of parallel chains (Rees et al., 1969).

Gelation of κ and ι -carrageenan can occur in water at concentration as low as 0.5% in the presence of potassium and calcium ions on cooling (Drohan et al., 1997). The higher the degree of sulfation of a carrageenan, the greater is its solubility in cold conditions. Total hydration of ι - and κ -carrageenans takes place by means of heating. Gelation occurs on cooling as the molecules approach each other to create junction zones involving a coil-helix transition followed by aggregation of helices (Morris, 1998). In these two types of carrageenan, the sulfate groups are placed on the external face of the spiral alone; therefore the internal faces of the chains can come together to form double spirals. Kappa-type gels are the strongest of the carrageenan gels. Extension of junction zones induces syneresis if other gums are not added. The unique effect of

potassium ion on κ -carrageenan is due to its small size in the hydrated state which allows it to overlap in the helix and partly to neutralize the sulfated groups. The double helices can form aggregates by coming together in absence of repulsive forces. This leads to hardening of the gel (brittle), shrinkage of the structure, exclusion of water, syneresis and opacity of the gel (Parker et al., 1993).

Iota carrageenan exhibits an intermediate behavior between λ and κ -carrageenans. With 2 sulfates for 2 galactose residues and a tendency to produce weaker gels than those obtained with a κ -carrageenan. Iota -type carrageenans gel best with calcium ions, and the resulting gel is soft and resilient with good freeze-thaw stability (Parker et al., 1993; Michel et al., 1997). Further, the gel does not synerese because iota-type carrageenans are more hydrophilic and form fewer junction zones than do kappa-type carrageenans.

Lambda carrageenan has 3 sulfates for 2 galactose units along with a pronounced anionic character and strong electrostatic repulsions. All salts of lambda-type carrageenans are soluble in cold systems and nongelling because they adopt coil conformations whatever the ionic and temperature conditions (Piculell, 1995). This is due to the fact that the sulfates are directed towards the interior or the exterior of the spiral and electrostatic repulsion prevent association of chains. Therefore this non-gelling macromolecule function as a thickening agent.

Carrageenans are most often used because of their ability to form gels with milk and water (Baeza et al., 2002). Another useful property of carrageenans is their reactivity with proteins, particularly those of milk. κ -Type carrageenans complex with kappa-casein micelles of milk, forming a weak, thixotropic, pourable gel. The thickening effect of κ -carrageenans in milk is 5-10 times greater than it is in water. This property is used in the preparation of

chocolate milk where the thixotropic gel structure prevents settling of cocoa particles. Such stabilization requires only about 0.025% gum.

Carboxymethylcellulose

Carboxymethylcellulose (CMC) has a backbone identical to that of cellulose. As a result, CMC solutions tend to be both highly viscous and stable. CMC interacts with caseins and other milk proteins at their isoelectric pH to yield a soluble complex stable to heat treatment and to storage. Hence milk products are stabilized against casein precipitation with CMC (Shenkenberg, 1971). CMC produces a smooth texture in ice-creams by regulating the growth of ice crystals and by preventing syneresis in gelled desserts (Dapia et al., 2003).

Xanthan gum

Xanthan gum is a heteropolysaccharide of high molecular weight ($M_w = 2 \times 10^6$). A trisaccharide side chain occurring in every alternate glucose residue, contains α -D-mannose, β -D-glucuronic acid and a terminal β -D-glucuronic acid and a terminal β -D-mannose unit. The presence of the glucuronic acid unit and pyruvate group impart anionic character to the xanthan gum. The presence of side chains on the xanthan molecules as well as their anionic character enhance the hydration (Pettitt, 1982). Therefore xanthan gum is soluble in cold water. But, because of interactions with calcium ions, a longer time of agitation is required to dissolve the gum in dairy medium. Xanthan gum is used as a food gum because it imparts a high and stable solution viscosity at low concentration in the temperature range from 0 to 100 °C and has a good freezing-thawing stability.

Gelling of pectin and interaction

The production of jam is the main use of industrially extracted pectins, using the ability of HMP to produce gels with sugar and acid. The high sugar contents of the jam create

conditions of low water activity which in turn favor chain-chain rather than chain-solvent interactions (Rees, 1972). The acid environment suppresses the negative charges on the carboxyl groups, thus reducing electrostatic chain repulsion and promoting hydrophobic association of methyl ester substituents (Doesburg and Grevers, 1960, Oakenfull and Scott, 1984, Evageliou et al., 2000.). Various factors impact the conditions of gel formation and the gel strength achieved. Gel strength increases with the molecular weight of the pectin used, whereas any treatment that depolymerized the pectin chains resulted in weaker gels (Christensen, 1954). However, the only analytical parameters that help prediction of gelling behavior are the degrees of esterification with methanol and acetyl. The degree of methylation of the HMP is believed to determine the setting temperature of a gel while acetyl group prevent gelation. These observations have led to the subdivision of HMP according to setting time or temperature.

LMP gel only in the presence of divalent cations such as calcium. Gelation in this case is due to the formation of intermolecular ionic Ca^{2+} bridges between two adjacent carboxyl groups of two different chains (Thibault and Rinaudo, 1986; Gilsenan et al., 2000). Gelation of LMP is influenced by various factors including degree of methylation, charge distribution, average molecular weight of the sample, ionic strength, pH and temperature (Narayanan et al., 2002). Speiser and Eddy (1946) reported that LM-pectins with free carboxyl groups in blocks were weaker than those produced from LM-pectin containing randomly distributed free carboxyl groups.

Gelation of calcium sensitive pectin

Calcium-sensitive pectins (CSP) gel in presence of calcium without the addition of sucrose as long as blocks of deesterified pectin are present (Joye and Luzio, 2000). Further, CSPs bind more water than LM pectins and therefore the gels produced are softer and more

appealing for many food applications including dietetic and acidic foods. Tailored calcium-sensitive pectins could be produced by modifying the degree of esterification (DE), the distribution of DE and the molecular weight of the pectin molecule (May, 1990).

Environmental factors influences on pectin modification and interaction

pH effect

At neutral pH, the pectin does not adsorb onto casein micelles in skim milk (Maroziene and de Kruif, 2000). Instabilities observed in dispersions under these conditions lead to a segregative phase separation by depletion flocculation mechanism. Depletion flocculation is brought about by the presence of non-adsorbing polymers in dispersion. The polymer, existing as a random coil, becomes excluded from the gap between two approaching particles. The resulting concentration gradient results in particle flocculation. At pH 5.3 the apparent particle size as a function of the pectin concentration had a typical maximum and then leveled off to a higher constant value. This behavior is characteristic of adsorption of pectin, leading to bridging flocculation. Bridging flocculation involves polymer chains sticking to multiple particles, making an aggregate large enough to settle out. Ambjerg and Jorgensen (1991) proposed that the binding of pectin to the casein micelle was electrostatic since it was influenced by pH.

However, Parker et al. (1994) found that stabilization effect of pectins is better explained by assuming a steric stabilization after adsorption since the zeta potential measured was too small. Steric stabilization of pectins stem from the fact that the high molecular weight polymer adsorb at casein molecule surface via their charged carboxylic groups in such a way that long loops and tails extend out into solution. Further, the steric shielding of pectin molecules prevents casein molecules from aggregation by repulsion.

The charge on casein micelle is caused by dissociation of acidic or basic groups on the casein micelle surface, or the adsorption of pectin molecules. The micelle charge is balanced by an equal and opposite charge carried by ions in the surrounding media. These counter ions tend to cluster around the micelles in diffuse clouds. This arrangement of particle surface charge surrounded by a diffuse cloud of counter charge is called the electrical double layer. The zeta potential represents the voltage difference between a plane a short distance from the particle surface and the solvent beyond the double layer. When two particles come so close that their double layer overlaps, they repel each other. The strength of this electrostatic force depends on the zeta potential. If the zeta potential is too small (typically lower than 25 mV) the repulsive force won't be strong enough to overcome the Van der Waals attraction between the particles, and they will agglomerate. The charge density of the pectins determined the strength of the complexation of HM-pectin and LM-pectin to β -lactoglobulin (Girard et al., 2002) and to casein (Pereyra et al., 1997; Marozienne and deKruif, 2000). LM-pectin with more carboxylic groups has more electrostatic binding sites and may adsorb onto the casein particle leading to sedimentation.

Concentration effect

At pH 5.3, pectin adsorbed onto to casein micelles in skim milk and an increase in concentration of the pectin led to a sequence of events: stability-bridging flocculation-stability-depletion flocculation-stable gel (Marozienne and de Kruif, 2000). At low concentrations of pectin bridging flocculation occurred. This instability is due to the fact that the adsorption of pectin is insufficient to yield full surface coverage. Under these circumstances, a pectin chain may adsorb onto two discrete particles, causing aggregation. Depletion flocculation however, occurs when adsorption of pectin onto the casein micelle is unfavorable. In this case, the center of mass of the polymer coil is displaced from the interface, creating a polymer depleted zone. The mechanism

responsible for this type of instability is called depletion flocculation (Jenkins and Snowden, 1996). Adding more pectin increased the coating of the casein surface and reduced attraction between the particles. At pectin concentration of 0.6%, LM pectin formed a stable gel. The quantity of pectin required for full coverage increased from HM-pectin < LMA- pectin < LM-pectin. A similar trend was observed by Langerdorff et al. (1997) with carrageenans ($\kappa < \iota < \lambda$ -carrageenan).

Enzymatic degradation

Pectinolytic enzymes are classified according to their attack on the galacturonan backbone of the pectin molecules. Two major groups should be distinguished: esterases (pectinesterases) and hydrolases (pectin depolymerases: polygalacturonases, pectate lyases and pectin lyases) (Rombouts and Pilnik, 1978). Pectinesterases split methanol from their substrate, transforming pectin into low methoxyl pectin and pectic acid. They are present in many fruits and vegetables and are particularly abundant in citrus fruits and tomatoes. They are also synthesized by many fungi and appear in varying amounts in commercial pectinolytic enzyme (pectinase) preparations which are commonly derived from *Aspergillus niger*. The optimum pH of plant pectinesterase is about 4.5 (Rombouts and Pilnik, 1978). Enzymatic deesterification of pectin proceeds linearly along the galacturonan chain, yielding free carboxyl groups, which make the polymer extremely calcium sensitive (Hotchkiss et al., 2002). The authors found that calcium sensitivity occurred with only a 6% reduction in the degree of esterification.

Polygalacturonase (galacturonanases) cleave the glycosidic linkages in the galacturonan chain by hydrolysis. Pectate and low methoxyl pectin are the preferred substrates. Both endo and exo-polygalacturonases exist. The action of the endo enzymes increases reducing groups and usually decreases the viscosity of the substrate solution. The exo-enzymes hydrolyze

galacturonic acid from either the reducing or the non-reducing end of the substrate chain and display a saccharifying, rather than a liquefying action. The optimum pH of polygalacturonases is in the range 4.0 to 5.5, and they are produced by fungi, bacteria, yeasts, and higher plants (Rombouts and Pilnik, 1978).

Pectate lyases split the glycosidic linkages in the galacturonan chain by β -elimination (Rombouts and Pilnik, 1978). The best substrates are low methoxyl pectin and pectate. Both exo and endo-enzymes exist, and their optimum pH is very high, from 8.0 to 9.5. Pectate lyases have an absolute requirement for calcium ions for their activity. These enzymes are synthesized by various bacteria and a few fungi. Pectin lyases cleave glycosidic linkages in the methylated galacturonan chain by beta-elimination. These enzymes are the only depolymerases which prefer highly methylated pectin as a substrate. The optimum pH is 5 to 6. However, at a pH lower than 5, these enzymes are still quite active on 75 to 85% esterified pectin. They are only produced by fungi (Rombouts and Pilnik, 1978). It is important to note that commercial polygalacturonase and pectinesterase preparations contain varying amounts of other enzymes such as cellulases, xylanases, arabanases, galactanases, glycosidases, proteases, esterases and oxido-reductases (Rombouts and Pilnik, 1978).

Enzymatic modification of pectin

Enzymatic modification of pectin by pectinesterase from higher plants and fungi produces a blockwise distribution of free carboxyl groups in the pectin molecule (Denes et al., 2000). However, microbial pectin methyl esterase leads to pectins with a random distribution of free carboxyl groups. Enzymatic deesterification of a commercial high-methoxy citrus pectin with PME isolated from Valencia orange peel produced a calcium sensitive pectin (CSP) without significant reduction of the pectin's weight-average molecular weight (M_w) (Savary et al., 2002;

Hotchkiss et al., 2002). CSP can gel without the addition of sucrose in the presence of calcium and such behavior was due to blocks of deesterified carboxylic groups in the pectins (Joye and Luzio, 2000). The functional properties of CSP result from the combined effect of modification of the degree of esterification and the high molecular weight of the mother's pectin (Hotchkiss et al., 2002).

Chemical modification

Native pectins have a high degree of esterification (HM pectins) around 70-75% and are modified by reducing the degree of esterification to prepare LM pectins (Ralet et al., 2001). Controlled acid hydrolysis is the most common process used to produce LM pectins; however alternative means, such as alkali or ammonia are used. Pectins with a random distribution of free carboxyl groups are obtained with acid or alkali treatment. If ammonia is used, amidated pectins with blockwise distribution of amide groups are produced (Thibault and Rinaudo, 1985). Chemical modification of pectin can result in reduced molecular weight of the pectin due to depolymerization of the pectin backbone via β -elimination (Renard and Thibault, 1996).

Environmental factors and whey protein interaction

Soluble high molecular weight polymers are formed when β -lactoglobulin is heated (Watanabe and Klostermeyer, 1976; Mulvihill and Kinsella, 1988). Reactions involving disulfide exchange contribute to the formation of polymers. Under the same conditions, whey protein isolate (10% w/v, pH 7, 80 °C for 30 min) do not gel unless salts are added (Kuhn and Foegeding, 1991a, b). Larger aggregates are formed by whey protein isolate than those formed by isolated β -lactoglobulin at higher temperatures (75 °C and 85 °C) compared to 65 °C (Kazmierski and Corredig, 2003). The authors also noticed that β -lactoglobulin denaturation occurred at a slower rate at 65 °C than that of α -lactalbumin. Various studies on UHT milk

(Oldfield et al., 1998) and heated WPC (Havea et al., 1998) solutions proved that almost all of the β -lactoglobulin was included into the aggregates via disulphide bonds and, to a considerable extent, via hydrophobic interactions.

Heat

The commonly used heat treatments such as pasteurization and sterilization cause denaturation of the whey proteins (de Wit, 1981). The protein structure dictates functional properties, which affect the utilization of whey as a functional ingredient. Heat denaturation unfolds the compact globular protein molecule, followed by protein aggregation and loss of protein solubility. Factors that determine heat stability of whey proteins in milk, whey or any other dairy products are ionic strength, pH, the rate of heating, the concentration of protein and lactose (Morr and Ha, 1993).

Sugars and polyhydric alcohols protect proteins against loss of solubility during heat treatment (Bernal and Jelen, 1985; Hillier and Lyster, 1979). The same authors found that lactose reduces whey protein aggregation during heat treatment, particularly in the isoelectric pH range. Thermal denaturation value of 82.2 ± 0.5 °C have been reported for β -Lg (Bernal and Jelen, 1985). Elfagm and Wheelock (1977) found that calcium reacts with the carboxyl groups of the proteins reducing the net charge to zero and thereby causing isoelectric precipitation.

BSA and IgG were cited by some authors (Hill, 1988) to be the most heat sensitive of the whey proteins, followed by β -Ig and α -lactalbumin. However, Bernal and Jelen (1985), De Wit et al. (1983) found α -lactalbumin to be the most heat sensitive of the whey proteins with denaturation temperature of 65 °C at pH 6.7. Ig G and other immunoglobulins are denatured when heated at 72 °C for 15 seconds at pH 6.7 (De Wit and Klareenbek, 1984). BSA denaturation temperature however, varies from 71.9 °C at pH 6.5 to 74 °C at pH 4.5 (Bernal and

Jelen, 1985). The thermal properties of β -lg are influenced by pH, calcium, the presence of chelating agents and phosphate (De Rham and Chanton, 1984). A slight conformational change of β -lg occurs at 40 °C and upon further heating to 50-60 °C it unfolds and expose thiols groups (Kella and Kinsella, 1988; McKenzie, 1971). Protein unfolding is favored by pH increase. Hegg (1980) showed that the T_d of β -Lg increased from 78 °C to 80 °C between pH 2 and pH 4, followed by a progressive decrease with increasing pH. The T_d value was 78 °C at pH 6; 67 °C at pH 7 and 60 °C at pH 8. At this latter pH value and at temperatures greater than 70 °C, the free thiol groups were exposed (Kella and Kinsella, 1988; Lyster, 1972) thereby enhancing disulphide interchange (De Wit and Klarenebek, 1984). The primary products are soluble high molecular weight polymers in which sulfhydryl-initiated disulfide exchange reactions play an important role at temperatures greater than 70 °C (Mleko and Foegeding, 2000). Thermally induced gels can be formed if the protein suspension has added salts (Kuhn and Foegeding, 1991a; Mulvihill and Kinsella, 1988). Between 60 °C and 70°C larger colloidal structures, independent of disulfide bonding, capable of producing a precipitable coagulum is formed.

Denaturation of α -lac is reversible at 65 °C. Heated at 100 °C for 10-30 minutes only 4% of the protein molecules revert back to their native state (Schnack and Klostermeyer, 1980). In addition, α -lac stability also depends on pH and denaturation is more severe at pH lower than 4.0 (De Wit and Klarenebek, 1984; Kronman et al., 1981). The least heat-sensitive pH range of the whey proteins lies between pH 2.5 and 3.5, where these proteins retain their good solubility (Modler and Emmons, 1977). However, the isoelectric pH range near 4.6 represents the most heat-sensitive region commonly used for the recovery of heat denatured whey-proteins (Morr et al., 1973; Morr and Foegeding, 1990).

Effect of pH

Disulfide-mediated polymerization of whey proteins occurs with increasing pH (Monahan et al., 1995). At pH 9 and 11, polymerization occurred at room temperature, while at pH 3, 5 and 7 polymerization was only evident after heating to 85, 75 and 70 °C respectively. Hoffmann et al. (1997) reported that at pH 8.0 increased number of aggregates with reduced size were formed. This is the reason why a second heating step is used at lower pH when formation of large WP-polymer formation is needed in WP-gels processing. Ju and Kilara (1998) produced whey protein gels by decreasing the pH of WP-polymer solutions from 7.0 to between 5.3-3.5 with glucono-delta-lactone (GDL). Gels obtained were opaque with a low degree of cohesiveness, indicating a particulate network structure. Mleko and Foegeding (2000) showed that a small decrease in pH (from 8.0 to between 6-6.5) was sufficient to produce weak gels.

Protein concentration

Work by Iametti et al. (1995) showed that irreversible modification of the tertiary structure of β -lg was not concentration dependent, however the temperature required for the occurrence of protein swelling, the initial step in the formation of aggregates by intermolecular disulfides was dependent on concentration only at temperatures below 75°C.

Shear effect

High mechanical shear including kneading, whipping, shaking or extrusion cause denaturation of proteins (Ohnishi and Asakura, 1976). Commercial 35% protein WPC solution heated at 70 °C followed by extrusion through a high shear orifice at high pressure gelled more rapidly than the control WPC solutions (Ker and Toledo, 1992). Further, scanning electron micrographs showed the development of a fine fibrous-like structure in the shear suspension, indicating numerous sites of protein-protein interaction. High hydrostatic pressure treatment of

mixtures of a whey protein isolate (WPI) and a high methoxy pectin (HMP) resulted in similar structure compared to a pure heat treatment of 75 °C (5-20 min) (Michel et al., 2001). However, noticeable changes were only visible in phase separating WPI/HMP mixtures if treated at 40 °C and at pressures greater than 400 MPa and after heat treatment at 85 °C for 10 min, where total WPI denatured above 60%. Under these circumstances the protein-enriched phase gelled and influenced the overall texture. However, in the presence of pectin, protein denaturation was decreased in both temperature and pressure treated samples.

Effect of ionic strength

Ionic strength affects the susceptibility of the whey proteins to heat induced denaturation and aggregation (Morr and Ha, 1993). Xiong (1992) reported that low ionic strengths of cations and low pH facilitated whey protein interaction, whereas phosphate ions (PO_4^{3-}) enhanced protein aggregation only in the presence of NaCl. Bryant and McClements (2000b) found that salt ions screen electrostatic interactions between charged whey proteins, however divalent ions have greater screening power combined with the ability to cross-link adjacent protein molecules. Hence, much lower concentration of calcium chloride was needed to induce gelation of heat denatured whey protein isolate compared to sodium chloride. McClements and Keogh (1995) reported that salt-induced gels produced by NaCl addition had a lower turbidity than the heat-induced gels, which indicated a more fine-stranded structure.

Assessment of protein carbohydrate interactions

Rheology

Rheological characterization is commonly conducted using shear stress versus shear rate ramps, which are used for characterizing the shear thinning nature of food systems (Da Silva and Rao, 1992). In weak gels the notion of structure point defined as an inflection observed in the

shear rate versus shear stress curve for a milk sample was used to determine the minimum concentration of stabilizer required for stabilization of cocoa particles in milk (Boomgaard et al., 1987). The viscoelastic nature is captured by parameters such as G' (storage modulus) and G'' (loss modulus). The storage modulus represents a measure of the elastic response of the liquid while the loss modulus is a measure of the viscous response. According to Barnes et al. (1989) in a frequency sweep for gels, G' was greater than G'' over a large frequencies and G' was relatively independent of frequency.

However before conducting such test the linear viscoelastic limit of the sample has to be determined. Conducting a torque sweep determine the largest amplitude that gives a linear relationship between displacement and torque (Rodd et al., 2000).

Visual

Using a Minolta chromameter, L, a, and b scaling system is used to determine the coloration of the gel samples. Opaque gels are indicative of particle gels or aggregates while translucent gels are composed of fine stranded microstructure (Errington and Foegeding, 1998, Turgeon and Beaulieu, 2001). In presence of cations or pH values near the isoelectric point, results in lower electrostatic repulsion allowing protein aggregation prior to gel formation. Under these circumstances opaque gels are produced. When electrostatic repulsions are important at pH below or above the isoelectric point of the whey proteins, translucent gels are formed.

Confocal laser scanning microscopy (CLSM) was used to study the ultrastructure of protein carbohydrate interactions (Bourriot et al., 1999, Turgeon and Beaulieu, 2001). The ultrastructure of micellar-casein (MC)- κ -carrageenan mixtures was described at 20°C using CLSM (Bourriot et al., 1999). The casein micelles were labeled with 8-anilino-1-naphthalene

sulfonic acid (ANS). The excitation was performed at 364 nm and the emission was recorded at 460 nm. Rhodamine isothiocyanate (RITC) with excitation-emission wavelengths of 543-590 nm was used to label κ -carrageenan. ANS is reported to bind to the hydrophobic zones of the proteins (Fitzgerald and Swaisgood, 1989). The double labeling with different excitation-emission wavelengths allowed localization of the protein and the hydrocolloid on the same micrograph. Observation of micellar-casein/ κ -carrageenan mixtures observed using different ionic forms of the carrageenan (Na^+ , K^+) in different ionic conditions and two temperatures revealed phase separations. Clear areas (protein-rich phase) were dispersed in dark areas (carrageenan-rich phase) at a low casein content (3%). At a casein concentration greater than 3% the phase separation process resulted in a casein continuous network within the carrageenan network. Gelation of the system occurred on cooling resulting in the inhibition of the separation of phases and formation of a biphasic system.

X-ray scattering properties was used to describe the size of the gel particles of biopolymers, the nature of the surrounding medium and formation of complexes (Eastoe, 1995). A decrease in scattering intensity was indicative of particles with larger size. Using this technique Mleko et al. (1997) found that for whey protein isolate and carrageenan mixture the scattering intensity decreased from pH 10, 7, and 3, respectively. At pH 10, both carrageenan and whey proteins have a negative charge and no complexes were formed. At pH 7 however the lower scattering intensity of the surrounding medium is consistent with carrageenan-whey proteins complexes formation. The scattering intensity was almost zero at pH 3 suggesting the presence of aggregates with size larger than 1000Å.

Titrimetric, potentiometric, ultrafiltration, Gel filtration

Gel filtration was used to ascertain protein-carbohydrate complexes formation through early elution of high molecular weight WPC-pectin complexes (Mishra et al., 2001). Potentiometric titrations of complex formation between protein and carbohydrate have been established by Mattison et al. (1998). Using the same basic principle Girard et al. (2002) determined complex formation between β -lactoglobulin and HM and LM-pectins. Two aliquots of the protein solution were prepared overnight for complete hydration. The pectin was added to one of the aliquot and both aliquots were titrated to pH 4.5 (predetermined pH corresponding to the pKa value of the pectin). A blank (pectin and β -lg free) solution was also titrated to take into account the titrant volume associated with the change in pH. Because the charge at the isoionic point is a function of ionic strength, the charge of β -lg was calculated relative to the isoionic point. The pH of soluble complex formation corresponded to the inflexion point on the titration curve. In this experiment, the effect of electrostatic interactions was evaluated by screening with a salt (NaCl), whereas urea was used to reduce hydrogen bonding without changing the ionic strength according to West et al. (1997). The importance of hydrophobic interactions was determined by raising the temperature of the samples from 4 °C to 40 °C. Quantification of complex formed was accomplished by centrifugation and ultrafiltration through a 100 kda molecular cut-off.

The non-complexed β -lactoglobulin in the permeate and total β -lactoglobulin were quantified using the bicinchoninic acid protein assay (BCA). The amount of β -lg complexed was determined by difference. Girard et al. (2002) found that at pH 4.5 the amount of β -lg complexed was greater with LM-pectin (96%) than HM-pectin (78%). Further, electrostatic interactions were more important than hydrogen bonding in the systems.

Sedimentation, phase separation

Mixed gels are made from blends with more than one gelling agent and can be classified into three groups: interpenetrating, coupled or complex coacervate, and phase-separated networks (Morris, 1986). When the two components form independent networks interpenetrating networks are obtained. Coacervate networks are produced in the presence of favorable intermolecular interactions between the different types of polymers. Phase separated gels are formed when the interaction between the different polymers are repulsive and/or when the two types of polymers display varying affinity toward the solvent (Tolstoguzov, 1991, 1995). At pH 8, in mixed whey protein/pectin systems phase separation was obtained after heat treatment and prior to gelation (Turgeon and Beaulieu, 2001). Whey protein and pectin are co-soluble due to electrostatic repulsions above pH 5.2 (Wang and Qvist, 2000). However, heat induced association of whey proteins was favored in a protein-enriched phase leading to the formation of spherical aggregates of WP with a low level of tridimensional network and lower hardness and deformation at fracture. Textural characteristics of the gel are affected by the aggregates and can be monitored by addition of sodium (up to 200 mM). The salt reduced electrostatic charges, WP and pectin were more compatible, and therefore phase separation was minimized before gelation resulting in smaller protein aggregates and more homogeneous gel network with increased hardness and deformation at fracture.

Texture profile analysis

Texture profile analysis (TPA) is used to determine the textural characteristics of gels. The gels are generally penetrated with a cylinder probe and a force-time curve is recorded at a fixed crosshead speed for a given displacement (Ju and Kilara, 1998). Using TPA, Beaulieu et al. (2001) found that the hardness of calcium (10 mM) added whey protein (8% w/w) gel was

higher than that of the calcium-free whey protein sample. Such difference was attributed to the electrostatic interactions between calcium and unfolded protein molecules (Kuhn and Foegeding, 1991b).

Beaulieu et al. (2001) produced a mixed gel by heating the whey protein solution with LM-pectin at 80 °C. The authors noticed that gel hardness increased with increasing amount of pectin and decreasing degree of esterification. A citrus pectin with degree of esterification (DE) value of 40 produced stronger gels than those with DE values of 28 or 35.

Casein micelle as an example

Transmission electron microscopy (TEM), scanning electron microscopy (SEM) and confocal laser scanning microscopic (CLSM) observations revealed that acid-induced skim milk gels are structured by a network of fused casein micelle clusters, immobilizing whey in pores of varying sizes (Hassan et al., 1995).

However, in the presence of polysaccharides (locust bean gum and xanthan gum), the primary casein network appears very compact with a decrease of pore size and a large increase in the porosity of the network at the supramolecular level (sponge-like morphology) (Sanchez et al., 2000). Confocal laser scanning microscopy observations requires labeling of the micellar-casein and of the polysaccharide by fluorescent probes. Casein is probed with 8-anilino-1-naphthalene sulfonic acid (ANS) which is adsorbed in the hydrophobic zones of the casein (Fitzgerald and Swaisgood, 1989), while the polysaccharide is covalently labeled with rhodamine isothiocyanate (RITC) (De Belder and Granath, 1973). This double labeling method allows localization of the polysaccharide and the casein by choosing a wavelength close to the wavelength of the maximum excitation of ANS (372 nm) such as 364 nm. Because RITC is not fluorescent at the

chosen wavelength (wavelength of maximum excitation of RITC = 543 nm), dark areas of the samples represents the polysaccharide while clear areas correspond to the proteins.

Summary

No study has been undertaken to study how the chemical composition of banana affects stability and rheology of acidified whey banana beverages. It is known that fruit pulps can differ in pectin, protein, amino acids, and phenolics. However, the implications of these differences in beverage systems have not been evaluated. Upon analyzing the composition of banana, mango, and orange pulps used in this study a clearer picture was drawn.

Model systems was formulated to study the interaction between components of the whey-banana beverages. It is commonly believed that protein-pectin interactions are of main importance in network formations and play an essential role in the stability and rheology of the final product (Benichou et al., 2002). By investigating the rheology and particle size, we hope to understand the contribution of any component or combination of components that impact the selected dependent variables the most.

Processing parameters including pH, temperature and homogenization affects the stability of the milk beverages. Electrostatic complexation between components of the acid milk beverage allows better interaction between oppositely charged particles (Dickinson, 1998). While reducing the particle size, homogenization also promotes interaction between components. The commonly used heat treatments such as pasteurization or sterilization cause denaturation and aggregation of whey proteins in milk drinks and can have adverse effect on sensory and physical characteristics on storage. Pectins prevent heat-induced aggregation of proteins. Our goal is to determine how time and temperature affect the physical characteristics of whey-banana beverages. By analyzing the serum and sediments of dispersions, our goal is to determine how

time and temperature affect the composition of the sediments harvested on storage. On the basis of the findings in this study, conditions for a stable whey fortified banana beverage was defined to ensure production of a beverage of superior quality.

References

- Ambjerg, P. and Jorgensen, B.B. Influence on the stability of casein solutions studied in dependence of varying pH and salt concentration. *Food Hydrocolloids* **1991**, 4, 323-328.
- Anet, E.R.L.J. 3-Deoxyglycosuloses and the degradation of carbohydrates. *Advances in Carbohydrate Chemistry* **1964**, 19, 18-188..
- Anon. "Rivella"- a new form of whey utilization. *Dairy Industry* **1960**, 25, 2, 113-117.
- Anon. Banana ripening guide. *CIRCULAR B, CSIRO*. **1972**.
- Anon. Tomorrow's pop bottles may be loaded –with protein. *Ind. Week* **1973**, 177, 4, 42-44.
- Anon. Bibliografia corta sobre jugos. UPEB (Union de Paises Exportadores de Banano), Panama. **1981**, 4.
- Areas, J.A.G.; Lajolo, F.M. Starch transformation during banana ripening: The Phosphorylase and Phosphatase behavior in *Musa acuminata*. *Journal of Food Biochemistry* **1981**, 5, 19-26.
- Askar, A. The importance of amino acids in fruit. Behavior of amino acids during ripening of bananas. *Gordian* **1973**, 73, 12-16.
- Baeza, R.I.; Carp, D.J.; Perez, O.E. and Pilosof, A.M.R. κ -Carrageenan-Protein Interactions: Effect of proteins on polysaccharide gelling and textural properties. *Lebensm.-Wiss.u-Technol* **2002**, 35, 741-747.
- Barnell, H.R. Hemicellulose metabolism of the banana fruit during storage and ripening. *Annals of Botany N. S.* **1943**, 7, 297.
- Barnes, H.A.; Hurton, J.F.; and Walters, K. An introduction to rheology, Amsterdam: Elsevier. **1989**.
- Beaulieu, M.; Turgeon, S. L.; and Doublier, J-L. Rheology, texture and microstructure of whey proteins/low methoxyl pectins mixed gels with added calcium. *International Dairy Journal* **2001**, 11, 961-967.
- Benichou, A; Aserin, A.; and Garti, N. Protein-polysaccharide interactions for stabilization of food emulsions. *Journal of Dispersion Science and Technology* **2002**, 23, 93-123.

- Bernal, V.; and Jelen, P. Thermal stability of whey proteins- a calorimetric study. *Journal of Dairy Science* **1985**, 11, 2847-2850.
- Boomgaard, T.; Van Den, T. ;and Van Hooydonk, A.C.M. Physical stability of chocolate milk. *International Journal of Food Science and Technology* **1987**, 22, 279-291.
- Boudhrioua, N.; Giampaoli, P. ;and Bonazzi, C. Changes in aromatic components of banana during ripening and air-drying. *Lebensm.-Wiss. u.-Technol.* **2003**, 36, 633-642.
- Bourriot, S.; Garnier, C.; and Doublier, J.L. Micellar-casein-kappa-carrageenan mixtures. I. Phase separation and ultrastructure. *Carbohydrate Polymers* **1999**, 40, 145-157.
- Brady, C. J.; Palmer, J. K.; O'Connell, P. B. H.; and Smile, R. M. An increase in protein synthesis during ripening of the banana fruit. *Phytochemistry* **1970**, 9, 1037.
- Bryant, C. M.; and McClements, D. J. Influence of xanthan gum on physical characteristics of heat-denatured whey protein solutions and gels. *Food Hydrocolloids*. **2000a**, 14, 383-390.
- Bryant, C.M.; and McClements, D.J. Influence of NaCl and CaCl₂ on cold-set gelation of heat-denatured whey protein. *Journal of Food Science* **2000b**, 65, 801-804.
- Bryce, D.J. ;and Greenwood, C.T. Thermal degradation of starch. *Starke* **1963**, 15, 5, 166.
- Caessens, P.W.J.R.; Visser, S.; and Gruppen, H. Method for the isolation of bovine β -lactoglobulin from a cheese whey protein fraction and physicochemical characterization of the purified product. *International Dairy Journal* **1997**, 7, 229-235.
- Calderon, P.; Buren, J.; and Robison, W. B. factors influencing the formation of precipitates and hazes by gelatin and condensed and hydrolysable tannins. *Journal of Agricultural Food Chemistry* **1968**, 16, 3, 479-482.
- Casimir, D.J.; and Jayaraman, K.S. Banana drink, a new canned product. *Food Research and Quality* **1971**, 31, 24-29.
- Christensen, P. E. Methods of grading pectin in relation to the molecular weight (intrinsic viscosity) of pectin. *Food Research* **1954**, 19, 163-170.
- Clark, W.S. Status of whey and whey products in the USA today, in *Trends in whey utilization*, Bulletin IDF 212, **1987**, p. 6.
- Collins, J.L.; and McCarty, I.I. Comparison of microwave energy with boiling water for blanching whole potatoes. *Food Technology* **1969**, 23, 337.
- Crowther, P.C. The processing of banana products for food use. *Tropical Products Institute Publication G122*, London **1979**.

Curl, A.L.; and Talburt, W.F. In Fruit and vegetable juice processing technology, D.K. Tressler and M.A. Joslyn, Eds. P. 421. AVI Publishing Co., Westport, CT **1961**.

Dalgleish, D; Law, A.J.R. pH-Induced dissociation of bovine casein micelles. I. Analysis of liberated caseins. *Journal of Dairy Research* **1989**, 55, 529-538.

Dapia, S.; Santos, V.; and Parajo, J.C. Carboxymethylcellulose from totally chlorine free-bleached milox pulps. *Bioresource Technology* **2003**, 89, 289-296.

Da Silva, J.A.L.; and Rao, M.A. Viscoelastic properties of food hydrocolloid dispersions. In M.A. Rao and Steffe. J.F. *Viscoelastic properties of foods* **1992**, 285-315.

Davies, D.T.; Law, A.J.R. The content and composition of protein in creamery milks in south-west Scotland. *Journal of Dairy Research* **1980**, 47, 83-90.

De Belder, A.N.; and Granath, K. Preparation and properties of fluorescein-labelled dextrans. *Carbohydrate Research* **1973**, 30, 375-382.

Deacon, W.; and Marsh, Jr. H.V. Properties of an enzyme from bananas (*Musa sapientum*) which hydroxylates tyramine to dopamine. *Phytochemistry* **1971**, 10, 2915.

Deckers, H.A.; Olieman, C. Calibration and application of high-performance size exclusion columns for molecular weight distribution of pectins. *Carbohydrate Polymers* **1986**, 6, 361-378.

DeMan, J.M. Proteins. In *Principles of Food Chemistry*; Van Nostrand Reinhold: New York, NY, **1990**.

Denes, J.M.; Baron, A.; Catherine, Renard, M.G.C.; Pean, C. and Drilleau, J.F. Different action patterns for apple pectin methylesterase at pH 7.0 and 4.5. *Carbohydrate Research* **2000**, 237, 385-393.

Devkota, K. Protein-pectin interactions in cottage cheese whey. Master's thesis, Edmonton, Alberta. **1991**.

Demott, B. J. Acceptability of flavored drinks made with cottage cheese whey produced by the direct acidification process. *Journal of Milk and Food Technology* **1975**, 691-692.

De Rham, O.; and Chanton, S. Role of ionic environment in insolubilization of whey protein during heat treatment of whey products. *Journal of Dairy Science* **1984**, 67, 5, 939-949.

De Swardt, G.H.; and Maxie, E.C. Pectin methylesterase in the ripening banana. *South African Journal of Agricultural Science* **1967**, 10, 501.

De Vries, J. A.; Voragen, A. G. J.; Rombouts, F. M.; and Pilnik, W. Extraction and purification of pectins from alcohol insoluble solids from ripe and unripe apples. *Carbohydrate Polymers* **1981**, 1, 117-127.

De Wit, J. N. Structure and functional behaviour of whey proteins. *Netherlands Milk Dairy Journal* **1981**, 35, 47-64.

De Wit, J. N.; Klareenbek, G.; and Hontelez, b. E. Evaluation of functional properties of whey protein concentrates and whey protein isolates. 1. Isolation and characterization. *Netherlands Milk Dairy Journal* **1983**, 37, 37-49.

De Wit, J. N.; and Klareenbek, G. Effect of various heat treatments on the structure and solubility of whey proteins. *Journal of Dairy Science* **1984**, 67, 11, 2701-2710.

Dickinson, E. Stability and rheological implications of electrostatic milk protein-polysaccharide interactions. *Trends in Food Science and Technology* **1998**, 9, 347-354.

Doesburg, J. J.; and Grevers, G. Setting time and setting temperature of pectin jellies. *Food Research* **1960**, 25, 634.

Draudt, H.N.; and Huang, L. Effect of moisture content of freeze-dried peaches and bananas on changes during storage related to oxidative and carbonyl-amine browning. *Journal of Agriculture and Chemistry* **1966**, 14, 2, 123-128.

Drohan, D.D.; Tziboula, A.; McNulty, D.; and Horne, D.S. Milk protein-Carrageenan interactions. *Food Hydrocolloids* **1997**, 11, 101-107.

Dupaigne, P. ; and Dalnic, R. Boissons nouvelles a base de fruits. I. Bananes. *Fruits* **1965**, 20, 571-575.

Dupaigne, P. Quality control of dried bananas. *Fruits* **1967**, 22, 27-32.

Dupaigne, P. A propos de l'extraction d'un jus de banane, en vue de la production de la biere de banane. *Fruits* **1974**, 29, 821-827.

Eastoe, J. Small-angle neutron scattering and neutron reflection. In *New Physico-Chemical Techniques for Characterization of Complex Food Systems*, ed. E. Dickinson, **1995**, 268-295. Blackie Academic and Professional, Glasgow.

Eigel, W.N.; Butler, J.E.; Ernstrom, C.A.; Farell, H.M.; Harwalker, V.R.; Jenness, R.; and Whitney, R.M. Nomenclature of proteins of cow's milk, Fifth revision. *Journal of Dairy Science* **1984**, 67, 8, 1599-1631.

Elfagm, A. A.; Wheelock, J. V. Effect of heat on α -lactalbumin and β -lactoglobulin in bovine milk. *Journal of Dairy Research* **1977**, 44, 2, 367-371.

Ellis, G.P. The Maillard reaction. *Advances in Carbohydrate Chemistry* **1959**, 14, 63-70.

Embs, R.J.; and Markakis, P. The sulfur dioxide inhibition of enzymatic browning. Paper presented at the 25 th annual meeting, inst. Food Technol., Kansas City, Missouri **1965**.

Errington and Foegeding, E.A. Factors determining fracture stress and strain of fine-stranded whey protein gels. *Journal of Agricultural and Food Chemistry* **1998**, 46, 8, 2963-2967.

Evageliou, V.; Richardson, R.K.; and Morris, E.R. Effect of pH, sugar type and thermal annealing on high-methoxy pectin gels. *Carbohydrate Polymers* **2000**, 42, 245-259.

Farkye, N.Y.; and Imafidon, G. I. Thermal denaturation of indigenous milk enzymes. In P. F. Fox (Ed.), Heat Induced Changes in Milk, (2nd Edition), Brussels: *International Dairy Federation* **1995**, 331-348.

Feinberg, B.; Olson, R.L.; and Mullins, W.R. Prepeeled potatoes. In Potato processing, W.F. Talburt and O. Smith, ed. P. 491. AVI Publishing Co., Westport, CT. **1967**.

Finkle, B.J.; and Marri, M.S. Methylation of polyhydroxy-aromatic compounds by pampas grass o-methyl-transferase. *Biochemistry Biophysics Acta* **1964**, 85, 165.

Fitzgerald, R.J.; and Swaisgood, H.E. Binding of ions and hydrophobic probes to α -lactalbumin and κ -casein as determined by analytical affinity chromatography. *Archives of Biochemistry Biophysics* **1989**, 268, 239-243.

Fox, K.K.; Holsinger, V.H.; Posati, L.P.; and Pallansch, M.J. separation of β -lactoglobulin from other milk serum proteins by trichloroacetic acid. *Journal of Dairy Science* **1967**, 50, 1363-1367.

Freudenberg, K. die Chemie der Naturlichen garbstoffe, Springer-Verlag, Berlin, **1920**.

Fry, S. C. Phenolic components of the primary cell wall and their possible role in the hormonal regulation of growth. *Planta* **1979**, 146, 343-350.

Fry, S. C. Phenolic components of the primary cell wall. Feruloylated disaccharides of D-galactose and L-arabinose from spinach polysaccharides. *Biochemistry Journal* **1982**, 203, 493-498.

Galeazzi, M..A..M.; and Sgarbieri, V.C. Substrate specificity and inhibition of polyphenoloxidase (PPO) from a dwarf variety of banana (*Musa cavendishii*). *Journal of Food Science* **1981**, 46, 5, 1404-1410.

Garcia, E.; and Lajolo, F.M. Starch transformation during banana ripening: the amylase and glucosidase behavior. *Journal of Food Science* **1988**, 53, 1181-1188.

German, J.B.; Dillard, C.J.; and Walzem, R.L. U.S. Whey Products and Dairy Ingredients for Health: A review. U.S. Dairy Export Council. In press, **2001**.

Gilsenan, P.M.; Richardson, R.K.; and Morris, E.R. Thermally-reversible acid-induced gelation of low-methoxy pectin. *Carbohydrate Polymers* **2000**, 41, 339-349.

Girard, M.; Turgeon, S.L.; and Gauthier, S.F. Interbiopolymer complexing between β -lactoglobulin and low-and high-methylated pectin measured by potentiometric titration and ultrafiltration. *Food Hydrocolloids* **2002**, 16, 585-591.

Glahn, P.E. Hydrocolloid stabilization of protein suspensions at low pH. *Progress in Food and Nutrition Science* **1982**, 6, 171-177.

Glass, L.; and Hedrick, T.I. Nutritional composition of sweet and acid-type dry wheys. II. Vitamin, mineral and calorie contents. *Journal of Dairy Science* **1976**, 60, 2, 190-196.

Golding, J.B.; Shearer, D.; McGlasson, W.B.; and Wyllie, S.G. Relationships between respiration, ethylene, and aroma production in ripening banana. *Journal of Agricultural and Food Chemistry* **1999**, 47, 1646-1651.

Griffiths, L.A. Detection and identification of the polyphenoloxidase substrate of banana. *Nature* **1959**, 184, 58-65.

Hagerman, A.E.; and Butler, L.G. Protein precipitation method for the quantitative determination of tannins. *Journal of Agricultural and Food Chemistry* **1978**, 26, 4, 809-812.

Hagerman, A. E.; and Butler, L. G. Condensed tannins purification and characterization of tannin associated proteins (Tannin from sorghum grain and pinto beans). *Journal of Agricultural and Food Chemistry* **1980**, 28, 5, 947-952.

Hannigan, K. Anyone for citrus milk? *Food Engineering* **1978**, 14-16.

Hansen, P. M. T. Food hydrocolloids in the dairy industry. In *Food Hydrocolloids: Structures, Properties, and Functions*, eds K. Nishinari and E. Doi. Plenum Press, New York, pp. **1994**, 211-224.

Harborne, J.B.; and Simmonds, N.W. The natural distribution of phenolic aglycone, in *Biochemistry of Phenolic Compounds*, Harborne, J.B., Ed., Academic Press, London, **1964**.

Harper, W.J. Biological Properties of Whey Components. A review. Chicago, IL: *The American Dairy Products Institute* **2000**.

Hassan, A.N.; Franck, J.F.; Farmer, M.A.; Schmidt, K.A.; and Shalabi, S.I. Formation of yogurt microstructure and three-dimensional visualization as determined by Confocal Scanning Laser Microscopy. *Journal of Dairy Science* **1995**, 78, 2629-2636.

Havea, P.; Singh, H.; Creamer, L. K.; and Campanella, O. H. Electrophoretic characterization of the protein products formed during heat treatment of whey protein concentrate solutions. *Journal of Dairy Research* **1998**, 65, 79-91.

Hegg, P. O. Thermal stability of β -lactoglobulin as a function of pH and relative concentration of sodium dodecyl sulfate. *Acta Agriculture Scandinavica* **1980**, 30, 4, 401-404.

- Hill, A. R. Thermal precipitation of whey proteins. *Milchwissenschaft* **1988**, 43, 9, 565-567.
- Hillier, R.M.; and Lyster, R.L.J. Whey protein denaturation in heated milk and cheese whey. *Journal of Dairy Research* **1979**, 46, 1, 95-102.
- Hiraoka, Y.; and Sugai, S. Thermodynamics of thermal unfolding of bovine apo- α -lactalbumin. *International Journal of Peptide and Protein Research* **1984**, 23, 535-542.
- Hoffmann, M.A.M.; Sala, G.; Olieman, C.; and De Kruif, K.G. Molecular mass distribution of heat-induced β -lactoglobulin aggregates. *Journal of Agricultural and Food Chemistry* **1997**, 45, 2949-2957.
- Holsinger, V.H.; Posati, L.P.; and DeVilbiss, E.D. whey beverages: A Review. *Journal of Dairy Science* **1974**, 57, 8, 849-859.
- Hope, G.W. The use of antioxidants in canning apple halves. *Food Technology* **1961**, 15, 548-556.
- Holt, C.; and Horne, D.S. the hairy casein micelle: Evolution of the concept and its implications for the dairy technology. *Netherlands Milk Dairy Journal* **1996**, 50, 85-111.
- Holtermann, A. The browning reaction. *Starke* **1966**, 16, 319-325.
- Horne, D.S. Casein interactions: casting light on the black boxes, the structure in dairy products. *International Dairy Journal* **1998**, 8, 171-177.
- Hotchkiss, A.T.; Savary, B.J.; Cameron, R.G.; Chau, H.K.; Brouillette, J., Luzio, G.A.; and Fishman, M.L. Enzymatic modification of pectin to increase its calcium sensitivity while preserving its molecular weight. *Journal of Agricultural and Food Chemistry* **2002**, 50, 2931-2937.
- Hultin, H.O.; and Levine, A.S. Pectin methyl esterase in the ripening banana. *Journal of Food Science* **1965**, 30, 917-926.
- Hwang, J.; and Kokini, J. L. Contribution of the side branches to rheological properties of pectins. *Carbohydrate Polymers* **1992**, 19, 41-50.
- Iametti, S.; Cairoli, S.; De Gregori, B.; and Bonomi, F. Modifications of high-order structures upon heating of β -lactoglobulin: dependence on the protein concentration. *Journal of Agricultural and Food Chemistry* **1995**, 43, 53-58.
- Issenberg, P.; and Wick, E.L. Volatile components of banana. *Journal of Agricultural and Food Chemistry* **1963**, 11, 1-8.

Iwamoto, Y.; Miyazaki, M.; Maeda, Y.; and Horio, T. Abnormal de-tinning of cans by nitrate in foods. III. Nitrate in banana and internal corrosion of canned banana drink. *Eiyo To Shokuryo* **1970**, 23, 213-217.

Jelen, P. Whey cheeses and beverages. In J.G. Zadow (Ed.), whey and Lactose Processing. New York: Elsevier Applied Science. **1992**, 157-193.

Jenkins, P.; and Snowden, M. Depletion flocculation in colloidal dispersions. *Advances in Colloid and Interface Science* **1996**, 68, 57-96.

Joslyn, M.A.; and Ponting, J.D. Enzyme catalyzed oxidative browning of fruit product. *Advances in Food Research* **1951**, 3, 1-5.

Joye, D.D.; and Luzio, G.A. Process for selective extraction of pectins from plant material by differential pH. *Carbohydrate Polymers* **2000**, 43, 337-342.

Ju, Z.Y.; and Kilara, A. Textural properties of cold-set gels induced from heat-denatured whey protein isolates. *Journal of Food Science* **1998**, 63, 288-292.

Kanazawa, K.; and Sakakibara, H. High content of dopamine, a strong antioxidant, in Cavendish banana. *Journal of Agricultural and Food Chemistry* **2000**, 48, 844-848.

Kayisu, K.; and Hood, L.F. Molecular structure of banana starch. *Journal of Food Science* **1981**, 46, 1894.

Kazmierski, M.; and Corredig, M. Characterization of soluble aggregates from whey protein isolate. *Food Hydrocolloids* **2003**, 1-8.

Kella, N.K.D.; and Kinsella, J.E. Enhanced thermodynamic stability of β -lactoglobulin at low pH: A possible mechanism. *Biochemistry Journal* **1988**, 255, 113.

Ker, Y.C.; and Toledo, R.T. Influence of shear treatments on consistency and gelling properties of whey protein isolate suspensions. *Journal of Food Science* **1992**, 57, 82.

Hannigan, K.J. Anyone for citrus milk? *Food Engineering* **1978**, 14-15.

Koeing, J.K.; Pierson, W.E.; Horike, M.; and Frank, R. a comparison of the pulmonary effects of 0.5 ppm and 1.0 ppm sulfur dioxide plus sodium chloride droplets in asthmatic adolescents. *Toxicol. Environ. Health* **1983**, 11, 129-136.

Koffi, E.K.; Sims, C.A.; and Bates, R.P. Viscosity reduction and prevention of browning in the preparation of clarified banana juice. *Journal of Food Quality* **1991**, 14, 209-218.

Konrad, G.; Lieske, B.; and Faber, W. A large-scale isolation of native β -lactoglobulin: characterization of physicochemical properties and comparison with other methods. **2000**, 10, 713-721.

- Kravtchenko, T.P.; Voragen, A.G.J.; and Pilnik, W. Studies on the intermolecular distribution of industrial pectins by means of preparative size exclusion chromatography. *Carbohydrate Polymers* **1992**, 19, 115.
- Kronman, M. J.; Sinha, S.K.; and Brew, K. Binding of Ca^{2+} and other metal ions to α -lactalbumin. *Journal of Biology and Chemistry* **1981**, 8582-8587.
- Kuhn, P. R.; and Foegeding, E. A. Mineral salts effects on whey protein gelation. *Journal of Agricultural and Food Chemistry* **1991a**, 39, 1013-1016.
- Kuhn, P. R.; and Foegeding, E. A. Factors influencing whey protein gel rheology: dialysis and calcium chelation. *Journal of Food Science* **1991b**, 56, 789-791.
- Kyamuhangire, W.; Myhre, H.; Sorensen H.T.; and Pehrson, R. 2002. Yield, characteristics and composition of banana juice extracted by the enzymatic and mechanical methods. *Journal of the Science of Food and Agriculture* **2002**, 82: 478-482.
- Lang, F.; and Lang, A. More advances in the manufacture of new milk-based food products. *Milk Industry* **1969**, 64, 6, 64-69.
- Langerdorff, V.; Cuvelier, G.; Launay, B.; and Parker, A. Gelation and flocculation of casein micelle/carrageenan mixtures. *Food Hydrocolloids* **1997**, 1, 35-40.
- Lee, C.N.; and Merson, R.L. Prefiltration of cottage cheese whey to reduce fouling of ultrafiltration membrane. *Journal of Food Science* **1976**, 41, 402.
- Len'kov, N.T. Beverage "Bodrost". Moloch. Prom. **1969**, 30, 11, 25-28.
- Lii, C.Y.; Chang, S.; and Young, Y.L. Investigation of the physical and chemical properties of banana starches. *Journal of Food Science* **1982**, 47, 1493.
- Lustre, A.O.; Soriano, M.S.; Morga, N.S.; Balagot, A.H.; Tunac, M.M. Physico-chemical changes in "SABA" bananas during normal and acetylene-induced ripening. *Food Chemistry* **1976**, 1, 125-132.
- Lyster, R. L. J. Review of progress of Dairy Science; Section C: chemistry of milk proteins. *Journal of Dairy Research* **1972**, 39, 2, 278-318.
- Mao, W.W. Banana fruit technology I. Dehydration of banana puree by drum drying. II. Properties of amylase in banana. Ph.D. thesis. Cornell University **1974**.
- Mao, W.W.; and Kinsella, J.E. Amylase activity in banana fruit: properties and changes in activity with ripening. *Journal of Food Science* **1981**, 46, 1400-1407.

- Maroziene, A.; and de Kruif, C.G. Interaction of pectin and casein micelles. *Food Hydrocolloids* **2000**, 14, 391-400.
- Maubois, J.L. New applications of membrane technology in the dairy industry. *Australian Journal of Dairy Technology* **1991**, 46, 91-98.
- Mate, J.; and Krochta, J.M. β -lactoglobulin separation from whey protein isolate on a large-scale. *Journal of Food Science* **1994**, 59, 1111-1114.
- Maubois, J.L.; Pierre, A.; Fauquant, J.; and Piot, M. Industrial fractionation of main whey proteins. *International Dairy Federation bulletin* **1987**, 212, 154-159.
- Mattison, K.W.; Dubin, P.L.; and Brittain, I.J. Complex formation between bovine serum albumin and strong polyelectrolytes: effect of polymer charge density. *Journal of Physical Chemistry* **1998**, 102, 3830-3836.
- Mavropoulou, I. P.; and Kosikowski, F. K. Free amino acids and soluble peptides of whey powders. *Journal of Dairy Science* **1973a**, 56, 9, 1135-1138.
- Mavropoulou, I.P.; and Kosikowski, F.V. Composition, solubility and stability of whey powders. *Journal of Dairy Science* **1973b**, 56, 9, 1128-1134.
- May, C. D. Industrial pectin: sources, production and applications. *Carbohydrate Polymers* **1990**, 12, 79-99.
- McCarthy, A.I.; Palmer, J.K.; Shaw, C.P.; and Anderson, E.E. Correlation of gas chromatographic data with flavor profiles of fresh banana fruit. *Journal of Food Science* **1963**, 28, 379-386.
- McCarthy, A.I.; Wyman, H.; and Palmer, J.K. Gas chromatographic identification of banana fruit volatiles. *Journal of Gas Chromatography* **1964**, 2, 121-128.
- McGugan, W.A.; Larmond, E.; and Emmons, D.B. Some observations on the flavor of acid whey. *Journal of the Canadian Institute of Food Science and Technology* **1979**, 12, 1, 32-35.
- McClements, D.J.; and Keogh, M.K. Physical properties of cold-setting gels formed from heat-denatured whey protein isolate. *Journal of the Science of Food and Agriculture* **1995**, 69, 7-14.
- McIntosh, G.H.; Royle, P.J.; Le Leu, R.K.; Regester, G.O.; Johnson, M.A.; Grinsted, R.L.; Kenward, R.S.; and Smithers, G.W. Whey proteins as functional food ingredients. *International Dairy Journal* **1998**, 8, 425-434.
- Mckenzie, H. A. β -lactoglobulin. In H.A. Mckenzie (Ed.). *Milk proteins*. **1971**, 2, 257-330. New York: Academic Press.

- Michel, A-S.; Mestdagh, M.M.; Axelos, M.A.V. Physico-chemical properties of carrageenan gels in presence of various cations. *International Journal of Biology of Macromolecules* **1997**, 21, 195-200.
- Michel, M.; Leser, M.E.; Syrbe, A.; Clerc, M.F.; Bauwens, I.; Bovetto, L.; Von Schack; M.L.; and Watzke, H.J. Pressure effects on whey protein-pectin mixtures. *Lebensmittel-Wissenschaft und-Technology* **2001**, 34, 41-52.
- Mishra, S.; Mann, B.; and Joshi, V.K. Functional improvement of whey protein concentrate on interaction with pectin. *Food Hydrocolloids* **2001**, 15, 9-15.
- Mleko, S; Li-Chan, E.C.Y.; and Pikus, S. interactions of κ -carrageenan with whey proteins in gels formed at different pH. *Food Research International* **1997**, 30, 6, 427-433.
- Mleko, S.; and Foegeding, E.A. pH induced aggregation and weak gel formation of whey protein polymers. *Journal of Food Science* **2000**, 65, 1, 139-143.
- Modler, H.W.; and Emmons, D.B. Properties of whey protein concentrate prepared by heating under acidic conditions. *Journal of Dairy Science* **1977**, 60, 177-186.
- Monahan, F.; German, J.B.; and Kinsella, J.E. Effect of pH and temperature on protein unfolding and thiol/disulfide interchange reactions during heat-induced gelation of whey proteins. *Journal of Agricultural and Food Chemistry* **1995**, 43, 46-52.
- Montgomery, M.W.; and Sgarbieri, M.W. Isoenzymes of the banana polyphenoloxidase. *Phytochemistry* **1975**, 14, 1245-1249.
- Morr, C.V.; Swenson, P.E.; and Richter, R.L. Functional characteristics of whey protein concentrates. *Journal of Food Science* **1973**, 38, 324-329.
- Morr, C.V. Functional properties of milk proteins and their use as food ingredients. In P.F. Fox (ed.), *Developments in Dairy Chemistry* 1, p 375. London, UK: Applied Science **1982**.
- Morr, C.V.; and Foegeding, E.A. Composition and functionality of commercial whey and milk protein concentrates and isolates: a status report. *Food Technology* **1990**, 44, 100-108.
- Morr, C.V.; and Ha, E.Y.W. Whey protein concentrates and isolates: processing and functional properties **1993**, 33, 6, 431-476.
- Morris, V.J. Multicomponent gels. In G.O. Philips, D.J. Wedlock and P.A. Williams, *Gums and stabilisers for the food industry*, London: Elsevier **1986**, 3, 87-99
- Morris, V.J. Gelation of polysaccharides. In : Hill, S.E., Ledward, D.A. and Mitchell, J.R. (Eds), *Functional Properties of Food Macromolecules*. Elsevier Applied Science, UK **1998**, 143-362.

Mulvihill, D.M.; and Kinsella, J.E. Gelation of β -lactoglobulin: effects of sodium chloride and calcium chloride on the rheological and structural properties of gels. *Journal of Food science* **1988**, 53, 231-236.

Mukhiddinov, Z. K.; Khalikov, D. Kh.; Abdusamiev, F. T.; and Avloev, Ch. Isolation and structural characterization of a pectin homo and rhamnogalacturonan. *Talanta* **2000**, 53, 171-176.

Mulvihill, D. M.; and Kinsella, J. E. Gelation of β -lactoglobulin: effects of sodium chloride and calcium chloride on the rheological and structural properties of gels. *Journal of Food Science* **1988**, 53, 231-236.

Mumyanganizi, B.; and Coppens, R. Extraction du jus de banane. *Industry Alimentaire et Agriculture* **1974**, 91, 185.

Muneta, P. Bisulfite inhibition of enzymatic blackening caused by tyrosine oxidation. *American Potato Journal* **1966**, 43, 397.

Myers, M.J.; Issenberg, P.; and Wick, E.L. vapor analysis of the production by banana fruit of certain volatile constituents. *Journal of Food Science* **1969**, 34, 504.

Narayanan, J.; Deotare, V.W.; Bandyopadhyay, R.; and Sood, A.K. Gelation of aqueous pectin solutions: a dynamic light scattering study. *Journal of Colloid and Interface Science* **2002**, 245, 267-273.

Ndi, E.E.; Swanson, B.G.; Barbosa-Canovas, G.V.; and Luedecke, L.O. Rheology and microstructure of β -lactoglobulin/sodium polypectate gels. *Journal of Agricultural and Food Chemistry* **1996**, 44, 86-92.

Nelson, F. E.; Brown, W.C.; and Taylor, R. R. Whey as a component of fruit flavoured drinks. *Cultured Dairy Products Journal* **1971**, 7, 1, 11-12.

Oakenfull, D.; and Scott, A. Hydrophobic interactions in the gelation of high methoxy pectins. *Journal of Food Science* **1984**, 49, 1093-1098.

Oh, H.I.; Hoff, J.E.; Haff, L.A.; and Armstrong, G.S. Hydrophilic interaction in tannin protein complexes. *Journal of Agricultural Food Chemistry*. **1980**, 28, 394-398.

Oh, H.I.; Hoff, J.E.; and Haff, L.A. Immobilized condensed tannins and their interaction with proteins. *Journal of Food Science* **1985**, 50, 6, 1652-1654.

Oh, H. I.; and Hoff, J. E. ph dependence of complex formation between condensed tannins and proteins. *Journal of Food Science* **1987**, 52, 5, 1267-1269.

Ohnishi, T.; and Asakura, T. Denaturation of oxyhemoglobin S by mechanical shaking. *Biochimica Biophysics Acta* **1976**, 453, 93-100.

Oldfield, D. J.; Singh, H.; Taylor, M. W.; and Pearce, K. N. Kinetics of denaturation and aggregation of whey proteins in skim milk heated in an ultra-high temperature (UHT) pilot plant. *International Dairy Journal* **1998**, 8, 311-318.

Palmer, J.K. Banana polyphenoloxidase preparation and properties. *Plant physiology* **1963**, 38, 508.

Palmer, J.K. The banana. In: The biochemistry of fruits and their products. Hulme, A.C. (Ed.). **1971**, Vol. 2, p. 65. Academic Press, New York.

Parker, A.; Brigand, C.; Miniou, C.; Trespoe, A.; and Vallee, P. Rheology and fracture of mixed ι - and κ -carrageenan gels two-step gelation. *Carbohydrate Polymers* **1993**, 20, 253-262.

Parker, A.; Boulenger, P.; and Kravtchenko, T. P. Effect of the Addition of High Methoxy Pectin on the Rheology and Colloidal Stability of Acid Milk Drinks. In *Food Hydrocolloids: Structures, Properties, and Functions*; Nishinari, K.; Doi, E., Eds.; Plenum Press: New York, NY, **1994**.

Pearce, R.J. Fractionation of whey proteins, in Trends in whey utilization, int. Dairy Fed., Brussels, Belgium, **1987**, 150-156.

Pearce, R. J. Thermal denaturation of whey protein. *International Dairy Federation Bulletin* **1989**, 238, 17-28.

Pereyra, R.; Schmidt, K. A.; Wicker, L. Interaction and stabilization of acidified casein dispersions with low and high methoxyl pectins. *Journal of Agricultural and Food Chemistry* **1997**, 45, 3448-3451.

Piculell, L. Gelling carrageenans. In A.M. Stephen, Food Polysaccharides and their applications. New York: Marcel Dekker. **1995**, 205-243.

Pilnik, W. Pectin-a many splendoured thing, gums and stabilizers for the food industry 5, (G. O. Philips, D. J. Wedlock and P. A. Williams, eds), IRL Press, Oxford, **1990**, 209-220.

Ponting, J.D. The control of enzymatic browning of fruits. In Food Enzymes, ch. 9. H.W, Schultz (Ed.) p. 105. AVI Publishing Co., Westport, CT. **1960**.

Prabha, T. N.; and Bhagyalakshmi. Carbohydrate metabolism in ripening banana fruit. *Phytochemistry* **1998**, 48: 915-923.

Ralet, M-C.; Dronnet, V.; Buchholt, H.C.; and Thibault, J-F. Enzymatically and chemically de-esterified lime pectins: characterization, polyelectrolyte behavior and calcium binding properties. *Carbohydrate Research* **2001**, 336, 117-125.

- Rao, K. L.; Eipeson, W. E.; Rao, P. N. S.; Patwardhan, M. V.; and Ramanathan, P. K. Rheological properties of mango pulp and concentrate. *Journal of Food Science and Technology* **1985**, 22: 30.
- Rawel, H. M.; Kroll, J.; and Hohl, U.C. Model studies on reactions of plant phenols with whey. *Nahrung* **2001**, 45, 2, 72-81.
- Rees, D.A.; Steele, I.W.; and Williamson, F.B. Conformational analysis of polysaccharides. III. The relation between stereochemistry and properties of some polysaccharide sulfates (1). *Journal of Polymer Science. Part C*, **1969**, 28, 261-276.
- Rees, D. A. Polysaccharide gels. *Chem. Ind. (London)* **1972**, 16, 630-638.
- Renard, C. M. G. C.; and Thibault, J.F. Pectins in mild alkaline conditions: β -elimination and kinetics of demethylation. In *Pectins and Pectinases*; Visser, J., Voragen, A. G. J., Eds.; Elsevier: New York, **1996**; pp 603-608.
- Reynolds, T.M. Chemistry of nonenzymic browning. II. *Advances in Food Research* **1965**, 14, 168-175.
- Ridley, B. L.; O'Neill, M. A.; and Mohnen, D. Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. *Photochemistry* **2001**, 57, 929-967.
- Robards, K.; Prenzler, P.D.; Tucker, G.; Swatsitang, P.; and Glover, W. Phenolic compounds and their role in oxidative process in fruits. *Food Chemistry* **1999**, 66, 401-436.
- Rodd, A.B.; Davis, C.R.; Dunstan, D.E.; Forrest, B.A.; and Boger, D.V. Rheological characterization of 'weak gel' carrageenan stabilized milks. *Food Hydrocolloids* **2000**, 14, 445-454.
- Rombouts, F. M.; and Pilnik, W. Enzymes in fruit and vegetable juice technology. *Process Biochemistry* **1978**, 13, 8-15.
- Rombouts, F.M.; and Thibault, J.F. Sugar-beet pectins: chemical structure and gelation through oxidative coupling, *Chemistry and Function of Pectins*, ACS Symposium Series 310, (M. L. Fishman and Jen, J.J, ds.), American Chemical Society, Washington, DC **1986**, p. 49.
- Salmon, B.; Martin, G.J.; Remaud, G.; and Fourel, F. Compositional and isotopic studies of fruit flavor. The banana aroma. *Flavour and Fragrance Journal* **1996**, 11, 353-395.
- Sanchez, C.; Zuniga-Lopez, R.; Schmitt, C.; Despond, S.; and Hardy, J. Microstructure of acid-induced skim milk-locust bean gum-xanthan gels. *International Dairy Journal* **2000**, 10, 199-212.

Savary, B. J.; Hotchkiss, A.T.; and Cameron, R.G. Characterization of a salt-independent pectin methylesterase purified from Valencia orange peel. *Journal of Agricultural and Food Chemistry* **2002**, 50, 3553-3558.

Sayavedra, L.A.; and Montgomery, M.W. Inhibition of polyphenoloxidase by sulfite. *Journal of Food Science* **1986**, 51, 1531-1536.

Schnack, V.; and Klostermeyer, H. Thermal behaviour of α -lactalbumin. *Milchwissenschaft* **1980**, 35, 206-208.

Shenkenberg, D.R.; Chang, J.C.; and Edmondsdon, L.F. Develops milk orange juice. *Food Engineering* **1971**, 97-98.

Shimokawa, K.; Ebihara, M.; Kinoshita, S.; and Murakami, H. Changes of acid content in *Musa sapientum* (banana) fruit during ripening. Bull. Fac. Agric., Miyazaki Univ. **1972**, 19, 329-334.

Siebert, K.J. Effects of protein-polyphenol interactions on beverage haze, stabilization, and analysis. *J. Agric. Food Chem.* **1999**, 47, 353-362.

Sienkiwicz, T.; and Riedel, C. Whey and Whey Utilization. Gielsekirchen-Buer, Germany: Verlag Th. Mann. **1990**.

Sims, C. A.; Bates, R. P.; and Areola, A. G. Color, polyphenoloxidase, and sensory changes in banana juice as affected by heat and ultrafiltration. *Journal of Food Quality* **1994**, 17, 371-379

Singleton, V. L. and Esau, P. Adv. Food Res. Suppl. **1969**, 1, 282.

Song, P.S.; and Chichester, C.O. Kinetic behavior and mechanism of inhibition in the Maillard reaction. *Journal of Food Science* **1967**, 32, 107-115.

Southgate, D.A.T. Determination of food carbohydrate. Ed.; Applied Science:London, **1976**.

Speiser, R.; and Eddy, C.R. Effect of molecular weight and method of deesterification on the gelling behavior of pectin. *Journal of the American Chemical Society* **1946**, 68, 287-293.

Sreekantiah, K.R.; Jaleel, S.A.; and Rao, R.T.N. Utilization of fungal enzymes in liquefaction of soft fruits and extraction and clarification of fruit juices. *Journal of Food Science* **1971**, 8, 201.

Stanley, N.F. Carrageenan. In P. Harris, Food gels **1990**, 79-119

Stover, R.H.; Simmonds, N.W. Bananas. 3rd Ed.; John, W. & Sons Inc: New York, **1987**.

Sugisawa, H.; and Edo, H. Thermal polymerization of glucose. *Chemistry Industry* **1964**, 40, 892.

Syrbe, A.; Fernandes, P. B.; Dannenberg, F.; Bauer, W. J.; and Klostermeyer, H. Whey protein-polysaccharide mixtures: polymer incompatibility and its application. In *Food Macromolecules and Colloids*, eds E. Dickinson and D. Lorient. Special Publication No 156. Royal Society of Chemistry, London, pp. **1995**, 328-339.

Syrbe, A.; Bauer, W. J.; and Klostermeyer, H. Polymer science concepts in dairy systems- an overview of milk protein and food hydrocolloid interaction. *International Dairy Journal* **1998**, 8, 179-193.

Swaigood, H.E. Chemistry of milk proteins, In *Developments in Dairy Chemistry*, vol 1 Proteins, Fox, P.F., Ed., Applied Science, New York, **1982**, 1-59.

Swaigood, H.E. characteristics of edible fluids of animal origin: milk, in *Food Chemistry*, Fennema, O. R., Ed., Marcel Dekker, New York, **1985**, 792.

Teranishi, R.; Hornstein, I.; Issenberg, P.; and Wick, E.L. *Flavor Research: Principles and Techniques*. Marcel Dekker, New York, **1971**.

Terra, N.N.; Garcia, E.; and Lajolo, F. M. Starch sugar transformation during banana ripening: The behavior of UDP glucose Pyrophosphorylase, Sucrose Synthetase and Invertase. *Journal of Food Science* **1983**, 48, 1097-1112.

Thibault, J. F.; and Rinaudo, M. Interactions of mono and divalent ions with alkali and enzyme-deesterified pectins. *Biopolymers* **1985**, 24, 2131-2140.

Thibault, J. F.; and Rinaudo, M. Chain association of pectic molecules during calcium-induced gelation. *Biopolymers* **1986**, 25, 455.

Tolstoguzov, V. B. Functional properties of food proteins and role of protein-polysaccharide interaction. **1991**, 4, 429-438.

Tolstoguzov, V.B. Some physico-chemical aspects of protein processing in foods. Multicomponents gels. *Food Hydrocolloids* **1995**, 9, 317-332.

Tressl, R.; and Drawert, F. Biogenesis of banana volatiles. *Journal of Agricultural and Food Chemistry* **1973**, 21, 4-13.

Turgeon, S.L.; and Beaulieu, M. Improvement and modification of whey protein gel texture using polysaccharides. *Food Hydrocolloids* **2001**, 15, 583-591.

Turquois, T.; Rinaudo, M.; Taravel, F.R.; and Heyraud, A. Extraction of highly gelling pectic substances from sugar beet pulp and potato pulp: influence of extrinsic parameters on their gelling properties. *Food Hydrocolloids* **1999**, 13, 255-262.

United Fruit Company. Unpublished data submitted to United Fruit Company by J.K. Palmer, MIT **1972**.

Vansoest, P. J.; and Robertson, J.B. Analytical problems of fiber. In : Carbohydrates and health, Ed.; AVI Publishing Co.: Westport, CT, **1977**, 69-76.

Van Sumere, C. F.; Albrecht, J.; Deodonder, A., Depooter, H.; and Pe, I. The Chemistry and Biochemistry of Plant Proteins. New York: academic Press. **1975**

Viquez, F.; Lastreto, C.; and Cooke, R.D. A study of the production of clarified banana juice using pectinolytic enzymes. *Journal of Food Technology* **1981**, 16, 115-122.

Von Loesecke, H.W. Banana. 2nd Ed.; Interscience: New York, **1950**.

Voragen, A. G. J.; Pilinik, W.; Thibault, J. F.; Axelos, M. A. V.; and Renard, M. G. C. Pectins. in Food polysaccharides and their Applications; Stephen, A. M., ed.; Marcel Dekker: New York, NY, **1995**.

Walker, J.B. The Biology of plant phenolic, Edward Arnold, London **1975**.

Walstra, P. Casein sub-micelles: do they exist? *International Dairy Journal* **1999**, 9, 189-192.

Wang, Q.; and Qvist, K.B. Investigation of the composite system of β -lactoglobulin and pectin in aqueous solutions. *Food Research International* **2000**, 33, 683-690.

Watanabe, K.; and Klostermeyer, H. heat-induced changes in sulphydryl and disulphide levels of β -lactoglobulin A and the formation of polymers. *Journal of Dairy Research* **1976**, 43, 411-418.

White, T. Tannins: their occurrence and significance. *Journal of the Science of Food and Agriculture* **1957**, 8, 377-384.

Wick, E.L.; Yamanish, T.; Kobayash, A.; Valenzuela, S.; and Issenberg, P. Volatile constituents of banana (*M. cavendishii*, Variety Valery). *Journal of Agricultural and Food Chemistry* **1969**, 17, 751-758.

Wills, R.B.H.; Lim, J.S.K.; and Greenfield, H. Changes in chemical composition of 'Cavendish' banana (*Musa acuminata*) during ripening. *Journal of Food Biochemistry* **1984**, 8, 69-76.

Wolform, M.L.; Sehnitz, R.D.; and Calvabieri, L.F. Discoloration of sugar solutions and 5-(hydroxymethyl)-fufural. *Journal of The American Chemical Society* **1948**, 70, 514-520.

Wong, N. P.; Lacroix, D. E.; and McDonough, F. E. Minerals in whey and whey fractions. *Journal of Dairy Science* **1978**, 61, 12, 1700-1703.

Wyman, H.; and Palmer, J.K. Organic acids in the ripening banana fruit. *Plant Physiology* **1964**, 39, 630-637.

Xia, J.; and Dubin, P. L. Protein-polyelectrolyte complexes. In *Macromolecular Complexes I. Chemistry and Biology*; Dubin, P. L.; Bock, J.; Davis, R.; Schulz, D. n.; Thies, C., eds.; **1994**, 247-271. Marcel Dekker: New York, N.Y.

Xiong, Y.L. Influence of pH and ionic environment on thermal aggregation of whey proteins. *Journal of Agricultural and Food Chemistry* **1992**, 40, 380-387.

CHAPTER 3
CHARACTERIZATION OF CLOUD COMPONENTS FROM SELECTED TROPICAL
FRUITS¹

¹Koffi, E. K., Wicker, L., and Phillips, R. To be submitted to the Journal of Food Quality

Abstract

The composition and characteristics of insoluble cloud matter (ICM) and alcohol insoluble solids (AIS) from banana, mango and orange was evaluated. The galacturonic acid content was significantly higher for mango and orange pectin (6.0%) compared to banana (5%). The degree of esterification of pectin in AIS was 78 ± 5 , 85 ± 6 , and 85 ± 2 for mango, orange, and banana respectively. Proteins in the fruit pulps were low molecular weight ($< 100,000$ Daltons). The relative proportion of the amino acids in the AIS and ICM were similar, with aspartic acid predominant while methionine and tyrosine contents were very low. Phenolic compounds were highest in banana pulp (138 ± 2 mg gallic acid equivalent/L = mg GA/L) compared to 102 ± 8 mg GA/L for orange. Phenolic compounds were about 10 fold less (15 ± 2 mg GA/L) in mango.

Keywords: banana; mango; orange; pectin; cloud; neutral sugars; phenolics; proteins

Introduction

Cloudiness in fruit juices is due to fine suspensions (0.4-5.0 μm) of heterogeneous matter including pectins, proteins, lipids and hesperidin (Baker and Bruemmer 1969; Yen and Song 1998). High molecular weight components including protein, pectin, hemicellulose and cellulose constitute 50% of the total citrus cloud by weight (Sinclair 1984). Pectic substances, proteins, and polyphenolic compounds produce stable cloud suspensions due to interaction of cloud components as well as chemical structure (Hoff et al. 1980). Oil droplets present in the citrus cloud decrease the average density of cloud particles and stabilize the suspension (Mizrahi and Berk 1970). Interaction between negatively charged pectin and positively charged proteins at pH values less than the isoelectric point of proteins stabilizes juice cloud (Yen and Song 1998). Further, covalently bound neutral sugars on pectin hydrogen bond with proteins to increase stability of the cloud (Shomer 1991). Klavons et al. (1991, 1994) found that pectin-protein stabilization of cloud was mainly due to soluble pectin (60% of cloud pectin). Pectin-hesperidin stabilization of cloud was attributed to neutral sugars rather than total amount of pectin (Ben-Shalom and Pinto 1999).

In apple juice, polyphenol- protein interactions lead to insoluble complexes through polymerization resulting in haze. Interaction of polyphenolic with proteins result in colloidal complexes that scatter light and sediment if sufficiently large (Siebert et al. 1996). Asano et al. (1982) found that polymeric phenols had greater affinity for proteins than simple phenolics. Further, the potential for immediate haze formation was higher for the polymeric phenolics in apple juice (Hsu et al. 1989). Proline-rich proteins enhance haze formation and are called haze active proteins (Asano et al. 1982). Siebert et al. (1996a) showed that the amount of haze depends on the concentration and ratio of polyphenol and protein. Hydrogen bonding was not

as important in the interaction between proteins and polyphenols as was hydrophobic interactions (Oh et al. 1980; Siebert et al. 1996). In another study Wu and Siebert (2002) found that the sizes of haze active proteins in apple juice were 28, 15 and 12 kDa.

Beveridge and Tait (1993) found that commercial apple juice haze contained from 11.4 to 29.0% protein (w/w). Denaturing electrophoresis destroyed the haze aggregates and revealed polypeptides ranging from 29 kDa to greater than 205 kDa. Staining techniques coupled with electron microscopy revealed the presence of spherical particles (proteins) embedded in polymerized matrix (phenolics).

However, a natural protective factor (HPF) was isolated from wine that stabilized protein and decreased haze formation (Waters and Wallace 1993). The HPF contained a polysaccharide component (96%) that was dominated by mannose (78%) and glucose (13%) and a protein component (4%) that was rich in serine (31%) and threonine (13%). In the absence of HPF, denatured grape proteins aggregated to form amorphous sediment or flocculated to produce haze.

A study of cloud and haze components is useful to understand the origin and stability of cloud. Tropical fruit juices and flavors provide consumer diversity of flavors. In addition, tropical fruits such as banana and mango are nutritious products containing carbohydrates, vitamins and micronutrients that may be lost during post harvest distribution. One way to use the overripe fruits may be to produce protein fortified beverages. However, consumers expect a stable cloud in most tropical fruit-based beverages (Jelen 1992). The basic information concerning the properties of the cloud components in banana, mango and orange pulps is not available. The objective of this study was to evaluate the cloud composition of banana, orange and mango for some factors that are likely to influence cloud stability of protein-fortified mango, banana and citrus beverages.

Materials and Methods

Materials

Cavendish bananas were donated by the University of Georgia Horticulture Department. Kent mangos were purchased from Brooks Brothers (Homestead, FL) and frozen concentrated Valencia orange pulp was donated by Citrus World (Lake Wales, FL). Bananas and mangos were allowed to ripen at ambient temperature. Ripeness was based on color of the fruits. Bananas were selected as ripe when the skin was extensively flecked with brown spots whereas the mangos were used when the skin was easily removed by hand. The fruit pulps were finely homogenized at a low speed using a Proscientific homogenizer (Pro 300 A, Pro Scientific, Inc., Monroe, CT) to form a puree.

Insoluble cloud matter and Alcohol Insoluble solids

Insoluble cloud matter (ICM) was prepared by the method of Klavons et al. (1994). Purees were boiled for 5 min in 4 volumes of deionized water, cooled and centrifuged at 21,000 g for 15 min at room temperature. The precipitate obtained (ICM) was redispersed in deionized water, vortexed, and centrifuged three times, freeze-dried and stored at -20°C.

Alcohol insoluble solids (AIS) were prepared from the puree by boiling in 4 volumes of 95% ethanol. After cooling to room temperature, the precipitate was filtered through a sintered glass funnel, washed sequentially with 6 parts of ethanol, and 4 parts of acetone. The residue was dried under the hood overnight at room temperature and stored at -20°C.

Degree of esterification

The degree of esterification of pectin from ICM and AIS was estimated by a modified HPLC method of Voragen et al. (1986) as described by Ackerley and Wicker (2003). About 50-100 mg of each sample was saponified in 2 mL water-isopropanol mixture (1:1) containing 0.4

N NaOH. The amount of methanol in the supernatant was determined from a regression curve. The pellets were dispersed in sulfuric acid and galacturonic acid content was estimated utilizing m-hydroxydiphenyl method (Blumenkrantz and Asboe-Hanson 1973). The % DE was estimated as the mole ratio of methanol to uronic acid.

Electrophoretic Analysis

Resolution of peptides in the AIS and ICM was obtained on 4 to 15 % gradient gels using the corresponding SDS-buffer strips on the PhastSystem^R (Amersham Pharmacia Biotech, Piscataway, NJ). An aliquot of 10 mg of ICM or AIS were dissolved in the SDS-Tris-HCl buffer with 5 % β -mercaptoethanol and 0.0125 % bromophenol blue, and heated for 5 min in boiling water bath. The samples were cooled to ambient temperature before centrifugation with an Eppendorf centrifuge 5412 (Brinkmann Instruments, Westbury, N.Y.). Approximately 5 μ l of the supernatant for each sample was loaded and run at 5 mA until 63 Vh was attained (about 45 min). Peptide bands were stained in 0.1 % Phastgel Blue^R dissolved in 30:10:60 methanol: acetic acid: water. Destaining of the gels was performed in 30:10:60 methanol: acetic: water. The molecular weight of proteins bands were estimated by comparison to low molecular weight calibration kit for electrophoresis (Amersham Pharmacia Biotech, Piscataway, NJ) using Bio-Rad's Image Analysis Systems (Bio-Rad laboratories, Hercules, CA).

Neutral sugar analysis

Neutral sugars of AIS or ICM were analyzed by gas chromatography (GC) according to a modified procedure by Blakeney et al. (1983). The derivatization and GC analysis was optimized to provide separation of the selected neutral sugars. About 40 mg of AIS or ICM were hydrolyzed in 5 mL of 2M trifluoroacetic acid at 120°C for 4 h on a Fisher Isotemp^R Dry Bath model 145 heating module (Fisher Scientific, Pittsburgh, PA). Samples were filtered through

glass wool. A total of 100 μ L of sample extract were added to GC vials and 64 μ g of the internal standard (phenyl β -D glucoside, crystalline from Sigma) was added. The samples were evaporated to dryness under a stream of nitrogen. An aliquot of 70 μ L of N, O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) in N, N-Dimethylformamide (DMF) (1:1, v/v) was added and the vials were capped. The monosaccharides were derivatized at 76C for 90 min on a Pierce Reacti-Therm Heating module (Wrightsville, PA). Samples were cooled to room temperature before analysis. The GC analysis was performed using a Hewlett-Packard 5890 A gas chromatograph (Gen Tech Scientific, Inc. Arcade, N.Y.) equipped with a flame ionization detector (FID) and a split injector. The GC-column was a 30 m x 0.32 mm (inner diameter) fused silica, DB-5, 0.25 μ m (film thickness) capillary column (J & W, Scientific Folsom, CA). The temperatures of the injector and detector were 250C and 300C, respectively. Oven temperature was initially held at 150C for 1 min and heated from 150C to 210C at 4C/min and held at 210C for 0.50 min, heated to 280C at 7C/min and held at 280C for 10 min. Helium was used as a carrier gas at a flow rate of 53.1 mL/min. A 3392 A integrator (Hi Tech Trade Inc., Abury Park, N.J.) was connected to the GC for the peak area measurement. Identification of each sugar was based on retention times using authentic standards (Sigma, St Louis, MO) including rhamnose, arabinose, xylose, mannose, galactose, glucose and fucose. The standards were dissolved in methanol (0.8 mg/mL) and run separately and together to determine the retention times of specific peaks.

Amino acid analysis

Predetermined weights of finely ground sample, approximately 500-600 μ g of protein were transferred into glass vials (10 x 100mm). A volume of 100 μ l of the internal standard (norleucine), 200 μ l of a 5% solution of 3, 3'-dithiodipropionic acid (DTDPA) in methanol and 1

mL of methanol were added to the vial. The contents of the sample vials were evaporated to dryness under a stream of argon and 0.05% phenol and 5mL of 6 M HCl was added and the sample was deaerated through alternating cycles of argon purging and vacuum (Barkholt and Jensen 1989). Samples were transferred to a mechanical oven (Linberg blue mechanical convection oven, model MO144OSC, Asheville, NC) and hydrolyzed at 110C for 18 h.

The hydrolysates were resuspended following the hydrolysis in 5 mL of 20 mM HCl solution and sonicated for 10 min. The suspension was filtered through a 0.2 μ m nylon membrane (Gelman Nylon Acrodisc 13, Gelman Sciences, Ann Arbor, MI). A filtrate volume of 20 μ l was derivatized with 6-amino quinolyl-N-hydroxysuccinamidyl carbamate (Cohen and Michaud 1993; van Wandelen and Cohen 1997). The derivative was transferred into limited volume inserts (LVI) fitted into 4 mL vials and placed in the Waters Intelligent Sample Processor (WISP) model 710 B.

Amino acid analysis was carried out according to Cohen and Michaud (1992). The amino acid analyzer consisted of the Waters Millipore AccQ-Tag chemistry package and a High Performance Liquid Chromatography (HPLC) system (Waters Alliance 2690 Separations Module, Milford, MA), equipped with a scanning fluorescence detector (Waters model 474, Milford, MA). Excitation and emission wavelengths were 256 nm and 395 nm, respectively. Chromatographic separation was performed on a NovoPakTM silica-bonded C₁₈, 4 μ m column (3.9mm x 150mm) thermostatted at 37C and operated at a flow rate of 1 mL/min. Elution of amino acids was performed by a gradient flow resulting from mixing acetate buffer (eluent A) pH 4.6 and 60% acetonitrile in deionized water (eluent B). The buffers were filtered through a 0.2 μ m nylon membrane.

Total phenolics

The total phenolics were determined according to the Folin-Ciocalteu method (Singleton and Rossi 1965). About 2g of puree were mixed with 8 mL of methanol and 2mL of water added. The tube was capped and shaken at 200 RPM, 60 C for 30 min in a water bath (Gyratory Water Bath Shaker, Model G76D, New Brunswick Scientific Co., Edison, NJ, USA). The tubes were removed, vortexed and centrifuged at 2,000 RPM using a Dynac II Centrifuge (Becton & Dickinson Company, Franklin Lakes, NJ) for 2 min. The samples were filtered through a 0.45 μ m Millipore syringe filter (Whatman Inc., Clifton, NJ). The total phenolics in the filtrate were determined colorimetrically. A 0.2 N Folin-Ciocalteu reagent (Sigma, St Louis ,MO) was freshly prepared by diluting a 2N stock solution with water. A volume of 100 μ L of filtrate was added to 900 μ L of distilled water, and 5 mL of 0.2 N Folin-Ciocalteu reagent were mixed. Saturated sodium carbonate (Sigma Chemical Co., St Louis, MO) (4 mL of a 75 g/L solution) was added and the mixture was vortexed. The tubes were incubated for 2 h at room temperature and the absorbance read at 765 nm with a UV-1601 Shimadzu spectrophotometer (Shimadzu Corporation, Kyoto, Japan). A standard curve was constructed using 100, 167, 250 and 500 mg/L gallic acid (Sigma, St Louis, MO) in water.

Statistical analysis

Experiments were run in duplicate and statistical analyses were performed using the SAS (SAS Institute Inc., Cary, NC 2000) software package. Analyses of variance were performed by ANOVA procedures. Significance level was defined as $P < 0.05$.

Results and Discussion

Neutral sugars analysis

Glucose was the predominant neutral sugar in both the AIS and ICM in fruit pulps except in orange AIS where galactose was the main sugar (Table 1). A representative chromatogram is depicted which shows the contents of neutral sugars in AIS and ICM of orange pulp (Figure 1a). The orange AIS contained galactose (10.40 ± 0.05 %), arabinose (4.55 ± 0.81 %), glucose (3.43 ± 0.10 %), mannose (3.87 ± 0.09 %), xylose (1.39 ± 0.05 %), and rhamnose (0.88 ± 0.0 %). The sugar composition profile of orange ICM is similar to that of the orange AIS with higher total neutral sugars content in the ICM (about 40 ± 4 %) compared to the AIS (about 25 ± 1 %). In Shamouti orange, Shomer et al. (1999) found that the total neutral sugar content in the AIS and the ICM was in the range of 34 ± 2 % and 35 ± 4 %, respectively.

Glucose was the predominant sugar in banana AIS (25.7 ± 0.7 %) and ICM (24.9 ± 2.2). There were 5-10 fold less quantity of galactose (5.06 ± 0.09 %), mannose (4.28 ± 0.05 %), xylose (2.10 ± 0.03 %) and arabinose (2.51 ± 0.03 %). See Figure 1b for a typical chromatogram. No rhamnose or fucose was detected in AIS or ICM of banana. The banana ICM had a similar sugar profile as the banana AIS. Prabha and Bhagyalakshmi (1998) also found in banana pulp pectic fractions that glucose had the highest concentration (6.2 mg/g fresh weight) whereas fucose was also not detected.

A representative chromatogram and composition for neutral sugars in mango AIS and ICM is shown in Figure 1c and Table 1. Mango AIS contained glucose (25.4 ± 0.03 %), arabinose (13.25 ± 0.03 %) and galactose (7.44 ± 0.03 %), xylose (1.23 ± 0.03 %), fucose (1.14 ± 0.25 %) and rhamnose (0.54 ± 0.03 %). The arabinose content in mango AIS (13.25 ± 0.03) was high compared to orange and banana. The mango ICM contained glucose (26.4 ± 0.71 %),

galactose ($5.37 \pm 0.27\%$), xylose ($4.35 \pm 0.07\%$) and mannose ($4.84 \pm 0.78\%$). Arabinose ($2.41 \pm 0.15\%$) was present in small amount while rhamnose and fucose were not detected in mango ICM.

Ratios of galacturonic acid to neutral sugars and galacturonic acid (GalA) to rhamnose (Rha) of the AIS are presented in Table 2. A lower ratio GalA/NS indicates the pectin is richer in neutral sugars and more branched or hairy (Voragen et al. 1995). Banana and mango AIS had two fold lower ratios GalA/NS than orange AIS, and is likely to be more highly branched than citrus AIS. The ratio GalA/Rha describes the kinks of the pectin molecules (Voragen et al. 1995). The higher the ratio the more linear the pectin chain. Rhamnose was not detected in AIS or ICM of banana pulp. Thus, banana pectin is likely to be more linear than mango or orange pectins. Orange pectins are likely to have more smooth regions ($\text{GalA/NS} = 0.23 \pm 0.01$) than mango ($\text{GalA/NS} = 0.12 \pm 0.0$) or banana ($\text{GalA/NS} = 0.10 \pm 0.02$) and be more kinked ($\text{GalA/Rha} = 6.4 \pm 0.2$) than mango ($\text{Gal A/ Rha} = 11.6 \pm 0.4$) or banana (no rhamnose). Ratios of galacturonic acid to rhamnose ranging from 2 to 10 are considered high (Kravtchenko et al. 1992a) and characteristic of rhamnose-rich hairy regions of pectins (de Vries et al. 1986; Saulnier and Thibault 1987). Similar results were observed for Lemon and apple pectin (Kravtchenko 1992b). The galacturonic acid content ranged from $6.3\% \pm 0.2$ in mango to $5.6\% \pm 0.2$ in orange and $4.5\% \pm 0.7$ in banana. The DE were not significantly different ($P \geq 0.05$) for orange ($85\% \pm 6$), banana ($85\% \pm 5.0$) and mango ($78\% \pm 5$). Based on DE values, banana, mango and orange pectins are high methoxyl pectins ($\text{DE} > 50\%$).

AIS, ICM and protein analysis

Denaturing electrophoresis (Figure 2) revealed protein fractions of 14,000 to 60,000 Daltons in banana AIS whereas those in the banana ICM ranged from 14,000 to 40,000. In

mango AIS, molecular weight of the denatured proteins ranged from 14,000 to 90,000 and from 14,000 to 30,000 in mango ICM. Proteins of orange AIS contained polypeptides in the range of 14,000 to 50,000 Daltons, and ranged from 14,000 to 40,000 Daltons in orange ICM. The protein molecular weights in orange compare to those found in Valencia orange AIS (20,000-52,000 Daltons) and ICM (17,000-50,000 Daltons) (Shomer et al. 1999). Some of these peptides likely participate in destabilization of juice cloud by catalysis and/or formation of complexes. Peptides at 13,000, 27,000, and 36,000 Daltons were identified in floc and pellet of clarified Valencia orange juice (Ackerley and Wicker 2003). Ackerley et al. (2002) proposed that the 36,000, 27,000, and 13,000 peptides influence the ability of PME to induce flocculation of citrus juice. Proteins of low molecular weight (21,000 Daltons) and high isoelectric point formed stable pectin complexes in guava puree (Yen and Song 1998). Proteins with molecular sizes of 28,000, 15,000, and 12,000 Daltons were reported in apple protein isolates (Wu and Siebert 2002) similar in size to the 30,000-20,000, 12,000 Daltons heat-unstable proteins of grapes (Hsu and Heaterbell 1987) and the 21,000-31,000 Daltons in Granny Smith apples (Hsu et al. 1989).

Amino acids composition of AIS and ICM and total phenolics

The ratio of amino acid to total amino acids is presented in Table 3 for the amino acid composition for the AIS and ICM of banana, mango and orange. A high ratio (AA/Tot sample) indicates a high level of a given amino acid. In general the amino acid composition of the AIS and ICM did not differ for a given fruit as shown by their AA/Tot ratio. Aspartic acid was predominant in the AIS and ICM of all fruits. Aspartic acid was also dominant in two major wine proteins (24,000 and 32,000 Daltons) that contribute significantly to grape wine ((Muscat of Alexandria brand) heat haze (Waters et al. 1992). All the samples (AIS or ICM) contained a

large proportion of leucine, lysine and glutamic acid. Proline, arginine, alanine and valine were present in a slightly lower amount while methionine and tyrosine levels were very low. Yen and Song (1998) also found that the major amino acids formed in guava puree were aspartic acid (dominant) and glutamic acid. Further, the authors observed an increase in the content of polar amino acids, such as aspartic, glutamic acid and serine by acidification of the puree.

The amino acid composition of banana, mango and orange AIS and ICM were slightly different from each other. Banana AIS or ICM contained larger proportion of histidine and lower proportion of lysine compared to mango or orange AIS or ICM. Orange AIS or ICM contained the highest proportion of aspartic acid.

The presence of proline in all fruit pulps is likely to enhance haze activity in presence of phenolics (Siebert 1996b). Banana pulp with high phenolic content (Table 2) is likely to be prone to haze formation compared to orange or mango with less phenolics. Heat unstable proteins in wine were high in serine, glycine and aspartic acid (Waters et al. 1992). The banana, mango and orange AIS or ICM contain a large proportion of most of the same type of amino acids that could react in a similar fashion with heat.

Conclusions

The variability of tropical fruit cloud composition has been analyzed through their alcohol insoluble solids and insoluble cloud matters. Low molecular weight and haze active polypeptides are present in both the alcohol insoluble solids and insoluble cloud matters. However, the abundance of phenolics in banana will most likely enhance interactions with proteins. Pectins present in banana, mango or orange will most likely interact with pulp-associated compounds in a unique way. Banana pectin with fewer neutral sugars and a linear backbone favor interchain interactions compared to more branched and neutral sugars rich

orange and mango pectins. Information about the composition of banana, mango and orange helped predict the performance of such fruits in producing cloud stable protein-fortified beverages.

References

- ACKERLEY, J., CORREDIG, M. and WICKER, L. 2002. Clarification of citrus juice is influenced by specific activity of thermolabile pectinmethylesterase (PME) and inactive PME-protein complexes. *J. Food Sci.* 67(7), 2529-2533.
- ACKERLEY, J. and WICKER, L. 2003. Floc formation and changes in serum soluble cloud components of fresh Valencia orange juice. *J. Food Sci.* 68(4), 1169-1174.
- ASANO, K., SHINAGAWA, K. and HASHIMOTO, N. 1982. Characterization of haze-forming proteins of beer and their roles in chill haze formation. *J. Am. Soc. Brew. Chem.* 40, 147-154.
- BAKER, R.A. and BRUEMMER, J.H. 1969. Cloud stability in the absence of various orange juice soluble components. *Citrus Ind. Proc. Fla. State Hort.* 82, 215-220.
- BARKHOLT, V. and JENSEN, A.L. 1989. Amino acid analysis: determination of cystine plus half-cystine in proteins after hydrochloric acid hydrolysis with a disulfide compound as additive. *Anal Biochem.* 177, 318-322.
- BEN-SHALOM, N. and PINTO, R. 1999. Natural colloidal particles: the mechanism of the specific interaction between hesperidin and pectin. *J. Agric. Fd. Chem.* 38, 179-182.
- BEVERIDGE, T. and TAIT, V. 1993. Structure and composition of apple juice haze. *Food Structure* 12, 195-198.
- BLAKENEY, A.B., HARRIS, P.J., HENRY, R.J. and STONE, B.A. 1983. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* 113, 291-299.
- BLUMENKRANTZ, N. and ASBOE-HANSEN, G. 1973. New method for quantitative determination of uronic acids. *Anal. Biochem.* 54, 484-489.
- COHEN S.A. and MICHAUD, D.P. 1993. Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate and its application for the analysis of hydrolysate amino acids via high performance liquid chromatography. *Anal Biochem.* 211, 279-287.
- DE VRIES, J.A., HANSEN, M., SODERBERG, J., GLAHN, P.E. and PEDERSEN, J.K. 1986. Distribution of methoxyl groups in pectins. *Carbohydr. Polym.* 6, 165-172.

- HOFF, J.E., ARMSTRONG, G.S. and HAFF, L.A. 1980. Hydrophobic interaction in tannin-protein complexes. *J.Agric. Food Chem.* 28, 394-397.
- HSU, J.C., HEATHERBELL, D.A. and YORGEY, B.M. 1989. Effects of fruit storage and processing on clarity, proteins, and stability of Granny Smith apple juice. *J. Food Sci.* 54, 660-662.
- JELLEN, P. 1992. Whey cheeses and beverages. In J.G. Zadow (Ed.), whey and lactose processing, pp. 157-193. New York: Elsevier Applied Science.
- KLAVONS, J.A., BENNETT, R.D. and VANNIER, S.H. 1991. Nature of the protein constituent of commercial orange cloud. *J.Agric. Food Chem.* 39, 1545-1548.
- KLAVONS, J.A., BENNETT, R.D. and VANNIER, S.H. 1994. Physical/chemical nature of pectin associated with commercial orange juice cloud. *J. Food Sci.* 59, 399-401.
- KRAVTCHENKO, T.P., VORAGEN, A.G.J. and PILNIK, W. 1992. Studies on the intermolecular distribution of industrial pectins by means of preparative ion-exchange chromatography. *J. Carbohydr. Polym.* 19, 115-124.
- MIZRAHI, S. and BERK, Z. 1970. Physico-chemical characteristics of orange juice cloud. *J. Sci. Food Agric.* 21, 250-253.
- PRABHA, T.N. and BHAGYALAKSHMI, N. 1998. Carbohydrate metabolism in ripening banana fruit. *Phytochemistry* 48(6), 915-919.
- OH, H.I., HOFF, J.E., ARMSTRONG, G.S. and HAFF, A. 1980. Hydrophobic interaction in tannin-protein complexes. 28, 394-398.
- SAULNIER, L. and THIBAUT, J.F. 1987. Enzymic degradation of isolated pectic substances and cell wall from pulp of grape berries. *Carbohydr. Polym.* 7, 345-360.
- SIEBERT, K.J., CARRASCO, A. and LYNN, P.Y. 1996a. Formation of protein-polyphenol haze in beverages. 44, 1997-2005.
- SIEBERT, K.J., TROUKHANOVA, N.V. and LYNN, P. 1996b. Nature of polyphenol-protein interactions. *J. Agric. Food chem.* 44, 80-85.
- SHOMER, I. 1991. Protein coagulation cloud in citrus fruit extracts. I. Formation of coagulates and their bound pectin and neutral sugars. *J. Agric. Food Agric.* 39, 2263-2266.
- SHOMER, I., YEFREMOV, T. and MERIN, U. 1999. Involment of proteins in cloud instability of shamouti orange [*Citrus sinensis* (L.) Osbeck] juice. *J. Agric. Food Chem.* 47, 2623-2631.
- SINCLAIR, W.B. 1984. The Biochemistry and physiology of the lemon p 411. Univ of California, Division of Agriculture & Natural Resources: Oakland, CASINGLETON, V.L. and

ROSSI, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* *16*, 144-158.

VAN WANDELEN, C. and COHEN, S.A. 1997. Using quaternary high-performance liquid chromatography eluent systems for separating 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate-derivatized amino acid mixtures. *Journal of Chromatography A*, *763*, 11-22.

VORAGEN, G.J., SCHOLS, M.A. and PILNIK, W. 1986. Determination of methylation and acetylation of pectins by HPLC. *Hydrocolloids I*, 65-70.

VORAGEN, A.G.J., PILNIK, W., THIBAULT, J.F., AXELOS, M.A.V. and RENARD, M.G. C. Pectins. In *Food Polysaccharides and Their Applications*; Stephen, A.M., ed., Marcel Dekker: New York, NY, 1995.

WATERS, E.J. and WALLACE, P.J. 1993. Isolation and characterization of a natural haze protective factor from wine. *J. Agric. Food Chem.* *41*, 724-730.

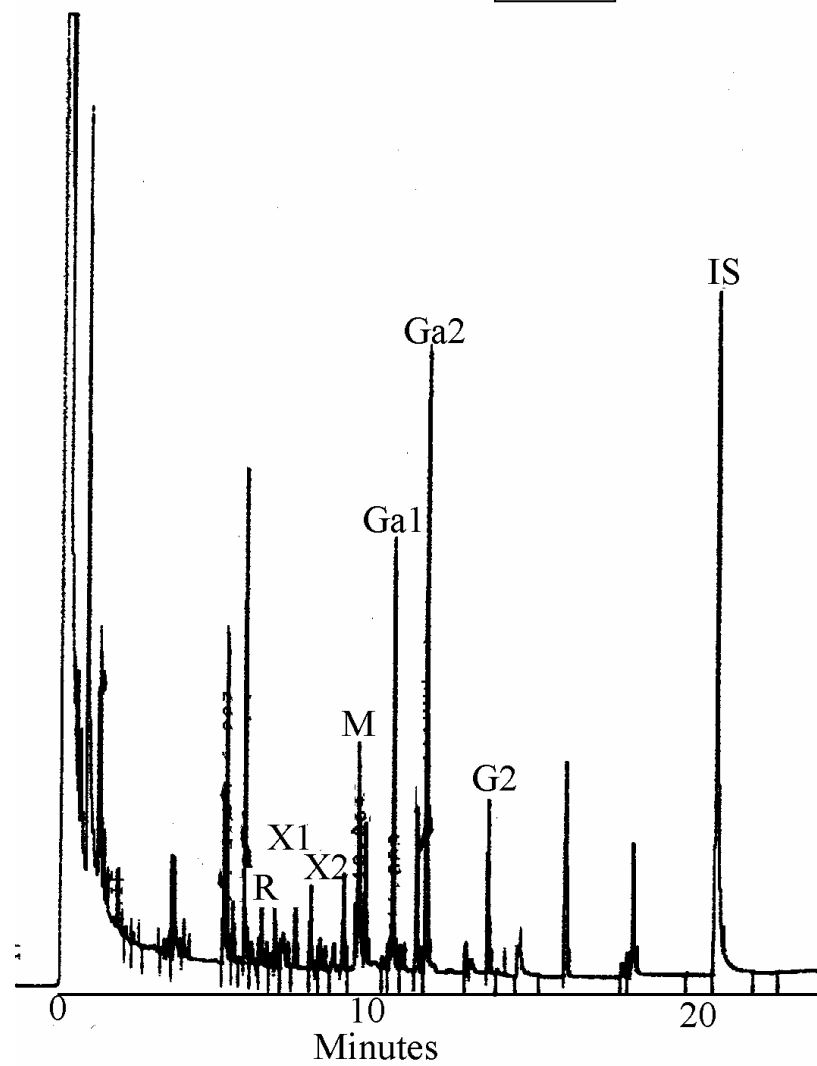
WATERS, E.J., WALLACE, W. and WILLIAMS, P.J. 1992. Identification of heat-unstable wine proteins and their resistance to peptidases. *J. Agric. Food Chem.* *40*, 1514-1519.

WU, L. and SIEBERT, K.J. 2002. Characterization of haze-active proteins in apple juice. *J. Agric. Food Chem.* *50*, 3828-3834.

YEN, G.C. and SONG, T.Y. 1998. Characteristics of clouding substances in guava puree. *J. Agric. Food Chem.* *46*, 3435-3439.

FIGURE 1a CONTENTS OF NEUTRAL SUGARS IN ALCOHOL INSOLUBLE SOLIDS (AIS) AND INSOLUBLE CLOUD MATTER (ICM) OF ORANGE PULP. (IS) INTERNAL STANDARD (PHENYL β -D GLUCOSIDE), (G1) (G2) α AND β -D GLUCOSE, (GA1) (GA2) α AND β -D GALACTOSE, (M) D-MANNOSE, AND β -D (X1) (X2) α XYLOSE,

AIS



ICM

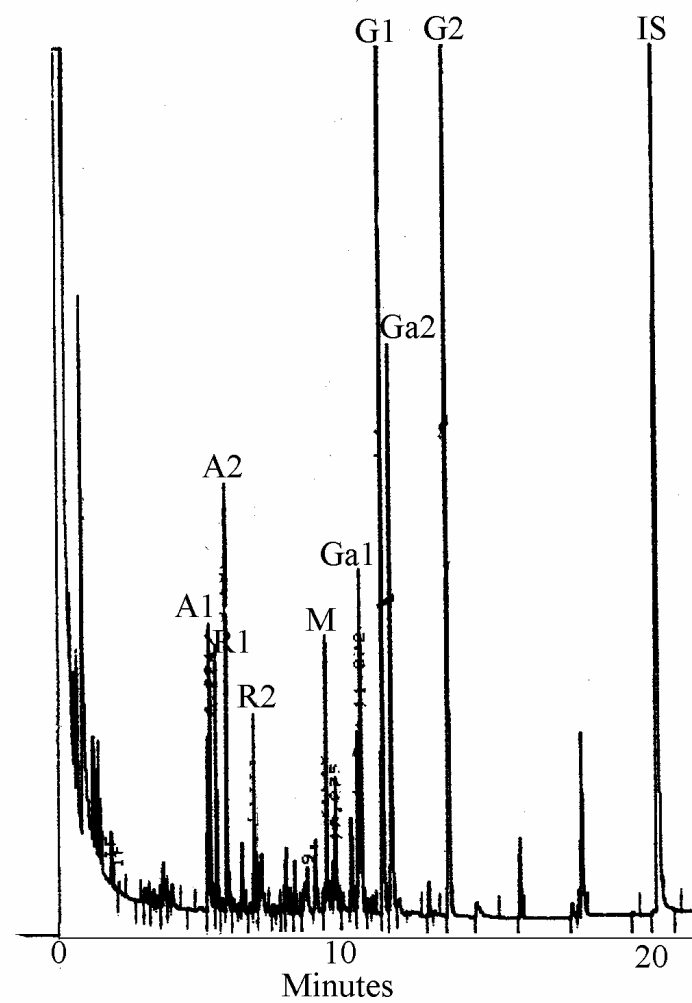


FIGURE 1 b. CONTENTS OF NEUTRAL SUGARS IN ALCOHOL INSOLUBLE SOLIDS (AIS) AND INSOLUBLE CLOUD MATTER (ICM) OF BANANA PULP (refer to page 87 for legend)

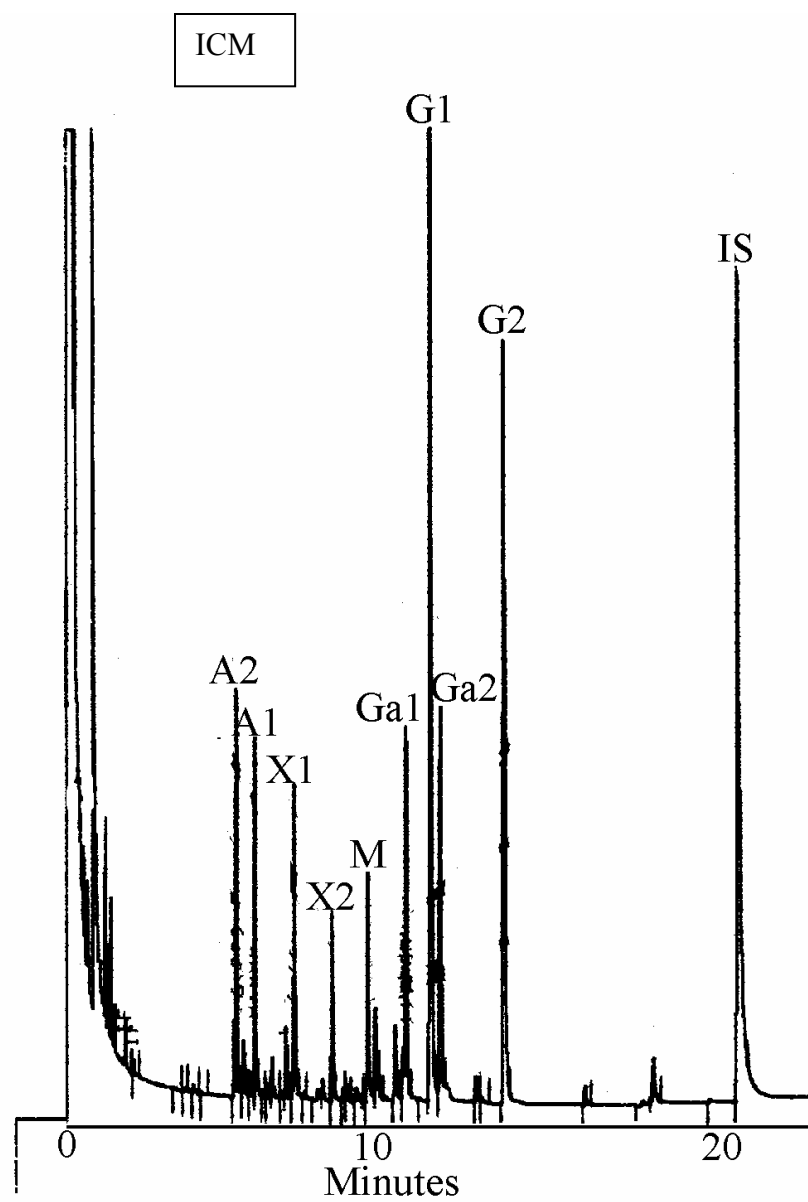
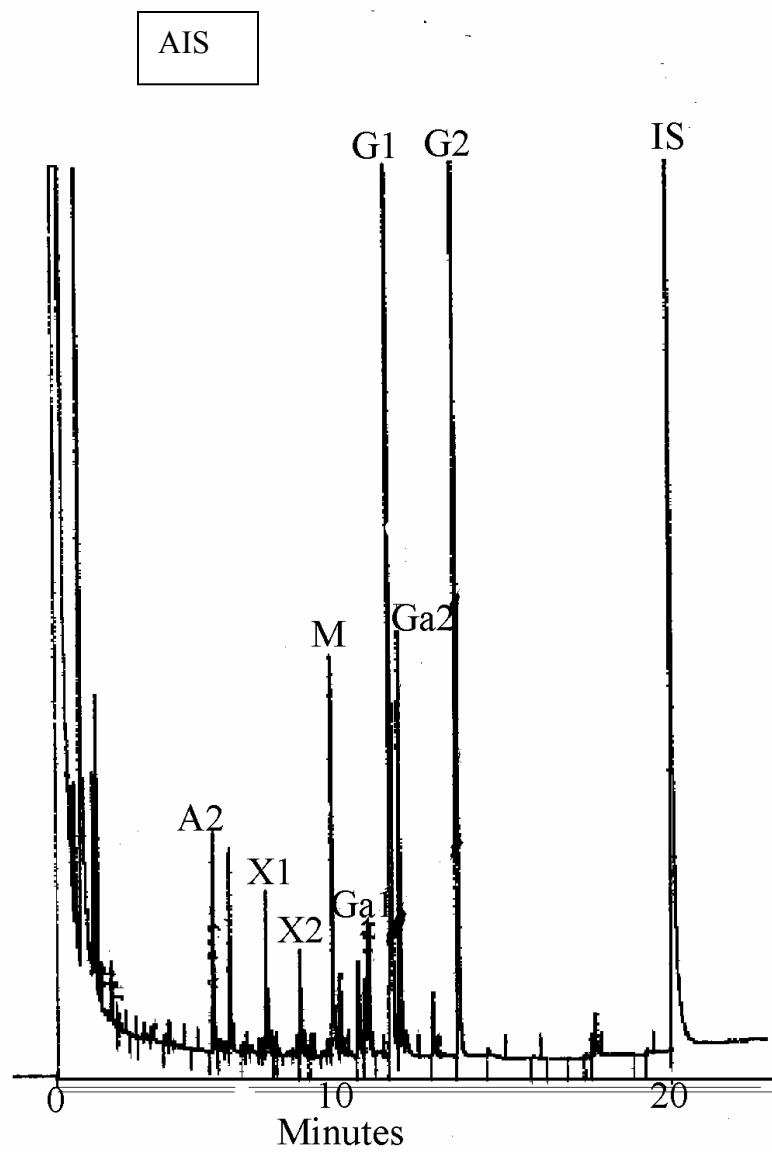


FIGURE 1 c. CONTENTS OF NEUTRAL SUGARS IN ALCOHOL INSOLUBLE SOLIDS (AIS)
AND INSOLUBLE CLOUD MATTER (ICM) OF MANGO PULP
(refer to page 87 for legend)

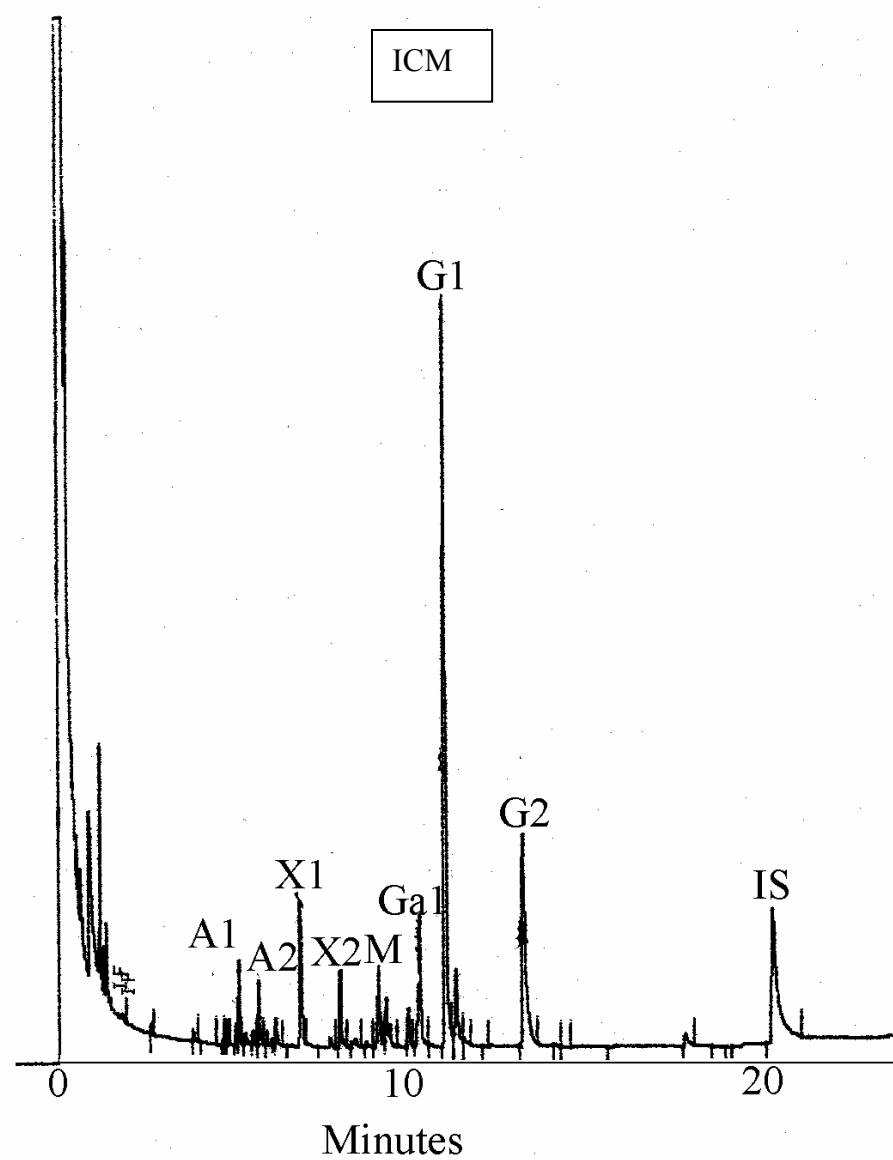
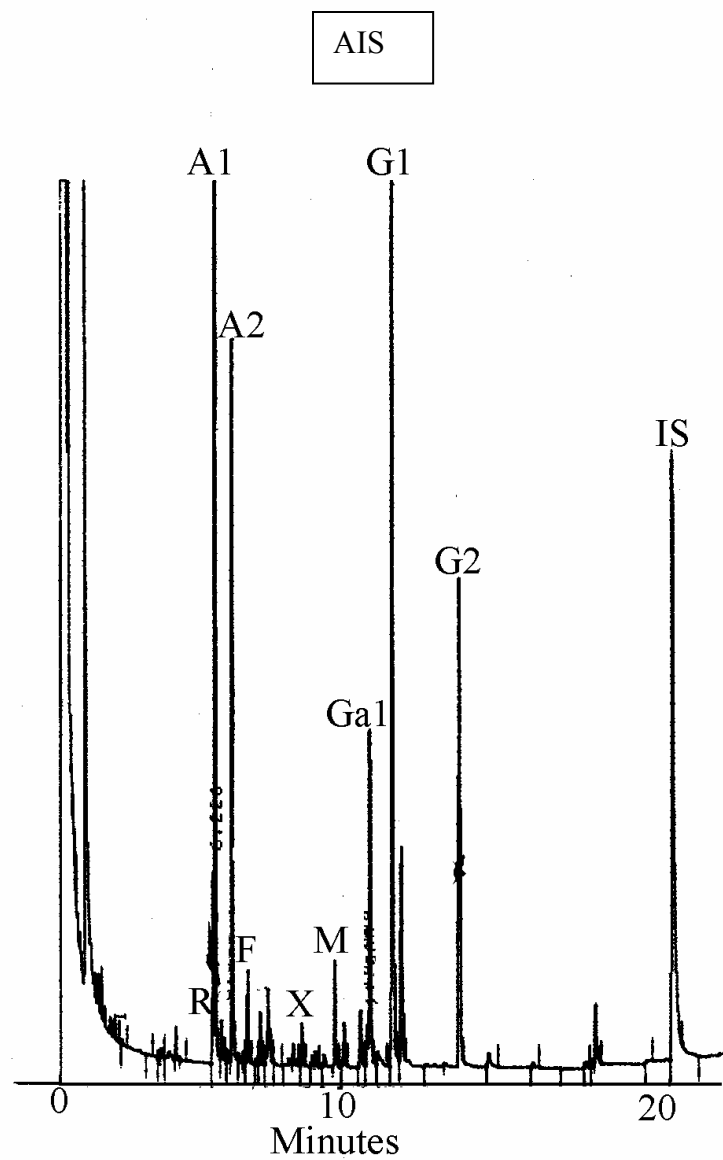


FIGURE 2.

DENATURING ELECTROPHORESIS (SDS-PAGE) PROFILES OF PROTEINS OF ALCOHOL INSOLUBLE SOLIDS (AIS) AND INSOLUBLE CLOUD MATTER (ICM) OF BANANA, MANGO (M) AND ORANGE (O) USING 4 TO 15% GRADIENT GEL AND THE PHASTSYSTEM^R. SAMPLES ARE S) STANDARD PROTEINS MARKERS, OI) ORANGE ICM, OA) ORANGE AIS, MI) MANGO ICM, MA) MANGO AIS, BI) BANANA ICM AND BA) BANANA AIS.

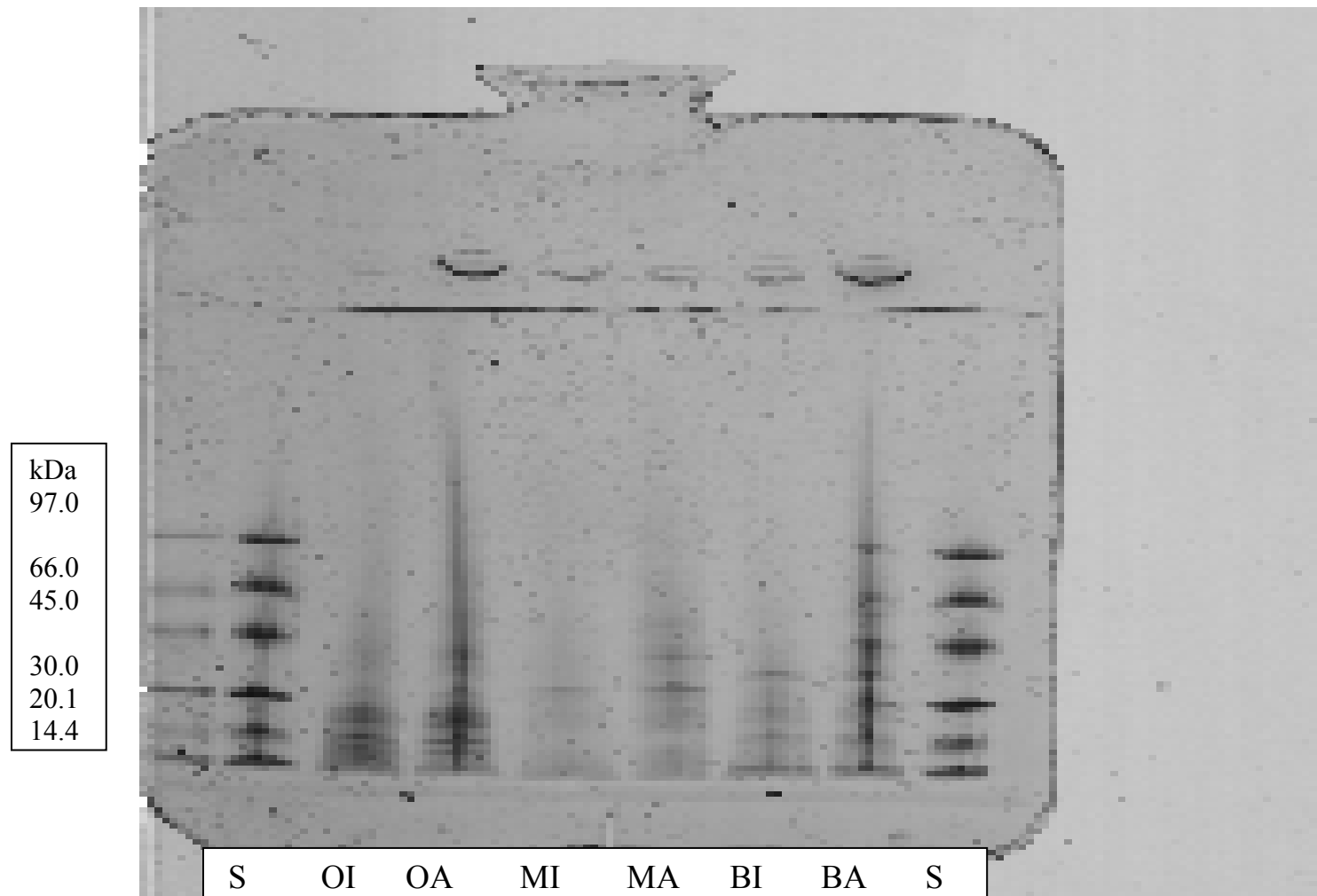


TABLE 1.
NEUTRAL SUGAR COMPOSITION (mg/100 mg) OF BANANA, ORANGE AND MANGO ALCOHOL
INSOLUBLE SOLIDS (AIS) AND INSOLUBLE CLOUD MATTER (ICM)

Sugar	Orange		Banana		Mango	
	AIS ^a	ICM ^b	AIS	ICM	AIS	ICM
Rhamnose	0.88 ± 0.0	2.78 ± 0.22	ND	ND	0.54 ± 0.03	ND
Arabinose	4.55 ± 0.81	4.68 ± 0.60	2.51 ± 0.03	5.05 ± 0.58	13.25 ± 0.03	2.41 ± 0.15
Xylose	1.39 ± 0.05	1.68 ± 0.50	2.10 ± 0.03	3.25 ± 0.88	1.23 ± 0.03	4.35 ± 0.07
Mannose	3.87 ± 0.09	2.25 ± 0.18	4.28 ± 0.05	4.02 ± 0.15	3.55 ± 0.03	4.84 ± 0.78
Galactose	10.40 ± 0.05	8.62 ± 1.19	5.06 ± 0.09	7.02 ± 0.59	7.44 ± 0.03	5.37 ± 0.27
Glucose	3.43 ± 0.10	19.90 ± 1.71	25.70 ± 0.71	24.90 ± 2.21	25.4 ± 0.03	26.4 ± 0.71
Fucose	ND ^c	ND	ND	ND	1.14 ± 0.25	ND

AIS^a alcohol insoluble solids. ICM^b insoluble cloud matter. ND^c not detected

TABLE 2.
TOTAL PHENOLIC CONTENTS, GALACTURONIC ACID, DEGREE OF ESTERIFICATION,
RATIO OF GALACTURONIC ACID TO RHAMNOSE OF BANANA, ORANGE AND
MANGO ALCOHOL INSOLUBLE SOLIDS

Fruit	^a Gal A (%)	^b DE (%)	Alcohol insoluble solids		^e Total phenolics
			^c Gal A/Ns	^d Gal A/Rha	
Banana	4.5 ± 0.03	85 ± 2	0.1 ± 0.0	ND	139 ± 1
Orange	5.6 ± 0.03	85 ± 6	0.2 ± 0.0	6.4 ± 0.2	102 ± 8
Mango	6.3 ± 0.03	78 ± 5	0.1 ± 0.0	11.6 ± 0.4	15 ± 2

^aContent of galacturonic acid mg/100 mg dry matter. ^bDegree of esterification. ^cRatio of galacturonic acid to neutral sugars. ^dRatio of galacturonic acid to rhamnose. ^eTotal phenolic content expressed in mg gallic acid equivalents/L. ND none determined

TABLE 3.
AMINO ACIDS PROFILE OF BANANA, MANGO AND ORANGE ALCOHOL INSOLUBLE SOLIDS AND
INSOLUBLE CLOUD MATTER^a (mg/g)

Amino acids	Banana				Mango				Orange			
	AIS ^b	AA/Tot	ICM ^c	^d AA/Tot	AIS	AA/Tot	ICM	AA/Tot	AIS	AA/Tot	ICM	AA/Tot
Pro	8.5	6.1	7.83	6.2	4.98	5.8	1.72	4.8	12.26	5.3	9.79	7.0
Ser	6.31	4.6	5.95	4.7	3.87	4.5	1.59	4.5	11.09	4.8	6.69	4.8
Glu	11.04	8.0	10.65	8.5	8.01	9.4	3.61	10.1	19.42	8.4	12.68	9.1
Gly	5.8	4.2	5.52	4.4	3.38	4.0	1.48	4.1	8.22	3.6	4.97	3.6
His	7.07	5.1	5.26	4.2	2.49	2.9	1.07	3.0	5.71	2.5	3.51	2.5
Arg	10.14	7.3	7.63	6.1	4.48	5.2	2.05	5.7	23.17	10.0	11.21	8.0
Thr	5.96	4.3	5.65	4.5	3.75	4.4	1.58	4.4	9.6	4.2	5.68	4.1
Ala	9.01	6.5	8.24	6.6	5.79	6.8	2.54	7.1	13.55	5.9	8.29	5.9
Asp	19	13.7	18.09	14.4	11.46	13.4	4.85	13.6	37.4	16.2	22.06	15.8
Lys	9.33	6.7	8.83	7.0	8	9.4	3.36	9.4	19.46	8.4	12.09	8.6
Tyr	5.15	3.7	3.81	3.0	2.73	3.2	1.02	2.9	7.49	3.2	4.28	3.1
Val	7.33	5.3	7.69	6.1	4.7	5.5	1.98	5.5	12.14	5.3	7.47	5.3
Met	2.29	1.7	2.06	1.6	1.4	1.6	0.62	1.7	3.3	1.4	2.36	1.7
Ile	6.68	4.8	6	4.8	4.21	4.9	1.79	5.0	10.33	4.5	6.32	4.5
Leu	12.28	8.9	11.46	9.1	7.92	9.3	3.25	9.1	18.12	7.9	11.77	8.4
Phe	5.27	3.8	4.76	3.8	3.22	3.8	1.33	3.7	8.31	3.6	5.05	3.6
Cysx ^e	7.44	5.4	6.33	5.0	5.08	5.9	1.93	5.4	11.03	4.8	5.8	4.1
Total	138.60		125.70		85.40		35.70		230.60		140.0	

^aThe data are mean values of triplicates and the % error was less than 10%. ^bAIS, alcohol insoluble solids. ^cICM, insoluble cloud matter. ^eCysx, cystine or cystine residues. ^dAA/Tot, ratio of amino acid to total amino acid multiplied

CHAPTER 4

INTERACTIONS OF WHEY PROTEINS WITH HIGH METHOXYL PECTINS IN ACIDIFIED BANANA BEVERAGES¹

¹Koffi, E.K., and Wicker, L. To be submitted to the Journal of Food Science.

Abstract

The interaction between whey proteins (10% w/w) and pectin (0.4%, w/w) was evaluated in acidified banana beverage model systems. Shear thinning water-pectin-whey-sucrose or banana juice-pectin-whey-sucrose dispersions had lower flow indexes ($n = 0.40 \pm 0.0$) than banana juice-pectin-sucrose or banana juice-whey-sucrose ($n = 0.98 \pm 0.01$). The consistency coefficients were higher for the four component systems ($K = 5.14 \pm 1.07 \text{ Pas}^n$ and $K = 8.10 \pm 2.26 \text{ Pas}^n$), than the three component systems ($K = 0.10 \pm 0.01 \text{ Pas}^n$ and $K = 0.04 \pm 0.0 \text{ Pas}^n$). The average particle size was not different among the three or four component systems ($D_{4,3} \cong 27 \mu\text{m}$) except for the water-pectin-whey-sucrose dispersion ($D_{4,3} \cong 106 \mu\text{m}$).

Keywords: whey proteins, polysaccharide-protein complexes, flow behavior, consistency index, particle size.

Introduction

Interactions of proteins and polysaccharides have a significant impact on rheology of beverages (Dickinson 1998; Syrbe and others 1998; Wang and Qvist 2000; Mishra and others 2001). As food thickening and gelling agents, as well as stabilizers for emulsions and foams, proteins and polysaccharides play key structural functions in food systems (Tolstoguzov 1991). They prevent emulsion separation during storage and contribute to the mouthfeel of products. Interaction between proteins and polysaccharides may result in their co-solubility, incompatibility and complex coacervation (Syrbe and others 1998). Attractive or repulsive interactions between milk proteins and polysaccharides are influenced by the chemical structure, molecular weight, protein/polysaccharide ratio, nature and density of charges, solution conditions such as pH and ionic strength (Dickinson 1998; Girard and others 2002).

Dairy products with pH lower than 5, are produced by acidification or bacterial fermentation. The addition of pectin to acid dairy beverages results in smoother texture and less tendency to sediment. High methoxyl pectins and small particle size prevent undesired flocculation of proteins during heat pasteurization and minimize whey separation (Glahn 1982; Parker and others 1994). High methoxyl pectin (HMP) is typically used to stabilize beverages especially at pH values between pH 3.7 and 4.2 where the casein and pectin have opposite net charges (Glahn 1982; Kravtchenko and others 1995, Dickinson 1998). At pH values near 4.0 the pectins are negatively charged and attract the positively charged milk proteins ($\text{pH} < \text{pI}$).

Electrostatic interaction of the whey proteins and pectins keeps them suspended in stable acidified dispersions at pH below the isoelectric point of β -lg (Wang and Qvist 2000, Girard and others 2002,). In aqueous dispersions (pH 3.5), β -lg (8-10% w/w) and HMP (3-4%) form soluble electrostatic complexes, which further associate into large aggregates via non-specific

interactions (Wang and Qvist 2000, Kazmierski and others, 2003). Under the same conditions, the viscosity of the HMP solution is significantly increased by the addition of β -lg (Wang and Qvist 2000).

Banana juice with excellent banana flavor is obtained by adding water to the banana puree before juice extraction by pressing (Kyamuhangire and others 2002). Compared to the enzymatic methods described previously (Koffi and others 1991, Viquez and others 1981), the mechanical method to produce banana juice is faster with lower energy input.

Whey-fruit beverages are typically made by mixing whey with fruit juice or fruit juice concentrate (Holsinger and others 1974). Interaction between whey components and fruit components (pectin, tannins, starch) results in turbidity, cloudiness, high viscosity and sedimentation (Devkota 1991). In whey-banana shakes, addition of xanthan gum (0.2% w/w) stabilized the dispersion for 24 hours (Shekilango and others 1997).

The development of a nutritional beverage using whey proteins and surplus bananas could be a better way to improve the nutritional status of populations in banana producing countries. The proteins in whey are of superior quality because they contain essential amino acids (McIntosh and others 1998). Understanding of the interactions between ingredients in the banana beverages is needed for optimization of formulations. The objective of this study was to characterize the physical properties of model systems of banana juice, water, whey protein concentrate (WPC), HMP and sucrose.

Materials and methods

Materials

Whey protein concentrate (80% protein) was obtained from Protient (Lot code LUV 1225 St. Paul, MN). High methoxyl pectin (50% uronic acid, 80% DE, Genu pectin type JMJ) was

provided by Hercules (Wilmington, DE) and acidified banana puree was donated by Chiquita (Chiquita Brands, Inc., Cincinnati, OH).

Preparation of banana juice

Banana juice (7 °Brix) was prepared from acidified banana puree with 1 kg of banana puree and 2 kg of deionized water. The ingredients were mixed for 10 min (Fisher Scientific, Dyna-Mix Pittsburgh, PA) and centrifuged at 1000 xg for 10 min (Sorvall RC-5B centrifuge; Dupont Instruments, Doraville, GA) and the pH of the supernatant was adjusted to pH 4.0 with 20% citric acid solution (see appendix for juice preparation flow chart).

Preparation of model dispersions

Sucrose was mixed with high methoxyl pectin and slowly added to banana juice under continuous mixing (Fisher Scientific, Dyna-Mix, Pittsburgh, PA). Whey protein concentrate was added gradually and pH of dispersions was re-adjusted to 4.0 using 20% citric acid. The dispersions were chilled to 4 °C and homogenized at room temperature (Gaulin Rannie APV Homogenizer Group, Wilmington, MA) at 3000 psi. The composition of the model system is shown in Table 1.

Particle size measurements

The particle size distribution of the model systems was determined by laser diffraction using a Malvern Mastersizer (Worcestershire, UK). Samples were introduced in the sample unit containing deionized water and pumped through the optical cell (code for optical properties of the particles, “presentation”, 3OHD) while stirring at 2,000 rpm with an obscuration of 20-30% as described by Barnes (2001). Size distribution as volume and surface area mean diameters were estimated and expressed as $D_{4,3}$ and $D_{3,2}$, respectively.

Rheological measurements

A Dynamic Stress Rheometer (Rheometrics, Piscataway, NJ) was used to determine the flow behavior and consistency indexes of the model systems. The couette geometry (cup diameter = 32.0 mm, bob diameter = 29.5 mm, and bob length = 44.5 mm) was used. A steady stress sweep test was conducted to determine the flow behavior data at 10 °C with an initial and final rate of 10 (sec⁻¹) and 100 (sec⁻¹) respectively with a measurement time of 10 seconds at each shear rate (Barnes 2001).

Statistical analysis

All experiments were conducted in duplicate and differences between treatment means were determined by Duncan's procedure at $p < 0.05$ using the Statistical Analysis System software (SAS Inc, NC).

Results and discussion

Banana juice with 0.4% high methoxyl pectin and 15% sucrose at pH 4.0 has close to Newtonian behavior with flow index of 0.98 (Figure 1). The consistency index is related to the magnitude of the viscosity (Morrison 2001). The consistency index of 0.10 Pasⁿ (Table 2) indicated that the dispersion had a low viscosity. High methoxyl pectin dispersions, up to approximately 0.5%, are nearly Newtonian and are not affected by the presence of calcium (Rolin and de Vries 1990).

The flow behavior of banana juice, 10% WPC and sucrose at pH 4.0 also exhibited near Newtonian behavior ($n = 0.98$) and the consistency index was low ($K = 0.04$ Pasⁿ) (Table 2). Khalil and others (1989) reported a near-Newtonian behavior for depectinized clarified and filtered banana juice over a wide range of concentrations (20-79.7° Brix) and temperatures (30-

70°C). Clarified fruit juice free from pulp in suspensions such as apple, pineapple and raspberry exhibit near-Newtonian flow (Krokida 2001).

WPC is a low viscosity protein (Morisson and Mackay 2001). Mohammad and Wiley (1996) found that whey protein concentrate solutions ($\leq 10\%$ w/w) exhibit near-Newtonian behavior at pH values of 4-8 and temperature range of 5-60 °C. The consistency index of banana juice, whey, and sucrose is lower (0.04 Pas^n) than that of the banana juice, pectin and sucrose mixture (0.04 Pas^n).

The plot of shear stress vs. shear rate for banana juice, 10% whey protein, 0.4% pectin and sucrose is depicted in Fig 1. The flow behavior index is 0.39 ± 0.06 . The dispersions had a high consistency index ($K = 8.10 \pm 2.2 \text{ Pas}^n$) and were shear thinning due to the reduction in the size of colloidal aggregates as the shear rate increased (Campanella and others 1995). A similar effect has been observed for other hydrocolloids, like carrageenan in sucrose added (17.5 °Brix) milk dispersions where the flow characteristics were $K = 6.5\text{-}13.2 \text{ mPas}^n$, $n = 0.71\text{-}0.98$ and $K = 70\text{-}145 \text{ mPas}^n$, $n = 0.56\text{-}0.68$ for κ -carrageenan and milk- κ -carrageenan solutions, respectively (Yanes and others 2002). The authors attributed the increase in pseudoplasticity to the formation of carrageenan casein micelle aggregates. High viscosity of about 100 s (as measured with an Ostwald viscometer) was observed in a model system consisting of pectin and bovine serum albumin (used as tomato protein analogue) at the same concentration as those found in tomato products at 10 °Brix (Takada and Nelson 1983). The authors concluded that pectin-protein interaction influences consistency of tomato products. Further, the sensitivity of the viscosity of the model tomato system to pH indicated electrostatic complex formation of pectin and protein. Presence of greater number of high molecular weight complexes (whey proteins and pectins) increase resistance to flow which, in turn increases the consistency index. Shear thinning is due

to the alignment of these complexes in the direction of the flow (Coia and Stauffer 1987). The apparent pKa for the galacturonic acid residues reported is 3.23 and the isoelectric points (pI) of the major whey proteins are in the region 4.5-5.2 (Zaleska and others 2000). At pH 4.0 ($< pI$) the proteins are positively charged while the pectin is slightly negatively charged thus an ionic interaction is likely between pectin and whey protein. Due to the lower proportion of carboxyl groups, high methoxyl pectins have a smaller region to interact with proteins, thus a more substantial portion of the molecule will interact with the solvent to increase its viscosity (Haylock and others 1995; Dickinson 1998; Pereyra and others 1997). Shear thinning in model acid milk drinks (from low fat milk) was caused by pectin adsorbed onto the casein aggregates in acid milk dispersions at pH 4 (Amice-Quemeneur and others 1994; Parker and others 1994). The shear thinning behavior observed with whey proteins dispersion is likely due to pectin-whey-sucrose interaction.

When water was substituted for banana juice (Figure 1) the dispersion remained pseudoplastic and the flow behavior and consistency indexes were $n = 0.45 \pm 0.04$ and $K = 5.14 \pm 1.07 \text{ Pas}^n$, respectively. It can be assumed that pectin-protein interaction contributed more to the rheology of the system than the protein-banana juice or pectin-banana juice because there was no significant change ($p > 0.05$) in the flow behavior and consistency indexes of dispersions in the presence of banana juice or water.

Bimodal distribution of particle size was obtained for the banana juice-pectin-sucrose dispersion (Figure 2). The volume and surface area mean diameters of particles were approximately $34 \mu\text{m}$ and $2 \mu\text{m}$, respectively. However, a unimodal distribution was observed for the banana juice-whey-sucrose dispersion with volume mean diameter values near $28 \mu\text{m}$. Chang and Powell (1994) have studied the effect of a bimodal size distribution on the rheological

behavior of concentrated suspensions using polystyrene spheres and polymethyl methacrylate beads sieved to obtain particle fractions in various size ranges. These simulation studies showed that at a fixed volume fraction, concentrated suspension with a bimodal size distribution of particles have lower shear viscosity than suspensions with a unimodal size distribution of particles. Our data show that the addition of whey protein to pectin shifted the particle size distribution from bimodal to unimodal with an increase in consistency from 0.10 to 8.10 Pasⁿ. (Figure 2, Table 2). Compared to banana juice-whey-sucrose dispersion, the particle size of banana juice-whey-pectin-sucrose was very similar (Figure 2) even though the consistency was higher and the flow behavior index was lower in the latter (Table 2). Whey contributes primarily to smaller, more uniform particle size. When water is substituted for banana juice in four component system, no significant change in flow behavior and consistency indexes was observed, but a large shift in particle size to 106 μm was observed. Thus, the loss of Newtonian behavior in the 4 component system is not due to banana juice but to pectin-whey components interactions.

In a dispersion of water, whey, pectin and sucrose a bimodal particle size distribution was obtained and $D_{3,2}$ and $D_{4,3}$ values of 106 and 5 μm , respectively (Figure 2). In the absence of sucrose, water-whey-pectin dispersion displayed a more unimodal distribution and smaller particle size ($D_{4,3} = 18 \mu\text{m}$) (Figure 2, Table 2). In the presence of sucrose the volume mean diameter of particles in the 4 component system (water-whey-pectin-sucrose) increased significantly ($106 \pm 6 \mu\text{m}$) compared to $18 \pm 3 \mu\text{m}$ for the non-sucrose containing system (Table 2). This increase in mean diameter in the presence of sucrose is likely to be due to pectin-pectin or sucrose-pectin interactions. Aggregate formation of pectin molecules due to cross-linking hydrogen bonds between the hydroxyl groups of pectin and those of sucrose (20%) have been

observed in pectin-sucrose solutions (Chen and Joslyn 1967; Michel and others 1984). In concentrated lemon juice low pH levels combined with high sugar concentrations (40 °Brix) promoted pectin-pectin attraction and loss of cloud due to irreversible flocculation (Epstein and Mizrahi 1975).

Conclusions

Consistency and flow behavior indexes of HMP-WPC dispersions increased drastically suggesting an interaction between the two components. Pectin-protein interaction plays a key role in the rheology of the whey-banana beverage. Whey protein, unlike pectin, contributes to particle size in similar fashion in three or four component systems. The loss of Newtonian behavior is not due to banana juice but to pectin-whey components and their interaction. Particle size is bigger in water. Sucrose increases particle size and decreases flow behavior index. Pectin-whey interaction is due to electrostatic as well as hydrogen bonding.

References

- Amice-Quemeneur N, Haluk JP, Hardy J. 1995. Influence of the acidification process on the colloidal stability of acidic milk drinks prepared from reconstituted nonfat dry milk. *J Dairy Sci* 78:2683-2690.
- Barnes BW. 2001. Pectin stabilization of acid milk dispersions. MS thesis, Athens, Georgia: Univ. of Georgia. 140 p.
- Campanella HO, Dorward NM, Singh H. 1995. A study of the rheological properties of concentrated food emulsions. *J Food Eng* 25:427-440.
- Chang C and Powell RL. 1994. Effect of particle size distribution on the rheology of concentrated bimodal suspensions. *J Rheol* 38: 85-98.
- Chen TS and Joslyn MA. 1967. The effect of sugars on viscosity of pectin solutions. I. Comparison of corn syrup with sucrose solutions. *J Coll Interface Sci* 23:399-406.
- Coia, KA and Stauffer, KR. 1987. Shelf life study of oil/water emulsions using various commercial hydrocolloids. *J Food Sci* 52:166-172.

- Devkota K. 1991. Protein-pectin interactions in cottage cheese whey. MSc thesis, University of Alberta, Canada 202 p.
- Dickinson E and Galazka VB. 1991. Emulsion stabilization by ionic and covalent complexes of beta-lactoglobulin with polysaccharides. *Food Hydrocoll* 5:281-296.
- Dickinson E. 1998. Stability and rheological implications of electrostatic milk protein-polysaccharide interactions. *Trends Food Sci Technol* 9:347-354.
- Epstein D, Mizrahi S. 1975. Effect of concentration process on cloud stability of reconstituted lemon juice. *J Sci Fd Agric* 26:1603-1608.
- Girard M., Turgeon SL, Gauthier SF. 2002. Interbiopolymer complexing between β -lactoglobulin and low-and high-methylated pectin measured by potentiometric titration and ultrafiltration. *Food Hydrocoll* 16:585-591.
- Glahn PE. 1982. Hydrocolloid stabilization of protein suspensions at low pH. *Progr. Food Nutr. Sci* 6:171-177.
- Hansen PMT, Hidalgo J, Gould IA. 1971. Reclamation of whey protein with carboxymethylcellulose. *J Dairy Sci.* 54:830-834.
- Haylock SJ, Towler C, Hewitt SA. 1995. Dairy Component Interactions in food Products. In: A.G. Gaonkay editor. *Ingredient interactions effects on food quality*. New York: Marcel Dekker. p 295-320.
- Holsinger VH, Posati LP, DeVilbiss ED. 1974. Whey beverages: A Review. *J Dairy Sci* 57:849-859.
- Kazmierski M, Wicker L, Corredig M. 2003. Interactions of β -Lactoglobulin and high-methoxyl pectins in acidified systems. *J Food Sci* 68:1673-1678.
- Khalil KE, Ramakrishna P, Nanjundaswamy AM, Patwardhan MV. 1989. Rheological behavior of clarified banana juice: effect of temperature and concentration. *J Food Eng* 10:231-240.
- Koffi EK, Sims CA, Bates RP. 1991. Viscosity reduction and prevention of browning in the preparation of clarified banana juice. *J Food Qual* 14:209-218.
- Kravtchenko TP, Parker A, Trespoey A. 1995. Colloidal stability and sedimentation of pectin-stabilized acid milk drinks. In: E. Dickinson, D. Lorient, editors. *Food Macromolecules and Colloids*. Cambridge: The Royal Society of Chemistry. p 349-355.
- Krokida MK, Maroulis ZB, Saravacos GD. 2001. Rheological properties of fluid fruit and vegetable puree products: compilation of literature data. *Int J Food Prop* 4:179-200.

- Kyamuhangire W, Myhre, H, Sorensen, HT, Pehrson R 2002. Yield, characteristics and composition of banana juice extracted by the enzymatic and mechanical methods. *J Sci Food Agric* 82:478-482.
- McIntosh GH, Royle PJ, Le Leu RK, Regester GO, Johnson MA, Grinsted, RL, Kenward RS and Smithers GW. 1998. Whey proteins as functional food ingredients. *Int Dairy J* 8:425-434.
- Michel F, Thibault JF, Doublier JL. 1984. Viscometric and potentiometric study of high-methoxyl pectins in the presence of sucrose. *Carbohydr. Polym* 4:283-297.
- Mishra S, Mann B, Joshi VK. 2001. Functional improvement of whey protein concentrates on interaction with pectin. *Food Hydrocoll* 15: 9-15.
- Mohammad RA and Wiley D. 1996. Non-Newtonian behavior of whey protein solutions. *J Dairy Res* 63:315-320.
- Morrison FA 2001. Generalized Newtonian fluids. In: G. F. Keith editor. *Understanding rheology*. New York, NY: Oxford University Press. p 230.
- Morison KR, Mackay FM. 2001. Viscosity of lactose and whey protein solutions. *Int J of Food Prop* 4:441-454.
- Parker A, Boulenger P, Kravtchenko TP. 1994. Effect of the Addition of High Methoxy Pectin on the Rheology and Colloidal Stability of Acid Milk Drinks. In *Food Hydrocolloids: Structures, Properties and Functions*; Nishinari, K.; Doi, E., eds.; Plenum Press: New York, NY
- Pereyra R, Schmidt KA, Wicker L. 1997. Interaction and stabilization of acidified casein dispersions with low and high methoxyl pectins. *J Agric Food Chem* 45:3448-51.
- Rolin C, De Vries J. 1990. Pectin. In: H. Peter, editor. *Food Gels*. Elsevier applied science. p 401-434.
- Shekilango A, Jelen P, Bagdan GC. 1997. Production of whey-banana beverages from acid whey and overripe bananas. *Milchwissenschaft* 52:209-212
- Syrbe A, Bauer WJ, Klostermeyer H. 1998. Polymer science concepts in dairy systems-an overview of milk protein and food hydrocolloid interaction. *Int Dairy J* 8:179-193.
- Takada N and Nelson PE. 1983. Pectin-protein interaction in tomato products. *J Food Sci* 48:1408-1411.
- Tolstoguzov VB. 1991. Functional properties of food proteins and role of protein-polysaccharide interaction. *Food Hydrocoll* 4:429-468.
- Viquez F, Laestro C, Cooke RD. 1981. A study of the production of banana juice using pectinolytic enzymes. *J Food Technol* 16:115-125.

Wang Q and Qvist KB. 2000. Investigation of the composite system of β -lactoglobulin and pectin in aqueous solutions. *Food Res Int* 33:683-690.

Yanes M, Duran L, Costell E 2002. Effect of hydrocolloid type and concentration on flow behavior and sensory properties of milk beverages model systems. *Food Hydrocoll* 16:605-611.

Zaleska H, Ring SG, Tomasik P. 2000. Apple pectin complexes with whey protein isolate. *Food Hydrocoll* 14:377-38

Table 1. Composition of whey-banana beverage model systems

Ingredients	Banana juice + 0.4% pectin	Banana juice +10% whey	Banana juice + 0.4% pectin +10% whey	Water + 0.4% pectin +10% whey	Water + 0.4% pectin +10% whey + sucrose
Banana juice (%)	84.6	75	74.6	0	0
Water (%)	0	0	0	89.6	74.6
Pectin (%)	0.4	0	0.4	0.4	0.4
Sucrose (%)	15	15	15	0	15
Whey (%)	0	10	10	10	10

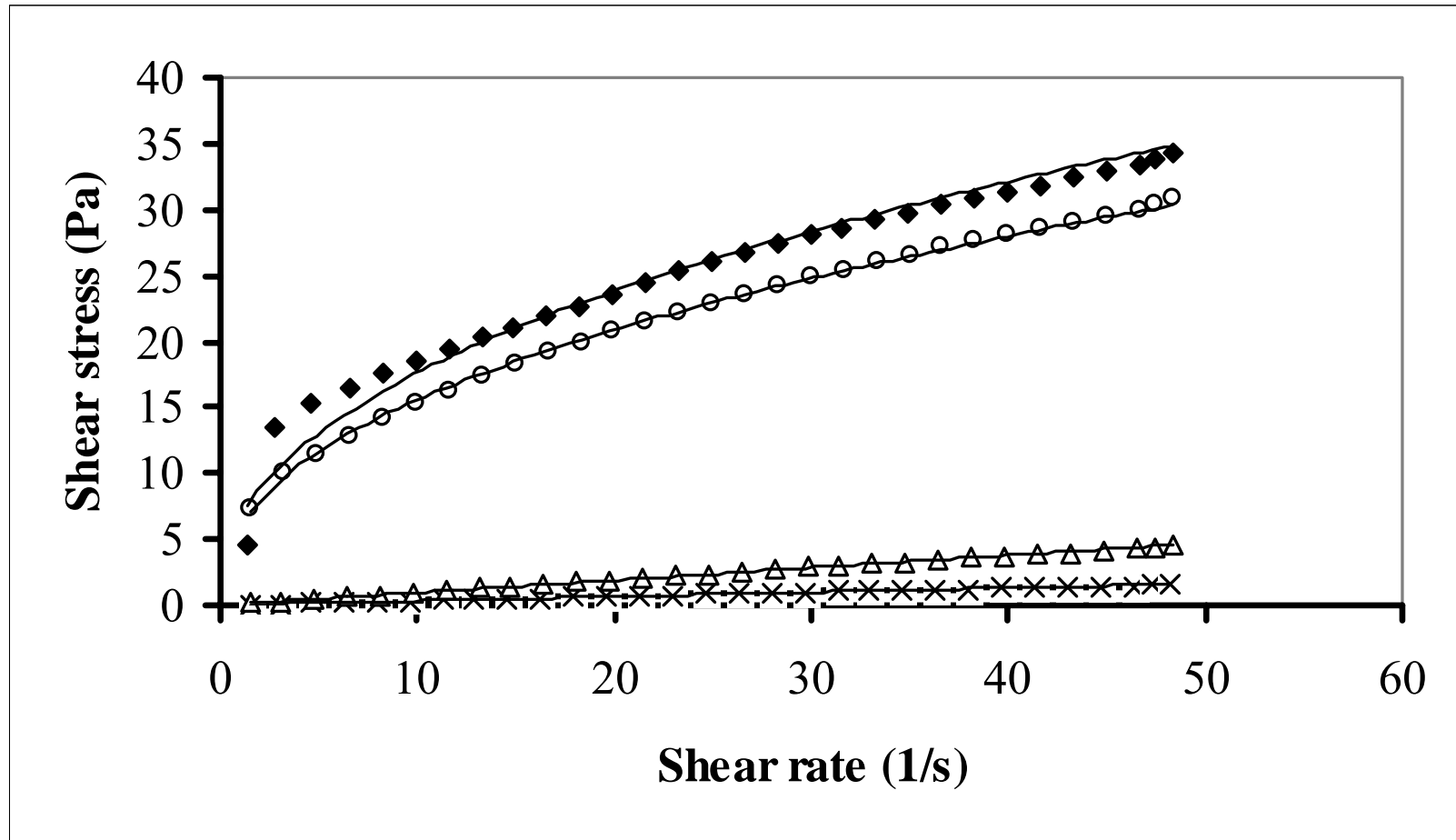


Figure 1-Flow curve for model systems: (■) banana juice, 10% whey, 0.4% pectin with 15% sucrose; (○) water, 10% whey, 0.4% pectin with 15% sucrose; (Δ) banana juice, 0.4% pectin with 15% sucrose; (×) banana juice, 10% whey with 15% sucrose. Experimental data points and power law fitting curves.

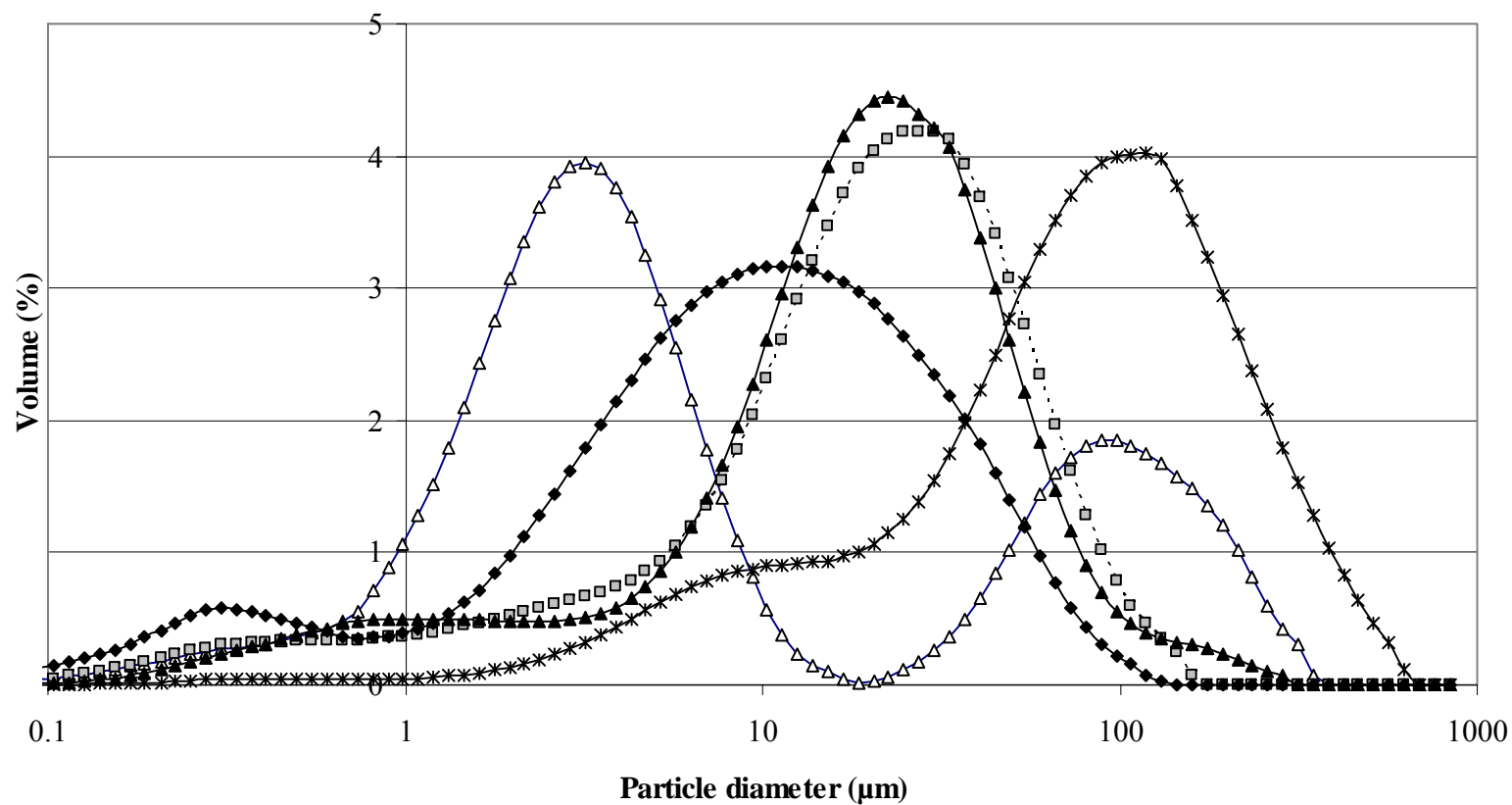


Figure 2-Average particle size distribution of the model systems

- △— Banana juice-pectin (0.4%)-sucrose (15%) - - - □ - - - Banana juice-whey (10%)-sucrose (15%)
- ▲— Banana juice-whey (10%)-pectin (0.4%)-sucrose (15%) —*— Water-whey (10%)-pectin (0.4%)-sucrose (15%)
- ◆— Water-whey (10%)-pectin (0.4%)

Table 2-Flow behavior indexes, consistency coefficients and particle sizes of model systems.

Sample designation	Flow behavior index	Consistency coefficients (Pas ⁿ)	Surface area mean diameter D _{3,2} (μm)	Volume mean diameter D _{4,3} (μm)
<i>3 component systems</i>				
Banana juice + 0.4% pectin + sucrose	0.98A	0.10C	2.2C	34.5B
Banana juice +10% whey + sucrose	0.98A	0.04C	3.6B	27.6B
<i>4 component systems</i>				
Banana juice +10% whey + 0.4% pectin + sucrose	0.39C	8.10B	4.3A	26.4B
Water +10% whey +0.4% pectin + sucrose	0.45C	5.14B	4.6A	106A
Water +10% whey +0.4% pectin Without sucrose	0.63B	0.47C	3.6D	18C

Means separation done by Duncan's procedure at $p < 0.05$. Means in a column with same letter are not significantly different

CHAPTER 5
STORAGE STABILITY AND SENSORY ANALYSIS OF UHT PROCESSED WHEY-
BANANA BEVERAGES¹

¹Koffi, E.K., Shewfelt, R.L. and Wicker, L., To be submitted to The Journal of Food Quality

Abstract

Selected characteristics of whey fortified banana beverages stored at 4, 20, 30 and 40C were monitored at specific time intervals over a 60-day storage period. The particle volume mean diameter (D_{43}) remained unchanged for the entire study. However the particle surface area mean diameter (D_{32}) changed only in the first week. The products remained light colored ($L^* \sim 71$) for 60 days, redness (a^*), yellowness (b^*) and saturation (C^*) increased with time at elevated storage temperatures. The Hue (H^*) was smaller at elevated temperatures. A sensory descriptive analysis panel generated terms to describe and quantify the sensory characteristics of the whey-banana beverage stored at 4C. The product was a sour, sweet, smooth beverage with distinctive banana flavor and minimum off-flavor. A consumer panel indicated that sourness and acidity were critical quality factors. Main differences were detected for sedimentation (greater at 40C) and serum separation (minimum at 4C).

Keywords: whey proteins, sensory analysis, whey-banana beverages, color dimensions, storage study

Introduction

Heat processing of whey beverage is required to eliminate microorganisms to make it suitable for human consumption. However, whey components undergo a number of heat induced physical and chemical changes, the magnitude of which is linked to the reaction conditions (pH, concentration, temperature, ionic strength) (de Wit 1981, Euston 2000, de la Fuente et al. 2002, Alting et al. 2000, Law and Leaver 2000, Lupano 1994, Jelen and Buccheim 1984, Rattray and Jelen 1997). Whey proteins aggregate at temperature above 60°C due to the heat sensitivity of β -lactoglobulin, α -lactalbumin and bovine serum albumin (BSA), all of which are ordered globular proteins (Wong et al. 1996, Euston et al. 2002).

Aggregation of whey proteins produces large particles that settle in whey drinks. Other components of the fruit also interact with proteins to induce sediments in protein fortified beverage. Shekilango et al. (1997) found that a blend of 3 parts (w/w) acid whey to 2 parts (w/w) banana was the most acceptable formula in terms of flavor, texture and viscosity. However, the beverage separates immediately when left standing due to protein interaction between fruit constituents, such as the banana pectins and tannins. The failure of whey-based beverages to perform well on the market is related to sedimentation problems (Jelen et al. 1987).

To overcome the sedimentation problems, high methoxyl pectins are used in acidified milk beverages (Parker et al. 1994). Processing parameters, including pH, temperature and homogenization, affect the stability of the milk beverages (Dickinson 1998). Steric stabilization of acid milk beverages at low pH by pectin is due to complexation of pectin with casein particles (Kravtchenko et al. 1995). Glahn and Rolin (1994) reported that the production of small casein particles and the presence of pectin above a critical pectin level prevent whey separation.

The high content of lactic acid in acid whey is associated with an undesirable flavor and protein aggregation in whey drinks so that researchers incorporate fruits like citrus and lemon to ameliorate the acid flavor note (Holsinger et al., 1974). Various other fruits have been used including mango, pineapple, guava, peach, passion fruit, apricot and kiwi (Jelen, 1992). Sensory evaluation of food is the only practical method available to investigate the human perception of flavor. However nutritious a food product, food choice is influenced by cultural, sensory, economic, environmental, and family factors (Hendricks and Badruddin 1992, Shepherd 1988). Further, to ensure product success in the marketplace consumer acceptance has to be considered.

In banana producing countries where malnutrition prevails, development of a nutritional shelf stable whey-banana beverage could be a way to utilize surplus banana fruit. Whey proteins possess more sulfur-containing amino acids than casein and a surplus of the essential amino acids, which are often limited in plant proteins (McIntosh et al. 1998). The objective of this research was to develop a cloud stable whey-banana beverage from sweet whey protein concentrate. Physical, chemical, and sensory analyses of the beverages stored at different temperatures were conducted to determine the effect of temperature and storage time on the stability of whey-banana beverage.

Materials and Methods

Materials

Whey protein concentrate (80% protein) was obtained from Protient (Lot code LUV 1225 St. Paul, MN). High methoxyl pectin (50% uronic acid, Genu pectin type JMJ) was provided by Hercules (Wilmington, DE) and Chiquita (Chiquita Brands, Inc., Cincinnati, OH) donated the acidified banana puree.

Preparation of whey-banana beverages

The formulation for the whey-banana beverages given in Table 1 was developed based on optimization results to prevent heat gelation (see appendix for optimization of amount of pectin results). A blend of 1 part of banana puree to 2 parts of acidified (20 % citric acid to pH 4.0) water was prepared in a surge tank with agitator and aseptic homogenizing valve of the N0-BAC Unitherm IV processing system (Cherry-Burrell Amc International, IA). Sucrose (15% w/w) and pectin (0.15% w/w) were mixed and added to the blend while stirring. The mixture was homogenized at 3000 psi and heated for 5-7s at 140C. The UHT processed beverages were filled into pre-sterilized 450 ml clear glass bottles under steam flush and stored in the dark at 4C, 20C, 30C and 40C for 1, 7, 15, 30 , 45 and 60 days for further analyses (see appendix for process flow chart).

Descriptive Analysis

A descriptive panel consisted of 12-14 students and employees from the Food Science and Technology Department at the University of Georgia. The judges were selected based on willingness to consume milk beverages and no history of negative allergic reactions. Panelists were further screened using triangle tests and ability to determine varying intensity of selected descriptors generated by the panel for milk based fruit beverages. Participation in all sessions was required. The training sessions (1-2h) were held twice a week for two months.

Unstructured scaling consisting of a horizontal 15 cm line with two anchor points on both ends was used. Each anchor point was labeled with a word or expression. The panelists generated six descriptors for whey-banana beverage sensory analysis (Table 2). Reference standards were provided to the panelists for each descriptor developed (Table 3). The samples used in the sensory sessions were refrigerated (4C) and served just before analysis. Drinking

water was used for mouth rinsing and crackers were served between samples to minimize carry over effect. Each sensory attribute was evaluated on a separate line and panelists scored by making a vertical line across the horizontal line at the point that best reflected their perception of the magnitude of that property. The ratings were then converted to numerical scores by measuring the distance of the marks from the left end of the line in units of 0.1 cm. A score of 1 was equivalent of 1cm on graphical scale (Meilgaard et al., 1991).

Consumer Panels

A consumer test using a three-point acceptability scale (tastes great, acceptable, unacceptable (Shewfelt et al., 1997, Dubost et al., 2003) was conducted using 86 students from the University of Georgia between the ages 18-25. The students were selected based on willingness to consume milk beverages and no history of negative allergic reactions. A sample volume of 30 mL of whey-banana beverage was served in a three-digit coded cup and presented to each student for evaluation. Students were asked to indicate their acceptability and to identify the descriptor (sweetness, acidity, smoothness, sourness, banana flavor) that contributed to their answer. Results were expressed as a percentage for each category (acceptable, tastes great, unacceptable).

Evaluation of storage stability

Whey-banana beverage samples were placed in 5 ml disposable pipettes sealed at both ends with parafilm and incubated at 4C, 20C, 30C and 40C to assess serum separation under gravity. The pipettes were inspected at various time intervals within 60 days. The volume of a layer of clear serum at the top was recorded as an indication of instability. The % volume of serum separated from the total volume was reported.

Sedimentation was assessed by centrifugation at 3000 x g (Marathon 3200, Fisher

Scientific, Pittsburgh, PA) for 20 min at ambient temperature. The relative weight of the pellets after draining the samples for 20 min was reported as an indication of stability. The sediments and supernatants were freeze-dried for uronic acid and protein analyses. Protein was determined in diluted (2.5 mg solids/mL in water) sediments and supernatant samples stored at 4C and 40C for 1 and 60 days using the bicinchoninic acid protein assay (Smith 1985). The freeze-dried sediments and supernatants were washed with ethanol and uronic acid content determined according to the colorimetric m-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen 1973).

Total phenolics

The total phenolics were determined according to the Folin-Ciocalteu method (Singleton and Rossi 1965). About 50 mg of freeze-dried sediments or supernatant samples were mixed with 1 mL of methanol, 1mL of water and 200 μ L of hydrochloric acid (1 M) and mixed using a vortex. The tube was capped and shaken at 200 rpm, 60 C for 30 min in a water bath (Gyratory Water Bath Shaker, Model G76D, New Brunswick Scientific Co., Edison, NJ, USA). The tubes were removed, vortexed and centrifuged at 2,000 rpm using a Dynac II Centrifuge (Becton & Dickinson Company, Franklin Lakes, NJ) for 2 min. The samples were filtered through a 0.45 μ m Millipore syringe filter (Whatman Inc., Clifton, NJ). The total phenolics in the filtrate were determined colorimetrically. A 0.2 N Folin-Ciocalteu reagent (Sigma, St Louis, MO) was freshly prepared by diluting a 2N stock solution with water. A volume of 100 μ L of filtrate was added to 900 μ L of distilled water, and 5 mL of 0.2 N Folin-Ciocalteu reagent were mixed. Saturated sodium carbonate (Sigma, St Louis, MO) (4 mL of a 75 g/L solution) was added and the mixture was vortexed. The tubes were incubated for 2 h at room temperature and the absorbance read at 765 nm with a UV-1601 Shimadzu spectrophotometer (Shimadzu

Corporation, Kyoto, Japan). A standard curve was constructed using 100, 167, 250 and 500 mg/L gallic acid (Sigma, St Louis, MO) in water.

Particle size analysis

Particle size analysis of the banana-beverages was performed at 1, 7, 15, 30, 45 and 60 days by integrated laser light scattering with a Malvern Mastersizer (Worcestershire, UK). Samples were introduced in the sample unit containing deionized water and pumped through the optical cell while stirring at 2,000 rpm with an obscuration of 20-30% as described by Barnes (2001). Size distribution as volume and surface area mean diameters were estimated and expressed as $D_{4,3}$ and $D_{3,2}$, respectively.

Color measurement

The color of the UHT whey banana beverages (a sample volume of 30 ml was placed in a test tube) were evaluated using a Minolta Chroma meter (CR-200/CR-231; Minolta, Japan) which determines the a^* (green/red), b^* (blue/yellow), c^* (chroma), H^* (hue) and L^* (lightness) values (Huang et al. 1990).

Statistical analysis

Results were analyzed as a factorial design with storage time and temperature as factors at 4 levels using the General Linear Model (PROC GLM) procedure of the Statistical Analysis System (SAS Institute, Cary, NC). Analysis of covariance was performed with temperature as a covariate to develop prediction models for physical measurements as functions of storage time. Significant differences in physical measurements among the beverages were determined by Tukey's mean separation ($p \leq 0.05$). Pearson correlation coefficients were evaluated to determine potential statistical relationships between physical variables.

Results and Discussion

Particle size

There was no significant change ($p > 0.05$) in particle volume mean diameter (D_{43}) for any of the whey banana beverages at any temperature and storage time (Figure 1A). This is in contrast to the findings of Williams et al. (2000) who found that UHT treatment increased the particle size of skim milk and blends. The analytical values for surface area mean diameter indicate that temperature had no effect ($p > 0.05$) on D_{32} (Figure 1B). However time had a significant effect ($p < 0.05$). Higher particle surface mean diameter was observed for day 1 and 7 ($6.85 \pm 1.05 \mu\text{m}$ and $8.79 \pm 1.52 \mu\text{m}$, respectively) compared to day 15, 30, 45 and 60 ($5.66 \pm 2.32 \mu\text{m}$, $4.41 \pm 0.56 \mu\text{m}$, $4.92 \pm 0.83 \mu\text{m}$ and $4.78 \pm 0.95 \mu\text{m}$ respectively). No changes however, were seen after 15 days. The D_{32} values had comparable values ($p > 0.05$) near $5\text{-}7 \mu\text{m}$ at all storage temperatures and storage period longer than 15 days.

The particle size distributions ($D_{43} \approx 60\text{-}70 \pm 8 \mu\text{m}$, $D_{32} \approx 5\text{-}10 \pm 1 \mu\text{m}$) in the whey-banana beverages were different from those reported by Barnes (2001) for acid milk dispersions made from skim milk powder ($D_{43} \approx 2\text{-}30 \mu\text{m}$, $D_{32} \approx 0.4\text{-}2 \mu\text{m}$). These differences may be due to the presence of banana pulp particles in the whey-banana beverages.

Color dimensions

The data in Figure 2 shows the plot of the color dimensions including Lightness (L^*), chroma (C^*) and Hue (H^*) as a function of time and temperature. The analysis of variance showed a main effect of time for L^* ($p < 0.001$) and significant time/temperature interaction effects for C^* ($p < 0.001$) and H^* ($p < 0.05$). Temperature was not significant for L^* . Small changes in C^* over time were observed at 4°C . A slight increase in C^* over time at 20 and 30°C was due primarily to an increase in b^* (yellow character, data not shown). However, a dramatic

increase in C^* over time at 40C was due to an increase in b^* (yellow character) and a^* (red character, data not shown).

Most decreases in H^* (hue) occur in the first 15 days at 4, 20 and 30C due to an increase in “+ b^* ” combined with a decrease in “- a^* ” (green character). Continued decrease in H^* after 15 days at 40C was due to increases in “+ b^* ” (yellow character) and a^* (red character). These changes were suggestive of browning. The compounds responsible for color during storage of milk products are produced by the Maillard reaction (Berg and Van Boekel 1994, Pellegrino et al. 1995). Sucrose present in the banana pulp as well as sucrose added will likely be hydrolyzed in presence of citric acid to produce glucose and fructose that are reducing sugars required for the Maillard reactions with whey proteins.

% Sediment

A significant ($p < 0.05$) effect was observed for time and temperature on sedimentation. The analysis of covariance showed that samples stored at higher temperatures produced higher % sediments (Figure 3). Tukey grouping revealed that significantly ($p < 0.05$) higher % sediments was observed for the samples stored at 40C. The % sedimentation of samples stored at 30C, 20C and 4C were similar. This is likely due to the activation of reaction between phenolics in banana pulp and the protein to form large aggregates at 40C. Glahn (1982) reported that large particles were more difficult to stabilize because repulsive forces were inadequate to prevent sedimentation.

Serum separation

The analysis of variance showed that time and temperature had significant ($p < 0.05$) effect on the volume of serum produced (Figure 4). The analysis of covariance showed that samples stored at higher temperature produced more serum. Tukey grouping revealed that the

samples stored at 4C had significantly ($p < 0.05$) lower serum production than those stored at 20C, 30C and 40C. The phenolic and protein content of the sediment collected was higher than that of the supernatant (Table 4). The ratio of total phenolics in the sediment to that of the serum at 4C and 40C at day 1 or after 60 days of storage was nearly 4. The ratio of protein in the sediment relative to serum was close to 2. However, the ratio of pectin (expressed as uronic acid) in the sediment relative to serum was close to 1. Pectin partitioning was the same in serum and sediment after storage regardless of temperature. The partitioning of protein and phenolics in the pellet is likely a result of protein-phenolic interactions. Banana pulp contains polyphenols that impart astringency in products (Forsyth 1981). Studies showed that tannins (polyphenols) precipitate proteins (Oh and Hoff 1987, Siebert 1999) and whey proteins formed high molecular fractions by reaction with plant phenols (Rawel et al. 2001).

Descriptive panel

After training, panelists used the vocabulary developed by the panel consistently. Occasional sessions were conducted for recalibration. There were minimum changes in the flavor and taste characteristics of the whey-banana beverage with storage time (Figure 5). The sourness and sweetness attributes were dominant in all sessions. No changes were observed between day 7 and day 60 for thickness, smoothness (lack of grainy texture), banana flavor and the off-flavor.

Consumer acceptability

Consumer acceptability was evaluated as the percentage of panelists that rated the whey-banana beverage as acceptable, tastes great or unacceptable (Dubost et al., 2003). Combining acceptable and tastes great data, half of the consumers (50%) found the whey-banana beverage an acceptable product (Table 5). Of the consumers who accepted the whey-banana beverage,

30% preferred the flavor, 44% the smooth texture, 19% sweetness, 5% the acidity and 2% for unknown reasons. These reasons were also identified by consumer population who found the whey-banana beverage unacceptable. Of the segment of the population who did not accept the whey-banana beverage, 51% disliked the flavor, 2% the smooth texture, 23% the sweetness, 19% the acidity and 4% for unknown reasons.

Conclusion

Color dimensions were most affected by storage. Sensory descriptive panel found no change during storage at 4 °C for 60 days. Consumer panel found the beverage acceptable. Therefore, the whey-banana beverage can be made and stored without apparent loss of quality. Physical and chemical measurements of quality were inadequate to predict shelf life. Color changes and serum separation were the best indicators of physical changes with storage.

References

- ALTING, A. C., HAMER, R. J., DE KRUIF, C. G. and VISSCHERS, R. W. 2000. Formation of disulfide bonds in acid-induced gels of preheated whey protein isolate. *J. Agric. Food Chem.* 48, 5001-5007.
- BARNES, BW. 2001. Pectin stabilization of acid milk dispersions. MS Thesis, University of Georgia.
- BERG, H. E., and VAN BOEKEL, M. A. J. S. 1994. Degradation of lactose during heating of milk. 1. Reactions pathways. *Neth. Milk and Dairy J.*, 48, 157-175.
- BLUMENKRANTZ, N. and ASBOE-HANSEN, G. 1973. New method of quantitative determination of uronic acids. *Anal. Biochem.* 54, 484-489.
- CIVILLE, G.V. and LYON, B.G. (Ed.) 1996. *Aroma and Flavor Lexicon for Sensory Evaluation*. ASTM Publication DS 66, PA. pp. 158.
- DE WIT, J. N. 1981. Structure and functional behavior of whey proteins. *Neth. Milk Dairy J.* 35, 47-64.
- DE LA FUENTE, M. A., SINGH, H. and HEMAR, Y. 2002. Recent advances in the characterization of heat induced aggregates and intermediates of whey proteins. *Trends in Food Sci. and Technol.* 13, 262-274.

DICKINSON, E. 1998. Stability and rheological implications of electrostatic milk protein-polysaccharide interactions. *Trends in Food. Sci. Technol.* 9, 347-354.

DUBOST, N.J., SHEWFELT, R.L. and EITENMILLER, R.R. 2003. Consumer acceptability, sensory and instrumental analysis of peanut soy spreads. *J. Food Qual.* 26, 27-42.

EUSTON, S. R. 2000. Aggregation kinetics of heated whey protein-stabilized emulsions. *Food Hydrocoll.* 14, 155-161.

EUSTON, S. R., FINNIGAN, S. R. and HIRST, R. L. 2002. Kinetics of droplet aggregation in heated whey protein-stabilized emulsions: effect of polysaccharides. *J. Food Hydrocoll.* 16, 499-505.

FORSYTH, W.G.C. 1981. Tannins in solid foods, in *The Quality of Foods and Beverages*, vol. 1, *Chemistry and Technology* (G. Charalambous and G. Inglett, eds.) Academic Press, New York, pp. 377-388.

GLAHN, P.E. 1982. Hydrocolloid stabilization of protein suspensions at low pH. *Progress. Food. Nutr. Sci.* 6, 171-177.

GLAHN, P.E.; ROLIN, C. 1994. Casein-pectin interaction in sour milk beverages. *Food Ingredients Europe* 1-5.

HENDRICKS, K. M. and BADRUDDIN, S. H. 1992. Weaning recommendations: The scientific basis. *Nutr. Rev.* 50, 125-133.

HOLSINGER, V.H., POSATI, L.P. and DE VILBISS, E.D. 1974. Whey beverages: A review. *J. Dairy Sci.* 57, 8, 849-859.

JELEN, P., and BUCCHEIM, W. 1984. Stability of whey protein upon heating in acidic conditions. *Milchwiss.* 39, 215-219.

JELEN, P., CURRIE, R., and KADIS, V. W. 1987. Compositional analysis of commercial whey drinks. *J. Dairy Sci.* 70, 4, 892-895.

JELEN, P. 1992. Whey cheeses and beverages. In J. G. Zadow (ed.), *whey and lactose processing* 157-193. New York: Elsevier Applied Science

KRAVTCHENKO, T. P., PARKER, A. and TRESPOEY, A. 1995. Colloidal stability and sedimentation of pectin-stabilized acid milk drinks, in *Food Macromolecules and Colloids* (Dickinson, E. and Lorient, D., eds), 349-355, Royal Society of Chemistry, Cambridge, UK.

LAW, A. J. and LEAVER, J. 2000. Effect of pH on the thermal denaturation of whey proteins in milk. *J. Agric. Food Chem.* 48, 672-679.

LUPANO, C. E. 1994. Effect of heat treatments in very acidic conditions on whey protein isolate properties. *J. Dairy Sci.* 77, 2191-2198.

McINTOSH G.H., ROYLE P.J., LE LEU R.K., REGESTER, G.O., JOHNSON, M.A., GRINSTED, R.L., KENWARD, R.S. and SMITHERS, G.W. 1998. Whey proteins as functional food ingredients. *Int. Dairy J.* 8, 425-434.

MEILGAARD, M., CIVILLE, G.V., and CARR, B.T., 1991. *Sensory Evaluation Techniques*. CRC Press, Inc., Boca Raton, Florida, 354 pp.

OH, H.I. and HOFF, J.E. pH dependence of complex formation between condensed tannins and proteins 1987. *J. of Food Sci.* 52, 5, 1267-1269.

PARKER, A., BOULENGER, P. and KRAVTCHENKO, T. P. 1994. Effect of the addition of High Methoxy Pectin on the Rheology and Colloidal Stability of Acid Milk Drinks. In *Food Hydrocolloids: Structures, Properties, and Functions*; Nishinari, K., Doi, E., Eds.; Plenum Press: New York, NY.

PELLEGRINO, L., RESMINI, P. and LUF, W. 1995. Assessment (indices) of heat treatment. In : Heat induced changes in milk. pp 409-457. *Bull. Int. Dairy Fed.*, Brussels, Belgium.

RATTRAY, W. and JELEN, P. 1997. Thermal stability of skim milk/whey protein solution blends. *Fd. Res. Int.* 30, 5, 327-334.

RAWEL, H.M., KROLL, J. and HOHL, U.C. 2001. Model studies on reactions of plant phenols with whey proteins. *Nahrung*, 45, 2, 72-81.

SHEKILANGO, S.A., JELEN, P. and BAGDAN, G.C. 1997. Production of whey-banana beverages from acid whey and overripe bananas. *Milchwis.* 52, 209-218.

SHEPHERD, R. 1988. Consumer attitudes and food acceptance. In D. M. H. Thomson (Ed.). *Food Acceptability* 253-265. London, UK, Elsevier Applied Science.

SHEWFELT, R.L., ERICKSON, M.C., HUNG, Y.C. and MALUNDO, T.M.M. 1997. Applying quality concepts in frozen food development. *Food Technol.* 51(2), 56-59.

SIEBERT, K.J. 1999. Effects of protein-polyphenol interactions on beverage haze, stabilization, and analysis. *J. Agric. Food Chem.* 47, 353-362.

SINGLETON, V.L. and ROSSI, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16, 144-158.

SMITH, P. K. 1985. Measurement of protein using bicinchoninic acid. *Anal. Biochem.* 150, 76-85.

WILLIAMS, R.P.W., PUVANENTHIRAN, A. and AUGUSTIN, M. A. 2000. Particle size and storage stability of UHT reconstituted skim milks with altered casein: whey protein ratio. *The Austr. J. Dairy Technol.* 55, 100-101.

WONG, D.W.S., CAMIRAND, W.M., and PAVLATH, A.E. 1996. Structures and functionalities of milk proteins. *Crit. Rev. Fd. Sci. Nutr.* 36, 807-844.

TABLE 1. WHEY-BANANA BEVERAGE FORMULATION

Ingredients	Wt (%)
Water	52.87
Banana puree	26.43
Sucrose	11.89
Whey protein concentrate ¹	5.86
Citric acid (20% solution)	2.80
Pectin ²	0.15

¹WPC with 80% protein

²High methoxyl pectin (50% uronic acid, Genu pectin type JMJ)

TABLE 2. SENSORY DESCRIPTORS GENERATED FOR WHEY-BANANA BEVERAGE

Descriptor	Definition
Sweet	Taste stimulated by sugars like fructose, sucrose
Sour	Taste stimulated by acids such as malic and citric acid
Thick	Perception of the viscosity of the beverage sample in the mouth
Grainy	Perception of particles against the roof of the mouth
Banana flavor	An overall integrated perception of taste and aroma associated with banana
Off flavor	A strange, extraneous type of aroma

Adapted from Meilgaard et al. (1991) and Civille and Lyon (1996).

TABLE 3. THE REFERENCE STANDARDS AND THEIR INTENSITIES FOR THE VARIOUS DESCRIPTORS

Descriptor	Reference standard	Intensity on 150-mm scale
Sweet	5% sucrose solution	50
	8% sucrose solution	80
	10% sucrose solution	100
Sour	0.05% citric acid solution	20
	0.08% citric acid solution	50
	0.15% citric acid solution	100
Thick	Water	10
	Ensure ^R	60
	Nestle ^R sweetened condensed milk	140
Grainy	Flavored vanilla yogurt	10
	900g flavored vanilla yogurt with 100 g Kroger ^R nutty nuggets	100
Banana flavor	Homogenized milk with 0.5 mL Kroger ^R imitation banana flavor	50
	Nestle ^R banana milk	30

Adapted from Meilgaard et al. (1991), Civille and Lyon (1996).

TABLE 4. PROTEINS, URONIC ACID AND TOTAL PHENOLICS COMPOSITION OF WHEY BANANA BEVERAGE SEDIMENTS AND SERUM

Samples	Proteins ($\mu\text{g/mL}$)	Ratio ¹ S/L	Uronic acid ($\mu\text{g/mL}$)	Ratio S/L	Phenolics (mg GA/L) ⁶	Ratio S/L
Day 1						
4S ²	117.5 ± 2.5	1.7	41.0 ± 1.0	1.0	1992 ± 7	3.8
4L ³	77.5 ± 0.0		35.0 ± 1.5		517 ± 7	
40S ⁴	85.0 ± 2.5	2.0	44.5 ± 1.5	1.0	1300 ± 4	3.9
40L ⁵	50.0 ± 5.0		43.5 ± 1.5		327 ± 10	
Day 60						
4S	92.5 ± 2.5	1.5	39.0 ± 1.5	1.0	2002 ± 11	3.8
4L	62.5 ± 2.5		37.5 ± 2.0		518 ± 10	
40S	60.0 ± 0.0	2.0	44.5 ± 1.5	1.0	1318 ± 2	3.8
40L	30.0 ± 0.0		42.5 ± 1.0		351 ± 7	

¹Ratio of the concentration of compound in sediment to that in the serum

²4S Sediments from whey-banana beverage samples stored at 4C

³4L Serum from whey-banana beverage samples stored at 4C

⁴40S Sediments from whey-banana beverage samples stored at 40C

⁵40L Serum from whey-banana beverage samples stored at 40C

⁶Phenolics expressed as mg gallic acid/ L

TABLE 5. CONSUMER ACCEPTABILITY DATA FOR WHEY-BANANA BEVERAGE

Descriptor	Category		
	Tastes Great	Acceptable	Unacceptable
Flavor	2	11	22
Smooth		19	1
Sweet		8	10
Acid	1	1	8
Other	1		2
Total	4	39	43

FIGURE 1. PARTICLE DIAMETERS OF WHEY-BANANA BEVERAGES AT 4C (◇), 20C (□), 30C (Δ) AND 40C (✱), FOR 60 DAYS. (A) VOLUME MEAN DIAMETER. (B) SURFACE AREA MEAN DIAMETER.

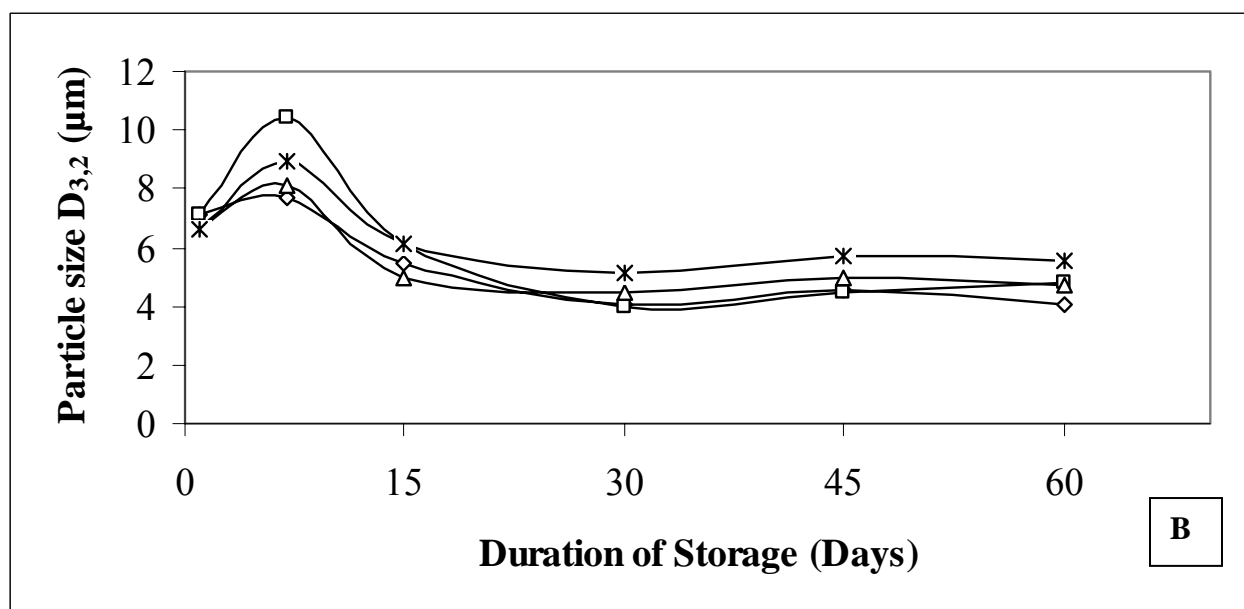
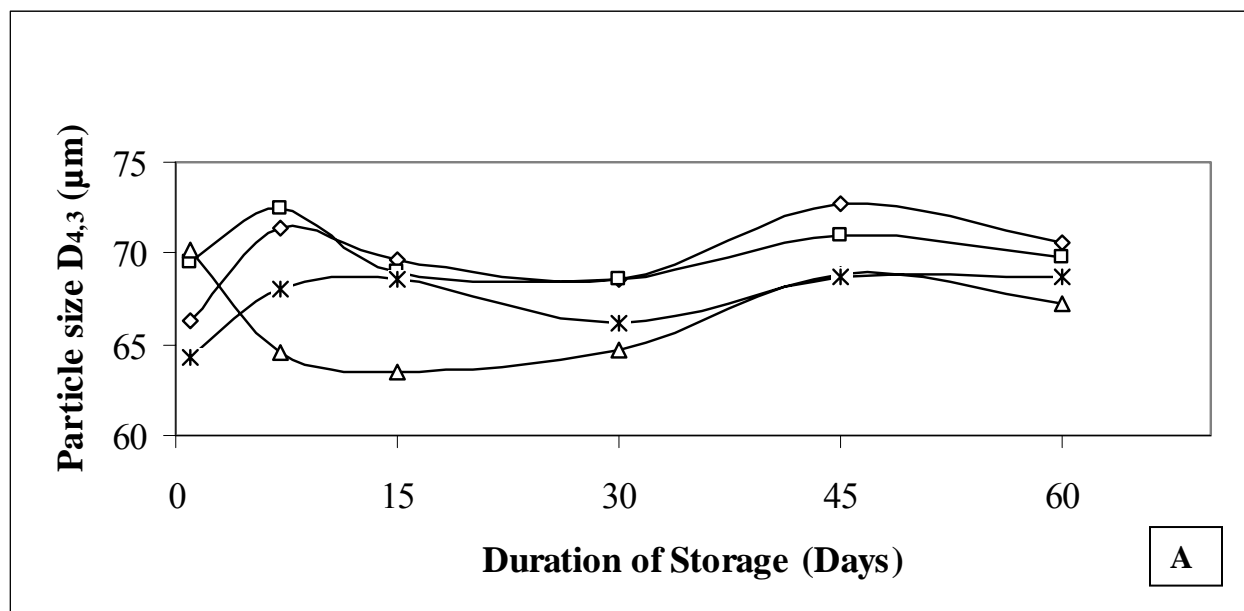


FIGURE 2. COLOR DIMENSIONS OF WHEY-BANANA BEVERAGES STORED FOR 60 DAYS AT 4C (\diamond), 20C (\square), 30C (Δ) AND 40C (\times). (A) CHROMA C^* . (B) LIGHTNESS L^* . (C) HUE H^* .

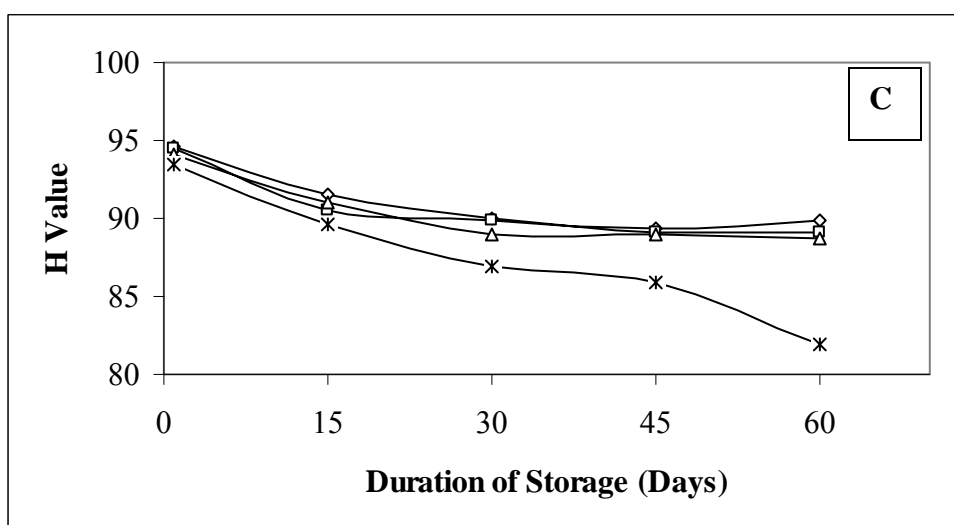
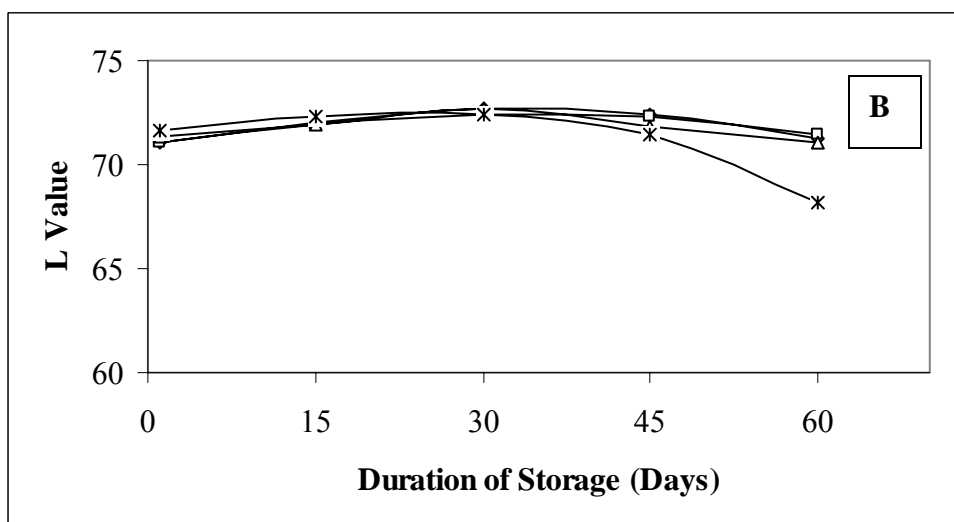
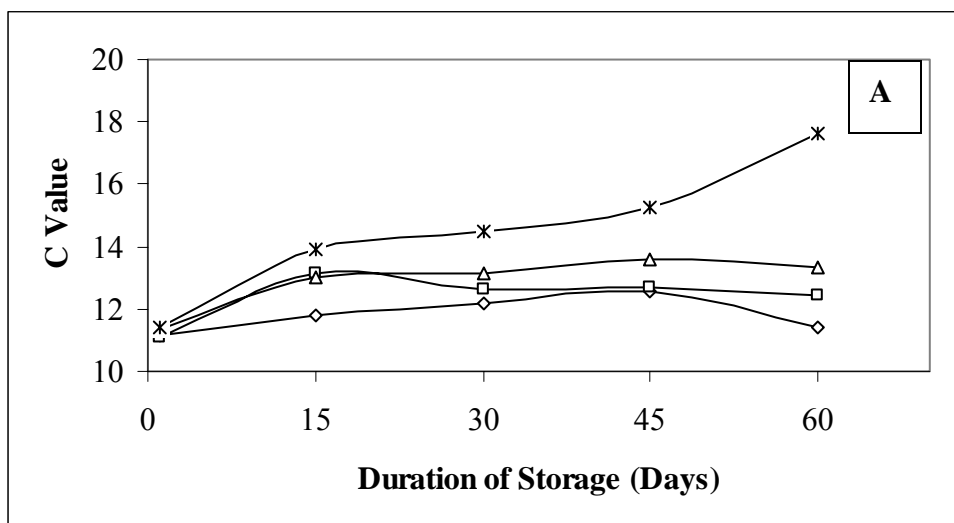


FIGURE 3. % SEDIMENT OF WHEY-BANANA BEVERAGES STORED FOR 60 DAYS AT 4C (◇), 20C (□), 30C (Δ) AND 40C (✱).

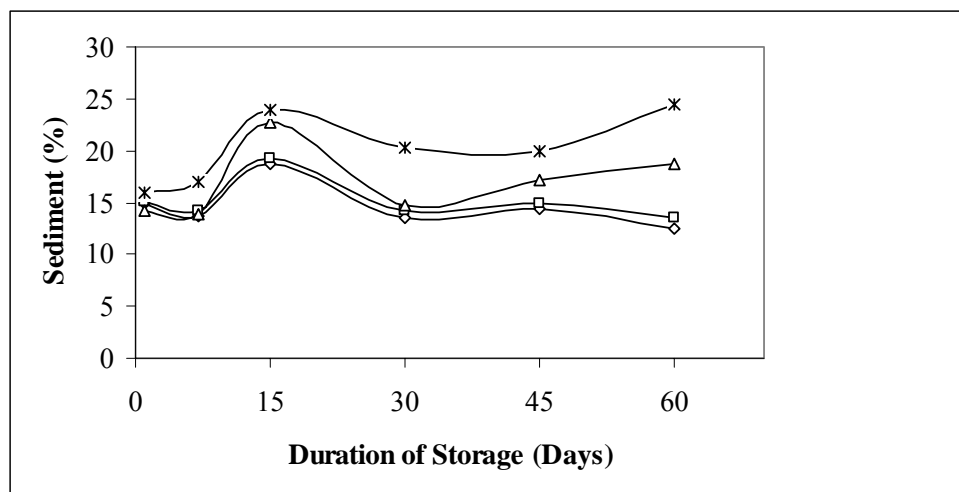


FIGURE 4. % SERUM SEPARATION OF WHEY-BANANA BEVERAGES STORED FOR 60 DAYS AT 4C (◇), 20C (□), 30C (Δ) AND 40C (✕).

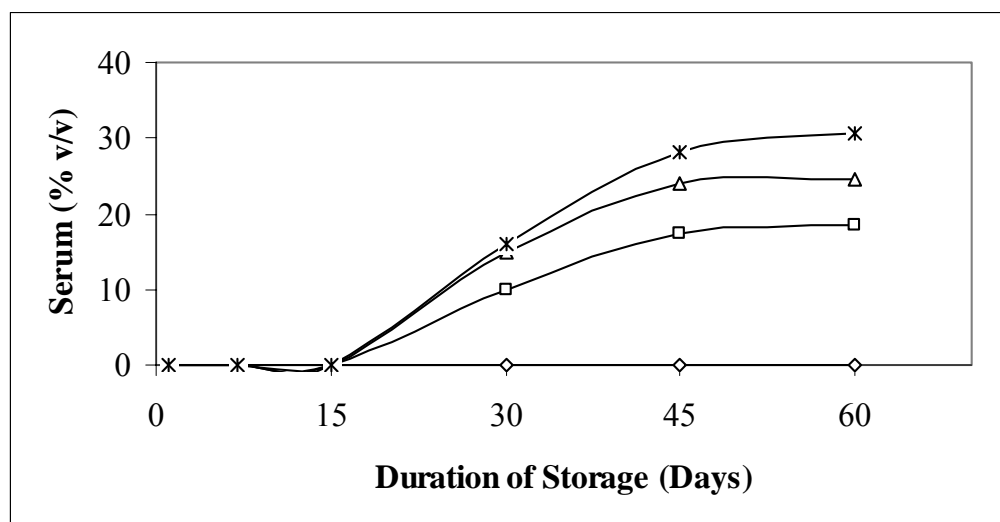
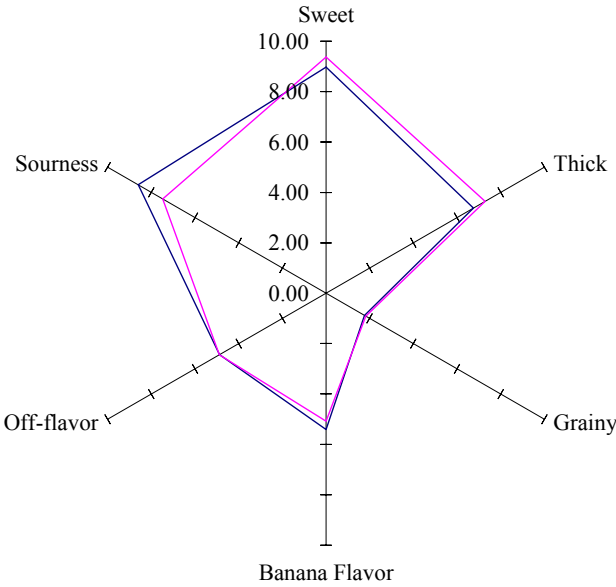
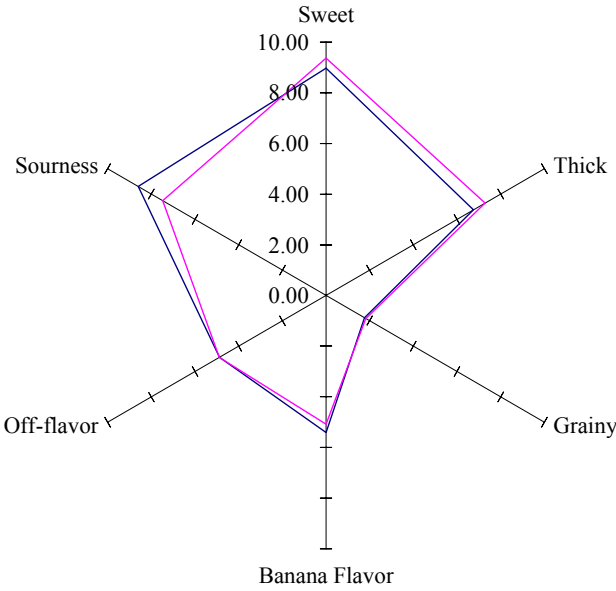


FIG. 5. SPIDER DIAGRAM FOR THE PANEL SCORES FOR THE SIX SENSORY ATTRIBUTES FOR WHEY-BANANA BEVERAGES AFTER 7 AND 60 DAYS STORAGE AT 4C (results for 2 replications).



Day 7



Day 60

CHAPTER 6

CHANGES IN THE PROPERTIES OF UHT WHEY-BANANA BEVERAGE DURING STORAGE¹

¹Koffi, E.K., and Wicker, L. To be submitted to the Journal of The Science of Food and Agriculture

Abstract

Changes in the physical characteristics of Ultra High temperature (UHT) and non-UHT treated whey-banana beverages were determined for samples stored for 1, 3, 10 and 17 days at 4, 20, 30 and 40C. Sedimentation was greater for the non-UHT whey-banana beverage and increased with increasing storage temperature. The flow behavior and consistency indexes were comparable for both UHT and non-UHT treated samples. While the flow behavior decreased with increasing storage temperature, the consistency increased. At 17 days, the volume mean diameter (D_{43}) was greater for the UHT beverages at all storage temperatures. However, the surface area mean diameter (D_{32}) was greater for the UHT beverages and independent of time and temperature. Except for the color value “H*” which was significantly higher for the non-UHT product, the color values a^* , b^* , c^* and L^* were greater for the UHT beverages over the ten day storage period at all four temperatures 4, 20, 30 and 40C.

Keywords: UHT processing, color, whey-banana beverages, polyphenols, browning, storage temperature, rheology

Introduction

Ultra high temperature (UHT) (~140-145°C/ 4-10 sec) treatment of whey products is required for sterility in aseptic packaging systems. However, whey proteins denaturation is inevitable, and precedes a number of changes taking place during storage ¹. Beta-lactoglobulin (β -lg) and alpha-lactalbumin (α -lac) are the most abundant whey proteins that influence the functional properties of the heated product. Several factors including protein concentration, pH, total solids and mineral contents influence the denaturation process ².

The effect of pH on the whey proteins has been studied and reports showed that β -lg was less stable at alkaline pH, whereas α -lac stability was independent in pH range 3 to 7.5 ³. However, sulfhydryl group-disulfide bond interchange reactions were demonstrated at ambient temperature and under acidic conditions (pH~ 4.5) ⁴. An increase in viscosity of skim milk upon heating and before gel formation during storage of milk products as well as factors affecting gel formation has been documented ⁵. All the sulphhydryl groups became reactive after UHT treatment ⁶. Intra or intermolecular reaction of β -lg with κ -casein or the proteins of the milk fat globule membrane can induce the formation of a three-dimensional protein network which causes the milk to thicken and gel. An increase in viscosity after heating milk product was due to unfolding of β -lg and polymerization products that acted as sticky agents between casein and stainless steel to form a fouling layer ⁷.

More severely heated milks (indirect heat) showed resistance to gelation due to the fact that processing mode takes place in the presence of oxygen which has been found to be a chain reaction break on thiol-disulfide exchange reactions ⁵. Thus, polymerization and denaturation of β -lg is slowed by oxidation of thiol groups to disulfides. However, the direct steam injection removes oxygen from the product and UHT milk using this method contains less denatured whey

protein attached to casein micelles (and gels faster) than indirectly heat-treated milk⁸. Changes in milk composition including protein concentration, pH, total solids concentration and minerals influence thermal stability and heat-induced interactions of whey proteins^{9,10,11}. Milk undergoes chemical changes during storage, via the Maillard reaction between lactose and the ϵ -NH₂ groups of lysine residues¹². The Maillard reaction proceeds during storage and affects the physical and nutritional properties of the product¹³.

Polyphenol-rich extracts of tea (green and black), red wine, cocoa powder, coffee, coconut shell and oak leaves increased the heat stability of skim milk and concentrated skim milk as well as the rennet coagulation time of milk^{14,15}. Pectins prevent protein aggregation during heat processing by adsorbing onto the surfaces of the proteins and acting as a dispersing agent in acid milk drinks¹⁶. Interaction between pectins and other components of heat processed beverages is likely to affect the physical properties of products. Previous work with UHT whey-banana beverages have shown that particle size and whiteness did not change over a 60 day storage period at 4, 20, 30 and 40°C even though sedimentation and serum separation was observed (Koffi et al. submitted)¹⁷. Protein aggregation and particle growth is a undesirable defect observed in milk beverages after heat processing. There is a need to better understand the contribution of UHT process to the stability characteristics of the whey-banana beverages. The objective of this work was to examine the effect of UHT treatment on the particle size, rheological, optical, properties and stability parameters of whey-banana beverages stored at varying temperatures between 4 and 40°C. A control with no heat treatment was used to provide information about the intrinsic developments in the beverages.

Materials and Methods

Materials

Whey protein concentrate (80% protein) was obtained from Protient (Lot code LUV 1225 St. Paul, MN). High methoxyl pectin (50% uronic acid, 80% DE, Genu pectin type JMJ) was provided by Hercules (Wilmington, DE) and acidified banana puree was donated by Chiquita (Chiquita Brands, Inc., Cincinnati, OH).

Preparation of UHT and non-UHT whey-banana beverages

A blend of 1 part of banana puree to 2 parts of acidified (20 % citric acid to pH 4.0) water and whey protein (5%) was prepared in a surge tank with agitator and aseptic homogenizing valve of the N0-BAC Unitherm IV processing system (Cherry-Burrell Amc International, IA) as described by Shekilango¹⁸ (1996). Sucrose (15% w/w) and pectin (0.15% w/w) were dry-mixed and added to the whey and citric acid while stirring. The mixture was homogenized at 3000 psi and heated for 5-7s at 140C with the indirect heating method. The UHT processed beverages were filled into pre-sterilized 450 ml glass bottles and stored at 4C, 20C, 30C and 40C for 1, 7 and 17 days for further analyses. The non-UHT product was processed and stored the same way without the heating step.

Color

The color of the UHT and non-UHT whey banana beverages (a sample volume of 30 ml was placed in a test tube) were evaluated using a Minolta Chroma meter (CR-200/CR-231; Minolta, Japan) which determines the a* (green/red), b* (blue/yellow), c* (chroma), H* (hue) and L* (whiteness or luminosity) values (Huang et al. 1990)¹⁹.

Rheology

A Dynamic Stress Rheometer (Rheometrics, Piscataway, NJ) was used to determine the flow behavior and consistency indexes of the UHT and non-UHT whey-banana beverages. The couette geometry (cup diameter = 32.0 mm, bob diameter = 29.5 mm, and bob length = 44.5 mm.) was used. A steady stress sweep test was conducted to determine the flow behavior data at 10 C with an initial and final rate of 10 (sec⁻¹) and 100 (sec⁻¹) respectively with a measurement time of 10 seconds at each shear rate²⁰

Particle size

Particle size distribution of the UHT and non-UHT whey-banana beverages was determined by laser diffraction using a Malvern Mastersizer (Worcestershire, UK); Samples were introduced in the sample unit containing deionized water and pumped through the optical cell (code for optical properties of the particles, “presentation”, 3ODH) while stirring at 2,000 rpm. Size distribution (volume and surface area mean diameters) were estimated and expressed as D_{4,3} and D_{3,2}.

Sediment

Centrifugation of the UHT and non-UHT whey-banana beverages were performed and supernatants were separated after centrifugation at 3000 x g (Marathon 3200, Fisher Scientific, Pittsburgh, PA) for 20 minutes at ambient temperature and weighed after draining for 10 minutes. The relative weight of the pellets was reported as an indication of stability.

Serum separation

Samples were placed in 5 ml disposable pipettes sealed at both ends and incubated at 4C, 20C, 30C and 40C to assess serum separation under gravity. The pipettes were inspected at various time intervals within 17 days. When sedimentation occurred, a layer of clear supernatant

was left at the top and volume of this was recorded as an indication of instability. The % volume of serum separated from the total volume was reported.

Statistical analysis

Analysis of variance of the data was applied according to a factorial design with temperature and storage time nested within processing mode (UHT / non-UHT treatment). Interaction between storage time used as covariate and the processing mode (treatment) was checked for significance before running the analysis. Differences between UHT and non-UHT treatment means were determined by the Tukey's procedure at $p < 0.05$ using the Statistical Analysis System software (SAS Inc., NC).

Results and Discussion

The analysis of variance for data collected at 1, 3, 10, and 17 days indicate that there were significant interactions ($p < 0.05$) between time, temperature, and heat treatment (Table 1). Therefore, comparisons between processing mode (UHT/no UHT) were made at each time and temperature.

Sediment

The data in Figure 1 shows the plot of sediment versus storage time and storage temperature for both UHT and non-UHT treated whey-banana beverages. The analysis of variance (Table 1) showed that heat treatment and storage temperature were the major factors influencing sedimentation. Storage time effect was not significant. The interactive effects of heat treatment-storage time, heat treatment-storage temperature, storage time-storage temperature, heat treatment-storage time-storage temperature were significant. Sedimentation was highest at 40°C for both UHT and non-UHT whey banana beverages. At day one the non-UHT whey-banana beverage exhibited significantly ($p < 0.05$) higher sedimentation than the UHT treated

whey-banana beverage at all storage temperatures. At day 3, the non-UHT beverage exhibited significantly ($p < 0.05$) higher sedimentation than the UHT beverages at 4, 20 and 30°C. Sedimentation was comparable in the non-UHT and UHT beverages stored at 40 °C for 3 days. At ten or seventeen days, the non-UHT beverage showed significantly ($p < 0.05$) higher sedimentation compared to the UHT beverages at any of the four temperatures 4, 20, 30 and 40 °C.

Destabilization is most likely to occur in pasteurized acid milk beverage compared to the non-pasteurized beverage ²¹. Storage time of whey-banana beverages did not significantly affect sediment generation in UHT milk, nor the non-UHT milk ($p > 0.05$). The difference in the amount of sediment for the UHT and non-UHT beverages could be due to a mechanism similar to the fouling phenomenon observed in whole milk during processing ²². According to this author, the material available for sedimentation in whole milk will appear in the carton only if it does not burn or deposit on the interior of the heat exchanger. Both UHT and non-UHT whey-banana beverages contain sedimentable material; however, possible fouling in the UHT system left less material available for sedimentation in the UHT treated beverages.

Rheology

The plot of the mean values for the flow behavior index for all data collected at each storage period and temperature for UHT and non-UHT treated whey-banana beverages is presented in Figure 2. The analysis of variance of the data (Table 1) showed that time and storage temperature were the major factors influencing flow behavior. The time effect was strongly dependent on the storage temperature. There was no significant effect due to heat treatment ($p > 0.05$). The interactive effects of heat treatment-storage period-temperature were

not significant ($p > 0.05$). The flow behavior of the UHT and non-UHT beverages stored at 40°C exhibited significantly ($p < 0.05$) lower flow behavior indexes (~ 0.4) than the beverages stored at all 3 lowest temperatures with flow indexes close to 0.6. Differences if any between UHT and non-UHT beverages was observed only at the beginning of storage (day one) and disappeared during the the rest of the storage period.

The decrease in flow behavior index at 40 °C compared to 4 °C, 20 °C or 30 °C could be due to the enhancement of the interaction between pectin and whey proteins at the elevated temperature. The difference between the flow behavior index of the UHT and the non-UHT treated beverage is likely due to the higher concentration of reactive sulfhydryl and disulfide generated by the UHT treatment ⁷ compared to the non-UHT treated beverage.

The data in Figure 3 shows the consistency index as a function of storage time and storage temperature for both UHT and non-UHT treated whey-banana beverages. The analysis of variance of the data (Table 1) showed that heat treatment, storage temperature, and storage time were the major factors influencing consistency. The storage time effect was strongly dependent on the storage temperature. The interactive effects of heat treatment-storage time, heat treatment-storage temperature and heat treatment-storage time-storage temperature were significant ($p < 0.05$). The increase in consistency of the UHT and non-UHT beverages at 40°C compared to the lower storage temperatures is likely due to structure building in the beverages enhanced by the elevated storage temperature. One of the important attributes of whey proteins is their ability to form complexes with themselves, other proteins and pectins ¹¹. No difference was observed between the UHT and non-UHT beverage at 40°C probably due to a slow rate of UHT-induced changes. Similar results have been reported for UHT whole milk ²³ while other authors have found increased viscosity after UHT ^{24,25}.

At day 1, the UHT and non-UHT beverages had comparable consistency at 4°C. However, the UHT beverages exhibited significantly ($p < 0.05$) higher consistency at 20°C, 30°C and 40°C. At day 3, the UHT beverage had significantly ($p < 0.05$) higher consistency than the non-UHT beverage at 4 °C. However, the UHT and non-UHT beverage had comparable consistency at 20 °C, 30 and 40°C. At day 10, the non-UHT beverage exhibited a higher consistency at 4 and 20 °C while the consistency was higher ($p < 0.05$) for the UHT beverage at 40°C compared to the non-UHT treated beverage. At day 17, the consistency was comparable for the UHT and non-UHT beverages stored at 4 and 40°C whereas the consistency was higher ($p < 0.05$) for the UHT beverage at 20 and 30°C compared to the non-UHT beverage.

Particle size

The data in Figure 4 shows the plot for the volume mean diameter (D_{43}) of particles as a function of storage time and temperature for both UHT and non-UHT treated whey-banana beverages. The analysis of variance of the data (Table 1) showed that heat treatment and storage temperature were the major factors influencing particle mean diameter. The heat treatment effect was strongly dependent on storage temperature. There was no significant ($p > 0.05$) effect due to storage time. However, the interactive effects of storage time-storage temperature and heat treatment-storage time-storage temperature were significant. At day 1, the particle volume mean diameter was significantly higher ($p < 0.05$) for the non-UHT beverages at 4 and 30°C whereas a significantly ($p < 0.05$) higher value was observed for the UHT beverage at 40 °C. Comparable particle mean diameters were obtained at 20 °C for the UHT and non-UHT beverages at day 1. At day 3, the non-UHT beverage stored at 4 and 20 °C had significantly ($p < 0.05$) higher particle volume mean diameter than the UHT beverage. Significantly ($p < 0.05$) higher particle volume mean diameter was observed for the UHT beverage at 40°C compared to the non-UHT beverage.

The UHT and non-UHT beverages stored at 30°C had comparable volume mean diameter of particles. At day 10, the UHT and non-UHT beverage exhibited comparable particle size at 4 and 20 °C. The particle volume mean diameter was significantly ($p < 0.05$) higher for the non-UHT beverage compared to the UHT beverage at 30 °C. However, the UHT beverage exhibited significantly ($p < 0.05$) higher particle size at 40 °C. At day 17, the non-UHT beverage exhibited higher ($p < 0.05$) particle size at 20, 30 and 40°C. The changes for this variable are not as clear cut. This could be due to the complex interaction between whey proteins, pectin and polyphenols present in the whey-banana beverages. Association and dissociation reaction between β -lactoglobulin and casein and κ -casein have been reported during age gelation of UHT milk ²⁶. Further, denatured proteins are also susceptible to aggregation via salt bridges and hydrophobic interactions ²⁷.

The data in Figure 5 shows the plot of surface area mean diameter (D_{32}) as a function of storage time and storage temperature for both UHT and non-UHT whey-banana beverages. The analysis of variance (Table 1) showed that heat treatment was the major factor ($p < 0.001$) influencing the surface mean diameter of particles. There was no significant effect ($p > 0.05$) due to storage temperature. Storage time and storage time-storage temperature effects were significant with minor effects ($p < 0.05$ and $p < 0.01$ respectively). The interactive effects of heat treatment-storage time, heat treatment-storage temperature and heat treatment-storage time-storage temperature were not significant ($p > 0.05$). The surface area mean diameter of particles was significantly higher ($p < 0.05$) for the non-UHT than the UHT beverages at all four storage temperatures 4 °C, 20 °C, 30 °C and 40 °C for the 17 day storage period. Further, storage time and storage temperature had no significant ($p > 0.05$) effect on the particle sizes. The smaller particle size of the UHT treated beverage could be due to the inhibitory effect of oxygen on

polymerization and denaturation of β -lg. Reports indicated that oxygen acted as chain reaction break on thiol-disulfide exchange reactions during the indirect UHT process⁷.

Color

The data in Figure 6 shows the plot for a^* values versus storage time and temperature for both UHT and non-UHT treated whey-banana beverages. The analysis of variance (Table 1) showed that heat treatment, storage time and storage temperature were the major factors influencing redness ($p < 0.0001$). The interactive effects of heat treatment-storage time, heat treatment-storage temperature, storage time-storage temperature, heat treatment-storage time-storage temperature were also significant ($p < 0.0001$). The UHT beverage exhibited higher redness value compared to the non-UHT beverage at all four temperatures 4 °C, 20 °C, 30 °C and 40 °C during the 10 day storage period.

The data in Figure 7 shows the plot of yellowness (b^* values) versus storage time and temperature for both UHT and non-UHT treated whey-banana beverages. The analysis of variance (Table 1) showed that heat treatment, storage temperature and storage time were the major factors influencing yellowness ($p < 0.0001$). The interactive effects of heat treatment-storage time, heat treatment-storage temperature, storage time-storage temperature, heat treatment-storage time-storage temperature were significant ($p < 0.0001$). The UHT beverage exhibited higher yellowness value compared to the non-UHT beverage at any of the four temperatures 4, 20, 30 and 40 °C during the ten days storage period. The higher yellowness of the UHT beverages is probably due to phenolics extracted from the banana pulp by the UHT process. Better extraction of phenolics was observed from apple pulp as the temperature of juice processing increased²⁸.

The data in Figure 8 shows the plot of saturation (c^* value) versus storage time and storage temperature for both UHT and non-UHT treated whey-banana beverages. The analysis of variance (Table 1) showed that heat treatment and storage temperature and storage time were the major factors influencing saturation ($p < 0.0001$). The interactive effects of heat treatment-storage time, heat treatment-storage temperature, storage time-storage temperature, heat treatment-storage time-storage temperature were significant ($p < 0.0001$). The UHT beverage exhibited higher saturation value compared to the non-UHT beverage at any of the four temperatures during the ten days storage period.

The data in Figure 9 shows the plot of hue (H^* value) versus storage time and temperature for both UHT and non-UHT treated whey-banana beverages. The analysis of variance (Table 1) showed that heat treatment, storage temperature and storage time were the major factors influencing hue ($p < 0.0001$). The interactive effects of these factors were significant ($p < 0.0001$). The non-UHT beverage exhibited higher hue value compared to the UHT beverage at any of the four temperatures during the ten days storage period.

The data in Figure 10 shows the plot of whiteness (L^* value) versus storage time and storage temperature for both UHT and non-UHT treated whey-banana beverages. The analysis of variance (Table 1) showed that heat treatment and storage temperature were the major factors influencing whiteness ($p < 0.0001$). Storage time effect was not significant ($p > 0.05$). The interactive effects of heat treatment-storage time, heat treatment-storage temperature, storage time-storage temperature, heat treatment-storage time-storage temperature were significant ($p < 0.01$). Usually the UHT beverage exhibited higher whiteness value compared to the non-UHT beverage at the four temperatures during the ten days storage period. The difference in whiteness between the UHT treated sample and the untreated control is due to particle sizes. The whiter

color of milk following heat treatment is due to more scattering particles produced by denaturation²⁹. Browning reactions occurring in the whey-banana beverage during storage could be responsible for the color differences between the UHT and the non-UHT whey-banana beverage. The Maillard reaction was faster when the temperature was above 20°C¹³. When ketoses or aldoses are heated in presence of amines, the reducing sugars produces a glycosylamine. After rearrangement (Amadori reaction or enolization), a derivative of 1-amino-1-deoxy-D-fructose is formed in the case of glucose. This derivative at pH 5 or lower yields a 2,3-enol derivative that is converted to 3-deoxyhexosulose. After dehydration a furan derivative which correspond to 5-hydroxymethyl-2-furaldehyde (HMF) is formed. Degradation of HMF yield organic acids and brown pigments the concentration of which increases overtime^{30,31}. Thus the non-UHT whey-banana beverage containing lactose and sucrose most likely caused the changes observed in the color parameters.

Conclusion

The physical characteristics of the whey-banana beverages were affected primarily by the UHT treatment, followed by storage temperature and storage time. During storage the particle size and L* remained constant, the color values a*, b*, c*, H* increased, the flow behavior decreased with increasing temperature and the consistency increased. Reliance on indicators other than particle size to study whey-banana beverage stability could be misleading.

References

- ¹Kocak, H.R. and Zadow, J.G. 1985. The effect of low-temperature-inactivation treatment on age gelation of UHT whole milk. *Australian Journal of Dairy Technology* 40, 53-58.0
- ²Donovan, M. and Mulvihill, D.M. 1987. Thermal denaturation and aggregation of whey proteins. *Irish Journal of Food science and Technology*, 11, 87-100.
- ³De Wit, J.N. and Klarenbeek, G. 1984. Effects of various heat treatments on structure and solubility of whey proteins. *Journal of Dairy Science* 67, 2701-2710.

- ⁴Vastbinder, A.J., Alting, A.C., Visschers, R.W. and de Kruif, C. G. 2003. Texture of acid milk gels: formation of disulfide cross-links during acidification. *International Dairy Journal* 13, 29-38.
- ⁵Hong, Y.H., Guthy, K. and Klostermeyer, H. 1984. On the influence of SH-groups in UHT milk during storage. *Milchwissenschaft* 39, 5, 285-287.
- ⁶Lyster, R.L.J. 1964. The free and masked sulphydryl groups of heated milk powder and a new method for their determination, *Journal of Dairy Research* 31, 1, 41-51.
- ⁷Theo, J.M., Jeurnink, and De Kruif, K.G. 1993. Changes in milk on heating: viscosity measurements. *Journal of Dairy Research* 60, 139-150.
- ⁸Corredig, M. and Dalgleish, D.G. 1996. Effect of different heat treatments on the strong binding interactions between whey proteins and milk fat globules in whole milk, *Journal of Dairy Research* 63, 3, 441-449.
- ⁹Elfagm, A.A., and Wheelock, J.V. 1978. Heat interactions between α -lactalbumin, β -lactoglobulin and casein in bovine milk. *Journal of Dairy Science*, 61, 159-163.
- ¹⁰Hillier, R.M., Lyster, R.L.J., and Cheeseman, G.C. 1979. Thermal denaturation of α -lactalbumin and β -lactoglobulin in cheese whey: Effect of total solids concentration and pH. *Journal of Dairy Research* 46, 103-111.
- ¹¹Oldfield, D.J., Singh, H., Taylor, M.W., and Pearce, K.N. 2000. Heat-induced interactions of β -lactoglobulin and α -lactalbumin with the casein micelle in pH-adjusted skim milk. *International Dairy Journal*, 10, 509-518.
- ¹²Moller, A.B., Andrews, A.T., and Cheeseman, G.C. 1977. Chemical changes in ultra-heat-treated milk during storage. *Journal of Dairy Research* 44, 267-275.
- ¹³Renner, E. 1988. Storage stability and some nutritional aspects of milk powders and ultra high temperature products at high ambient temperatures. *Journal of Dairy Research*, 55, 125-142.
- ¹⁴O'Connell, J.E., Fox, P.D., Tan-Kintia, R. and Fox, P.F. 1998. Effect of tea, coffee and cocoa extracts on the colloidal stability of milk and concentrated milk. *International Dairy Journal* 8, 689-693.
- ¹⁵O'Connell, E. and Patrick, F. 1999. Effects of phenolic compounds on the heat stability of milk and concentrated milk. *Journal of Dairy Research* 66, 399-407.
- ¹⁶Parker, A., Boulenguer, P., and Kravtchenko, T.P. 1994. *Food Hydrocolloids: Structures, Properties, and Functions*. Edited by K. Nishinari and E. Doi. Plenum Press, New York.

- ¹⁷Koffi, E. and Wicker, L. 2003. Storage stability and sensory analysis of UHT processed whey-banana beverages. *Journal of Food Quality* (submitted).
- ¹⁸Shekilango, S.A., Jelen, P. and Bagdan, G.C. 1997. Production of whey-banana beverages from acid whey and overripe bananas. *Milchwissenschaft* 52, 209-218.
- ¹⁹Huang, S., Hart, H. and Wicker, L. 1990. Enzymatic and color changes during post-harvest storage of lychee fruit. *Journal of Food Science* 55, 6, 1762-1763.
- ²⁰Barnes, B.W. 2001. Pectin stabilization of acid milk dispersions. MS Thesis, University of Georgia.
- ²¹Glahn, P.E. and Rolin, C. 1994. Casein-pectin interaction in sour milk beverages. *Food Ingredients Europe* 1-5.
- ²²Burton, H. 1968. Reviews of the progress of Dairy Science. Section G. Deposits from whole milk in heat treatment plant-a review and discussion. *Journal of Dairy Research* 35, 317-324.
- ²³Sur, A. and Joshi, V.K. 1989. Changes in viscosity, pH, oxygen content, sedimentation characteristics and fat separation in UHT milk during storage. *Indian Journal of Dairy Science* 42, 130-131.
- ²⁴Reddy, K.K., Nguyen, M.H., Kailasapathy, K. and Zadow, J.G. 1991. The effects of some treatments and storage temperatures on UHT whole milk. *Australian Journal of Dairy Technology* 46, 57-63.
- ²⁵Mittal, S.B., Hourigan, J.A. and Zadow, J.G. 1990. Effect of added sodium hexametaphosphate on certain technological aspects of UHT recombined milk. *Australian Journal of Dairy Technology* 45, 1-4.
- ²⁶Datta, N. and Deeth, H.C. 2001. Age gelation of UHT milk-A review. *Trans IChemE*, 79, Part C, 197-210.
- ²⁷Anema, S.G. and Yuming, L. 2003. Association of denatured whey proteins with casein micelles in heated reconstituted skim milk and its effect on casein micelle size. *Journal of Dairy Research* 70, 73-83.
- ²⁸Spanos, G.A., Wrolstad, R.E. and Heatherbell, D.A. 1990. Influence of processing and storage on the phenolic composition of apple juice. *Journal of Agricultural and Food Chemistry* 38, 1572-1579.
- ²⁹Walstra, P. and Jenness, R. In *Dairy Chemistry and Physics*; Wiley: New York, 1984.

³⁰Adhikari, A.K. and Singhal, O.P. 1991. Changes in the flavour profile of indirectly heated UHT milk during storage: effect of Maillard browning and some factors affecting it. *Indian Journal of Dairy Science* 44, 442-448.

³¹Zadow, J.G. 1970. Studies on the ultra heat treatment of milk. II. Measurement of browning reactions as influenced by processing and storage. *Australian Journal of Dairy Technology* 25, 123-126.

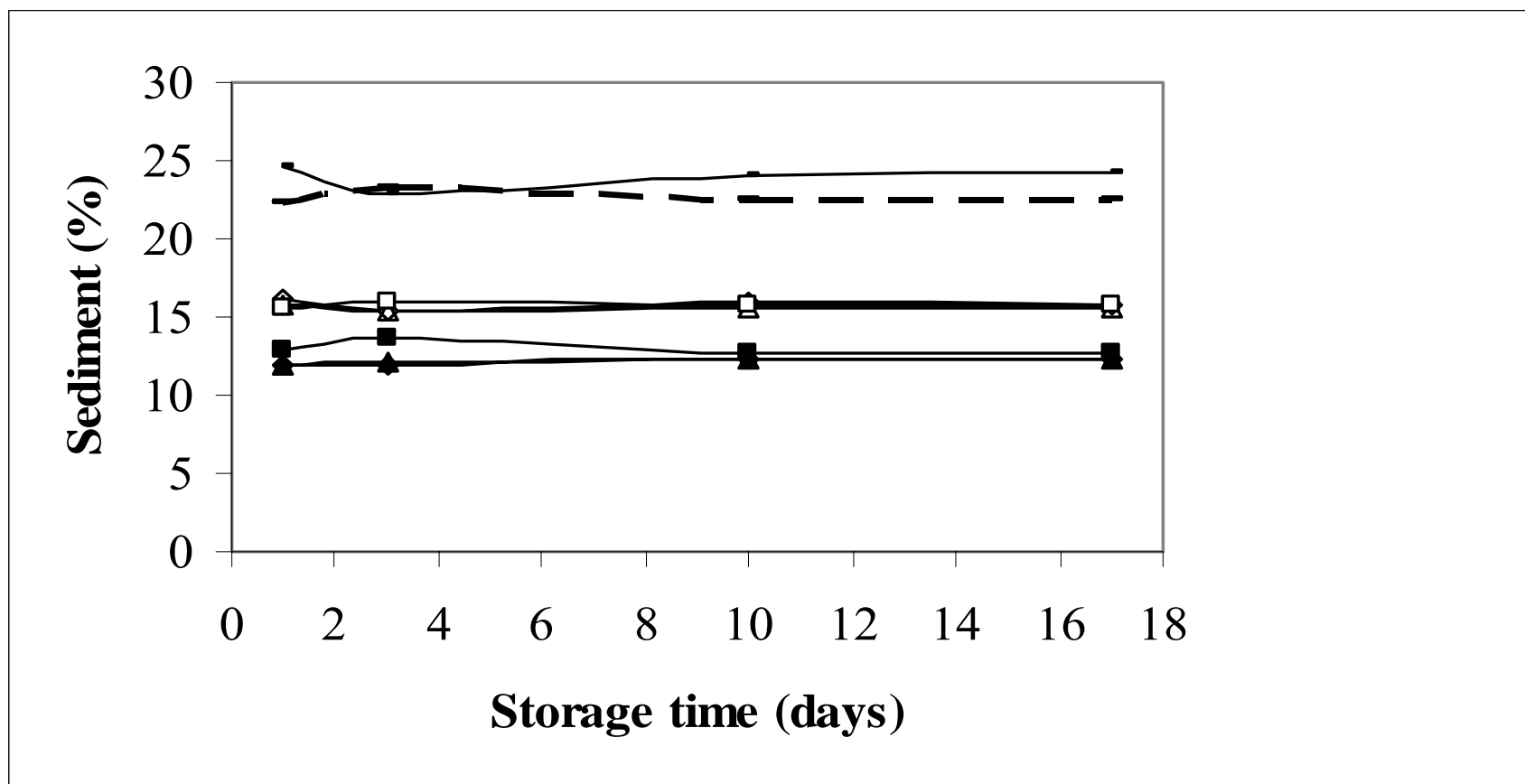


Figure 1. % Sediment measured after centrifugation (3000 x g, 20 min) of whey-banana beverage (12 g sample size) as a function of storage time. (◆) UHT treated whey-banana beverage stored at 4C, (◇) non-UHT treated whey-banana beverage stored at 4C, (▲) UHT treated whey-banana beverage stored at 20C, (△) non-UHT treated whey-banana beverage stored at 20C, (■) UHT treated whey-banana beverage stored at 30C, (□) non-UHT treated whey-banana beverage stored at 30C, (---) UHT treated whey-banana beverage stored at 40C, (—) non-UHT treated whey-banana beverage stored at 40C.

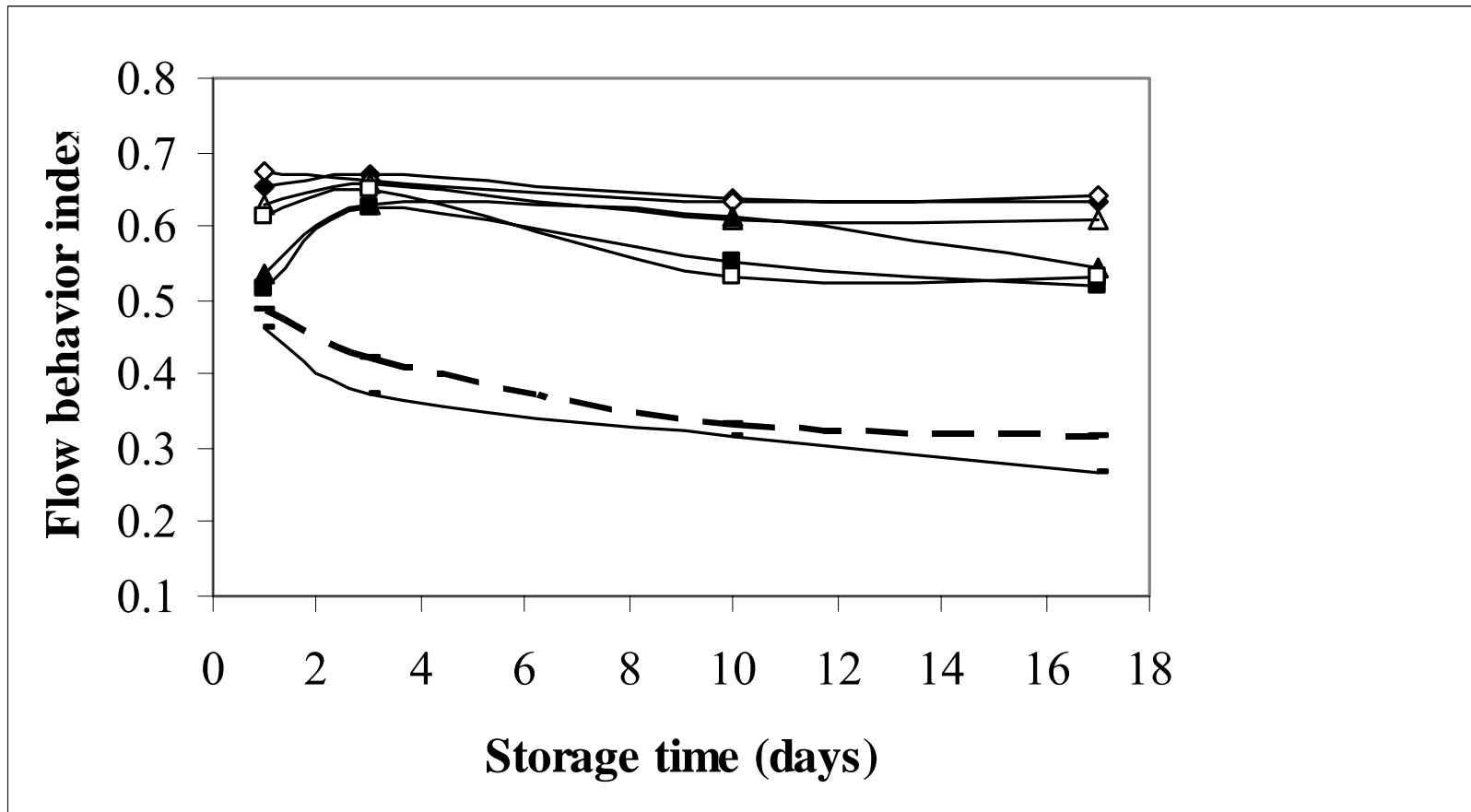


Figure 2. Flow behavior index of whey-banana beverages as a function of temperature and storage time.
(Refer to Figure 1 for legend)

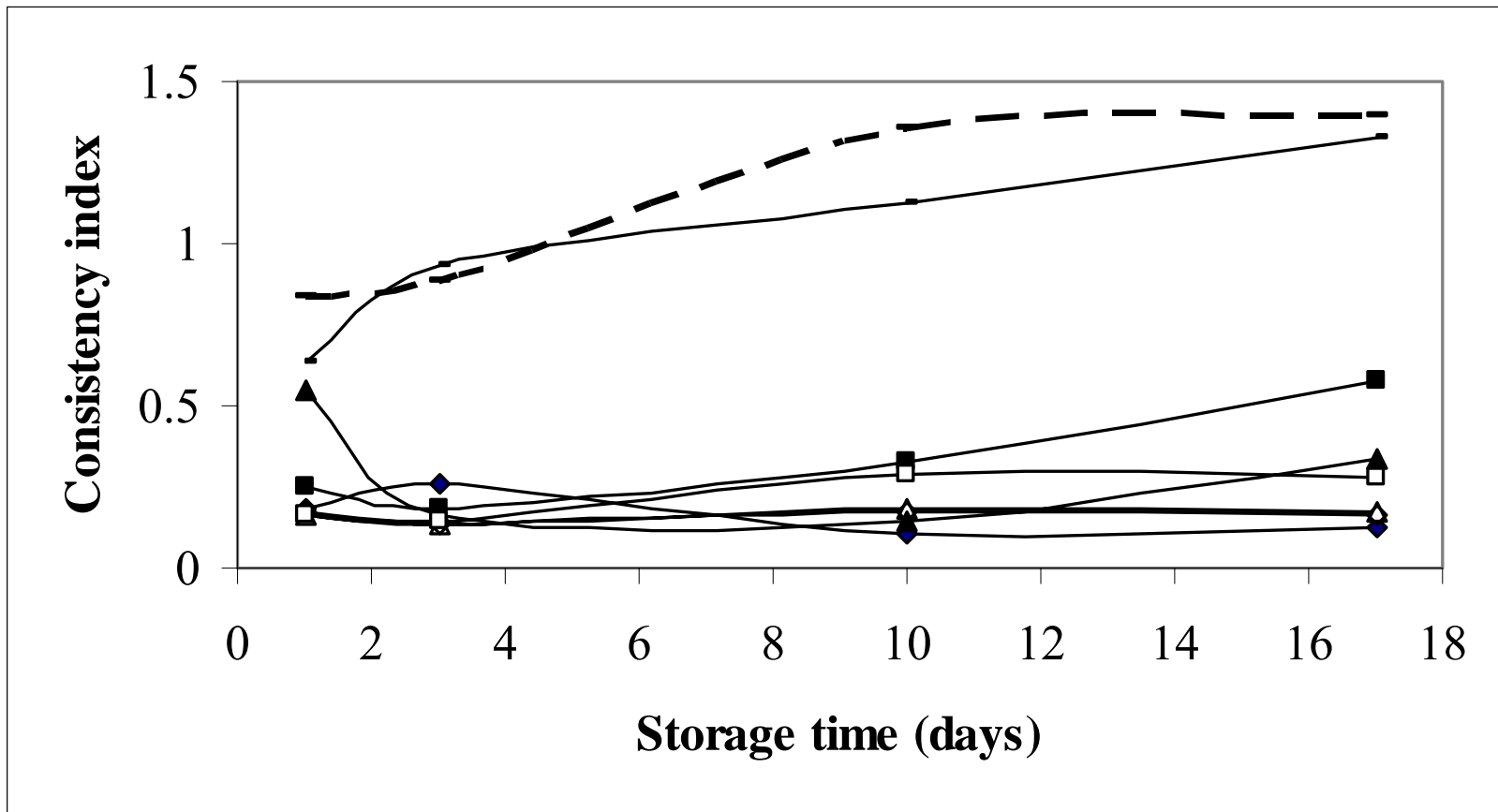


Figure 3. Consistency index of whey-banana beverages as a function of storage temperature and time
(Refer to Figure 1 for legend)

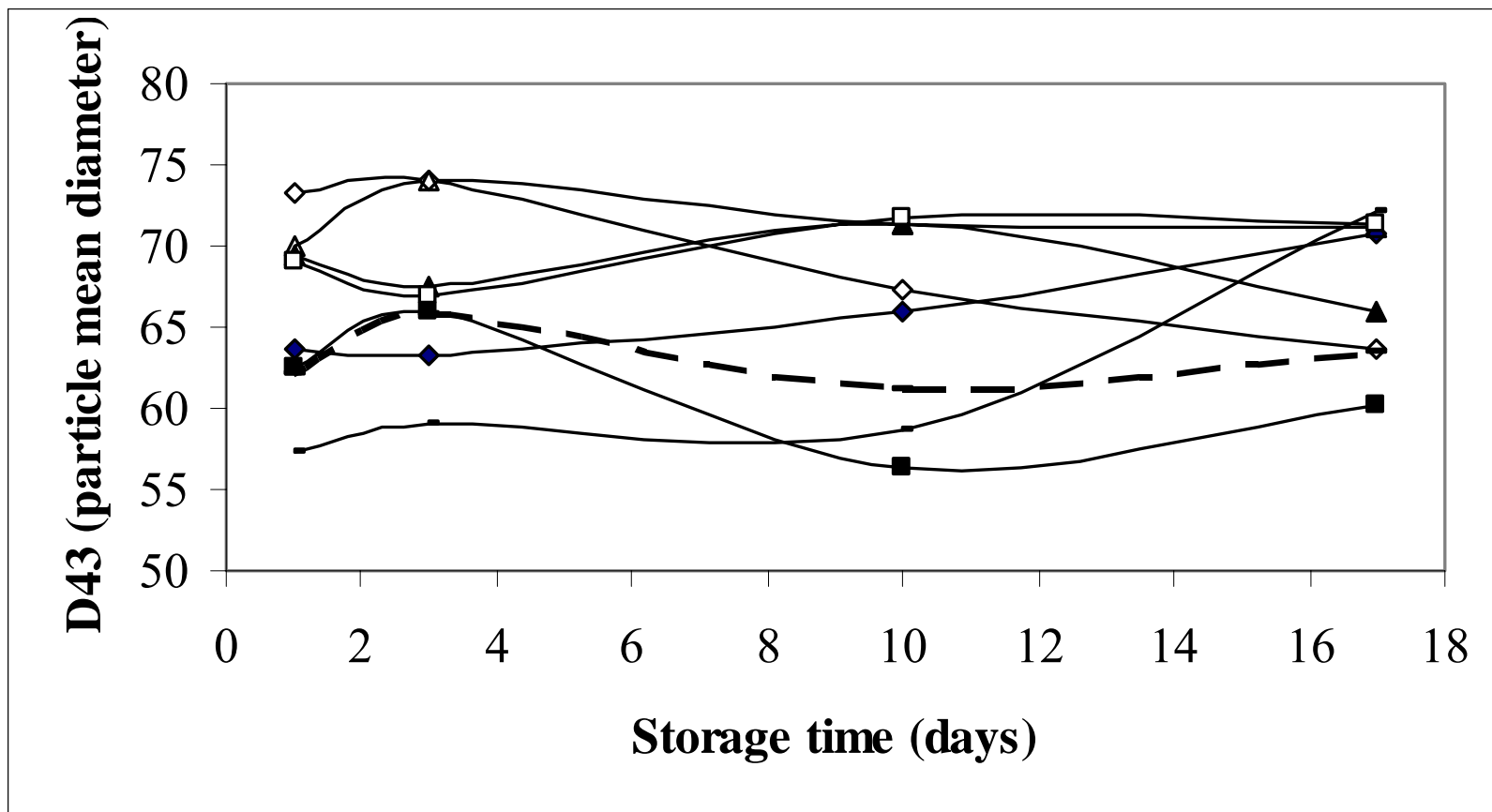


Figure 4. Average of volume mean diameter obtained from Malvern Mastersizer of whey-banana beverages as a function of storage temperature and time. (Refer to figure 1 for legend).

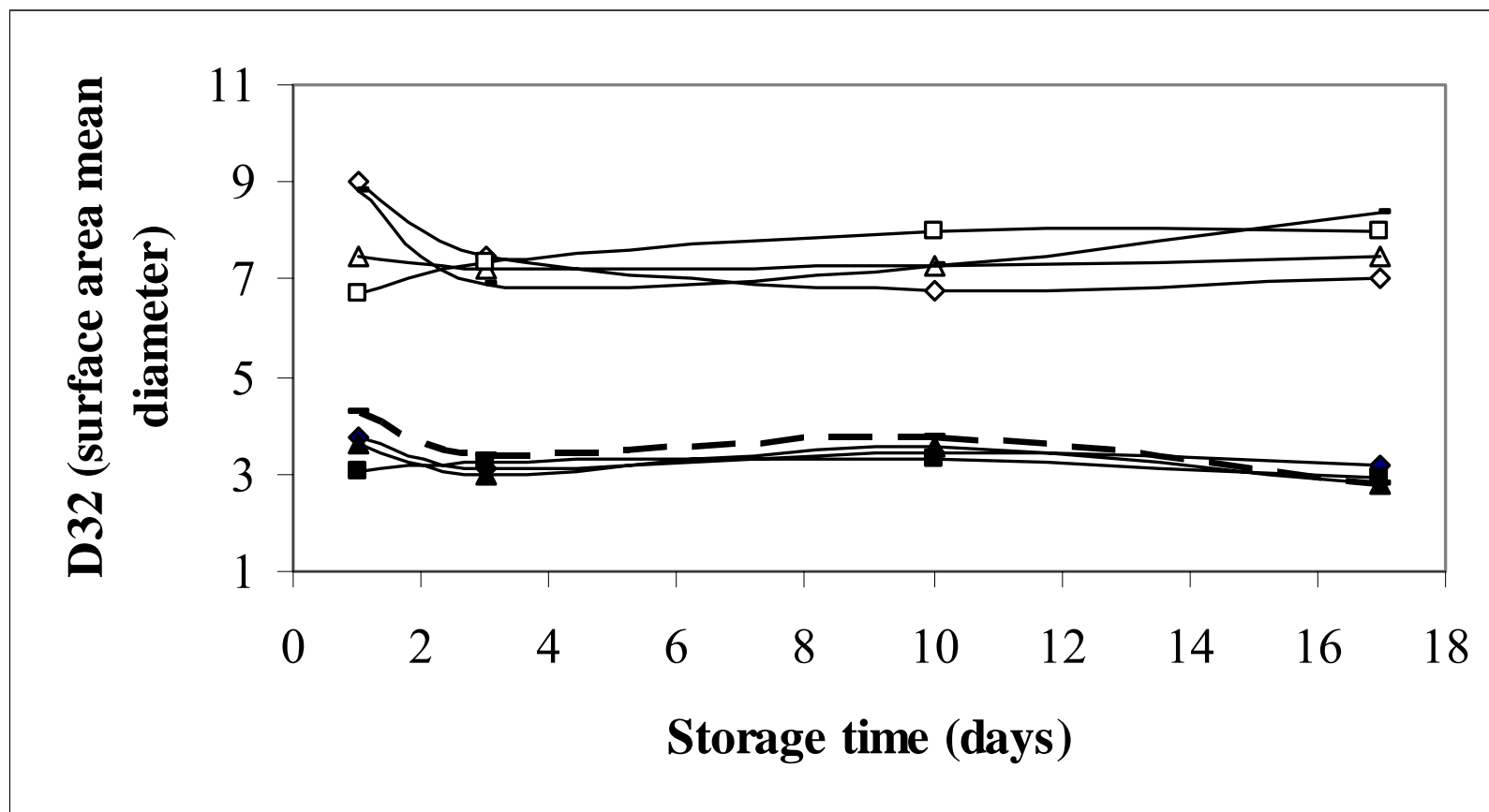


Figure 5. Average of surface area mean diameter of particles obtained from Malvern Mastersizer of whey-banana beverages as a function of storage temperature and time. (Refer to Figure 1 for legend).

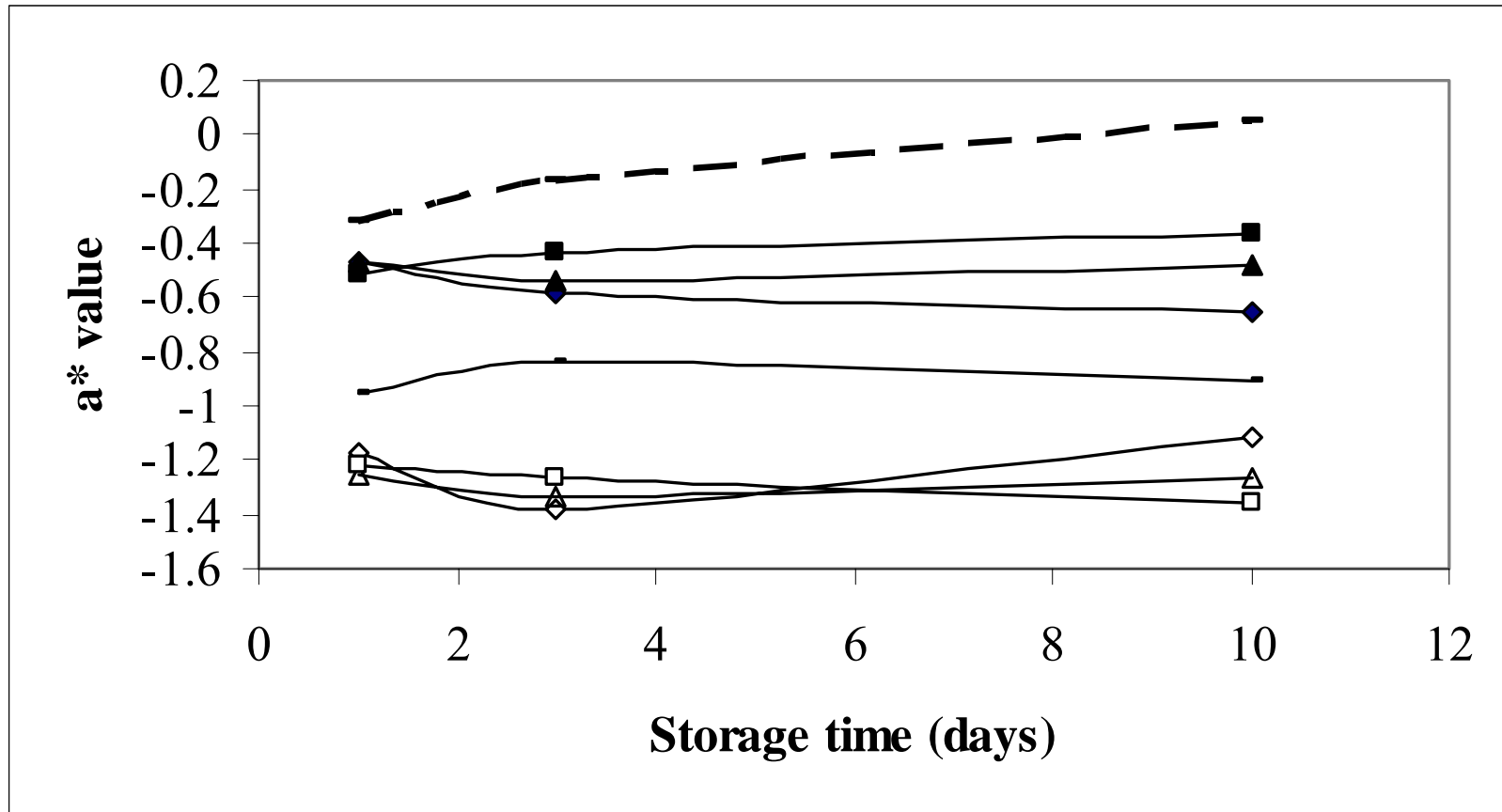


Figure 6. Color measurement of whey-banana beverages (a^* -value) as a function of storage temperature and time. (Refer to Figure 1 for legend).

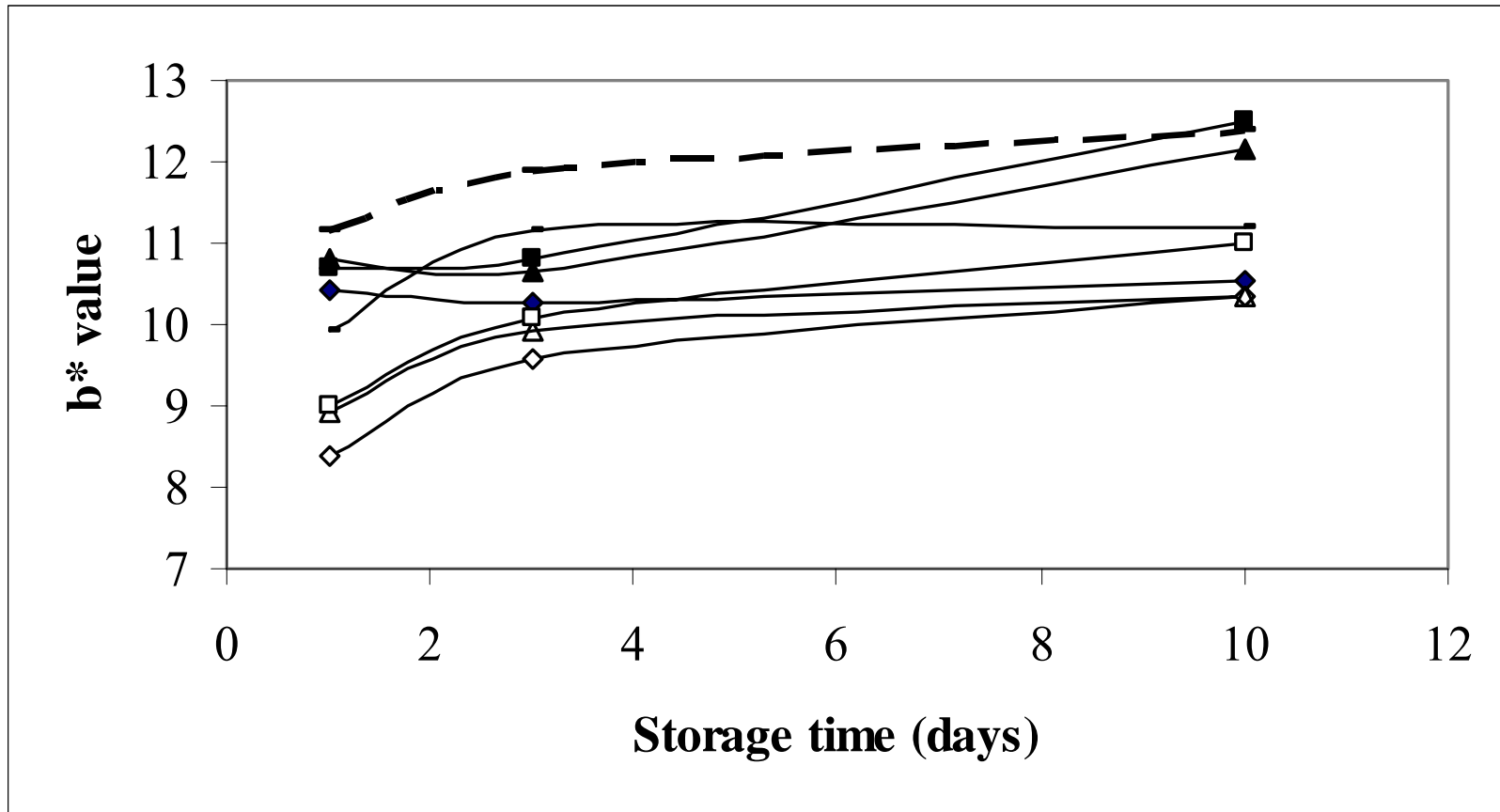


Figure 7. Color measurement of whey-banana beverages (b^* -value) as a function of storage temperature and time. (Refer to Figure 1 for legend).

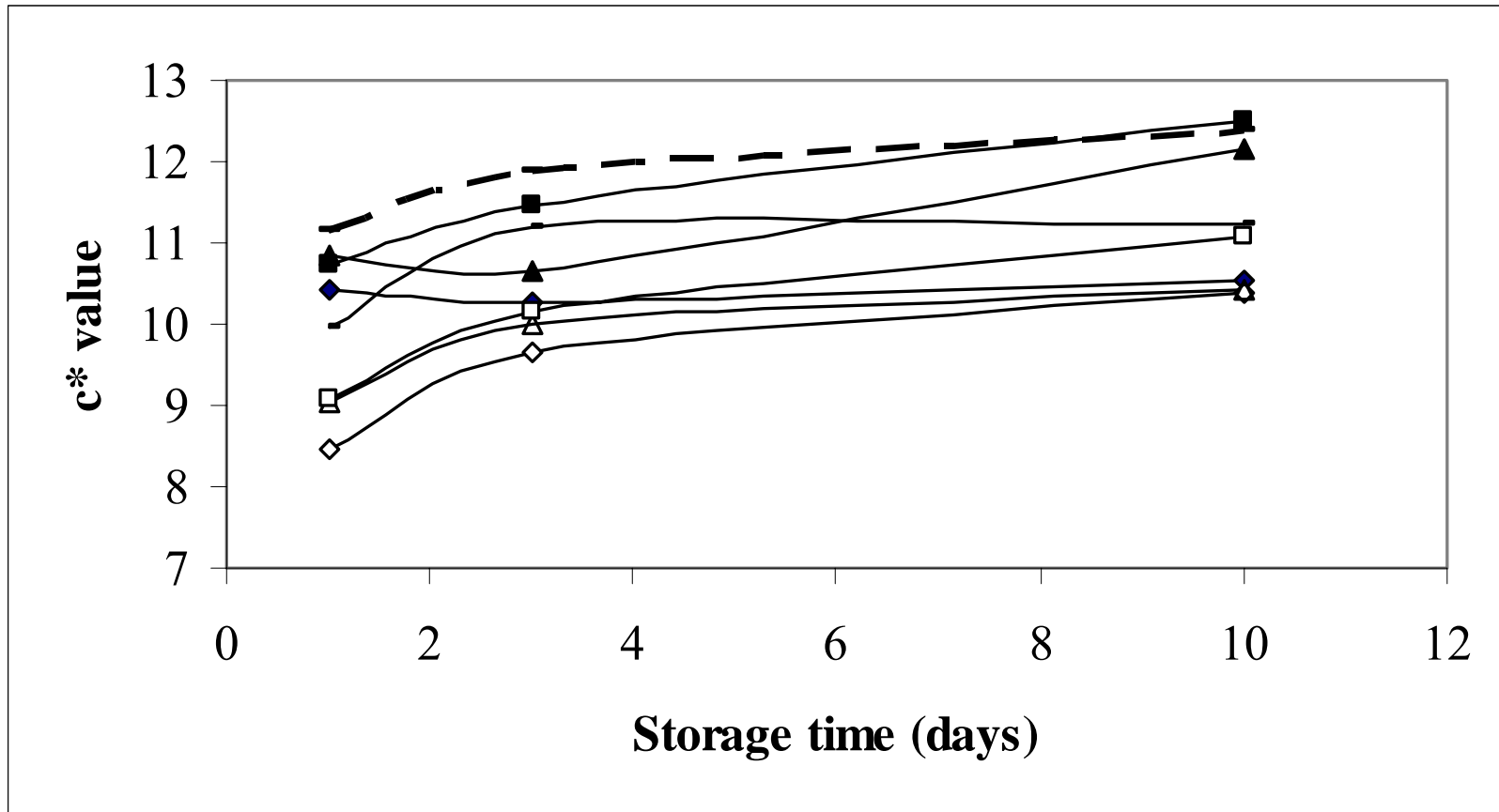


Figure 8. Color measurement of whey-banana beverages (c^* -value) as a function of storage temperature and time. (Refer to Figure 1 for legend)

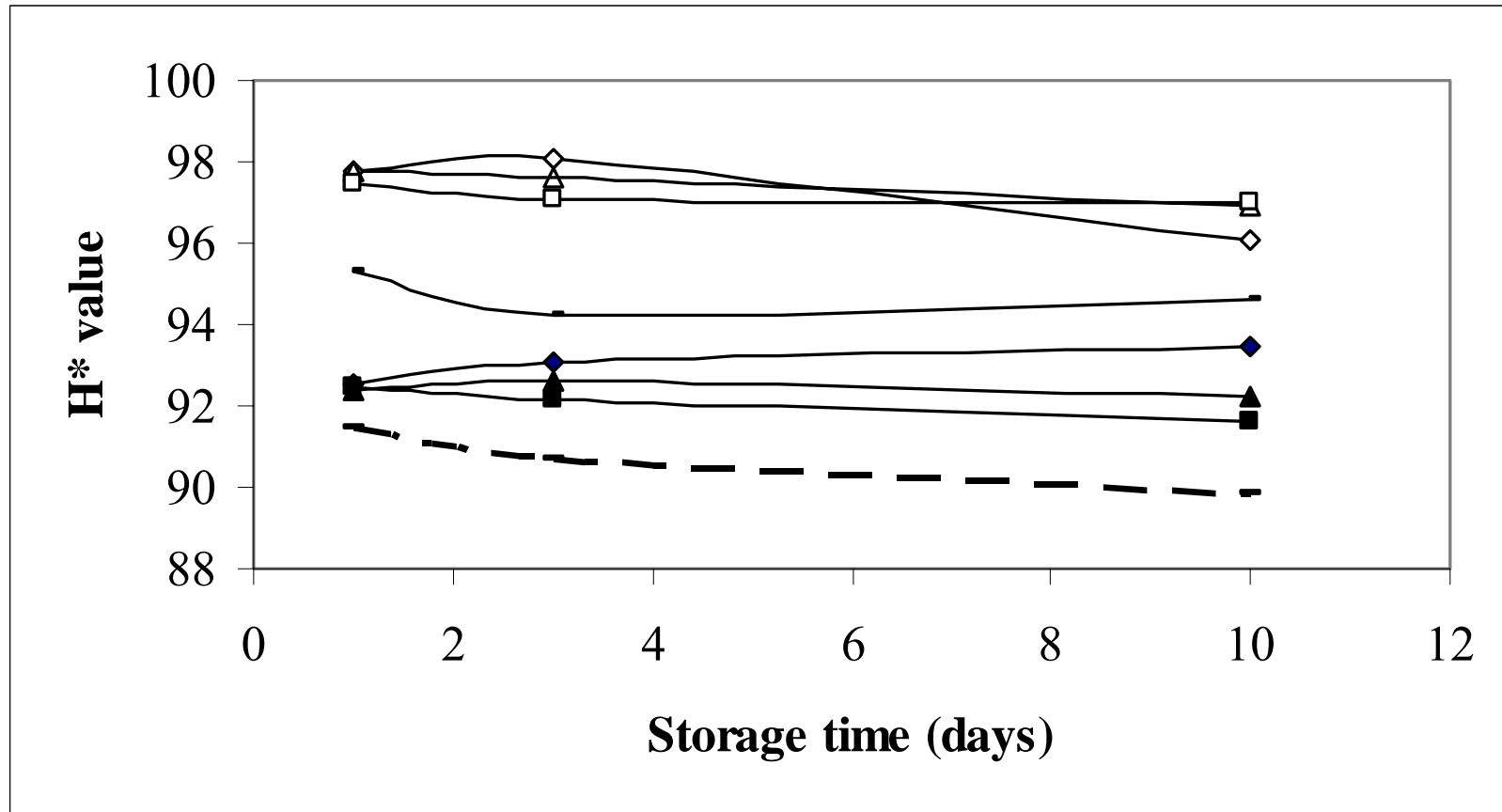


Figure 9. Color measurement of whey-banana beverages (H^* -value) as a function of storage temperature and time. (Refer to Figure 1 for legend).

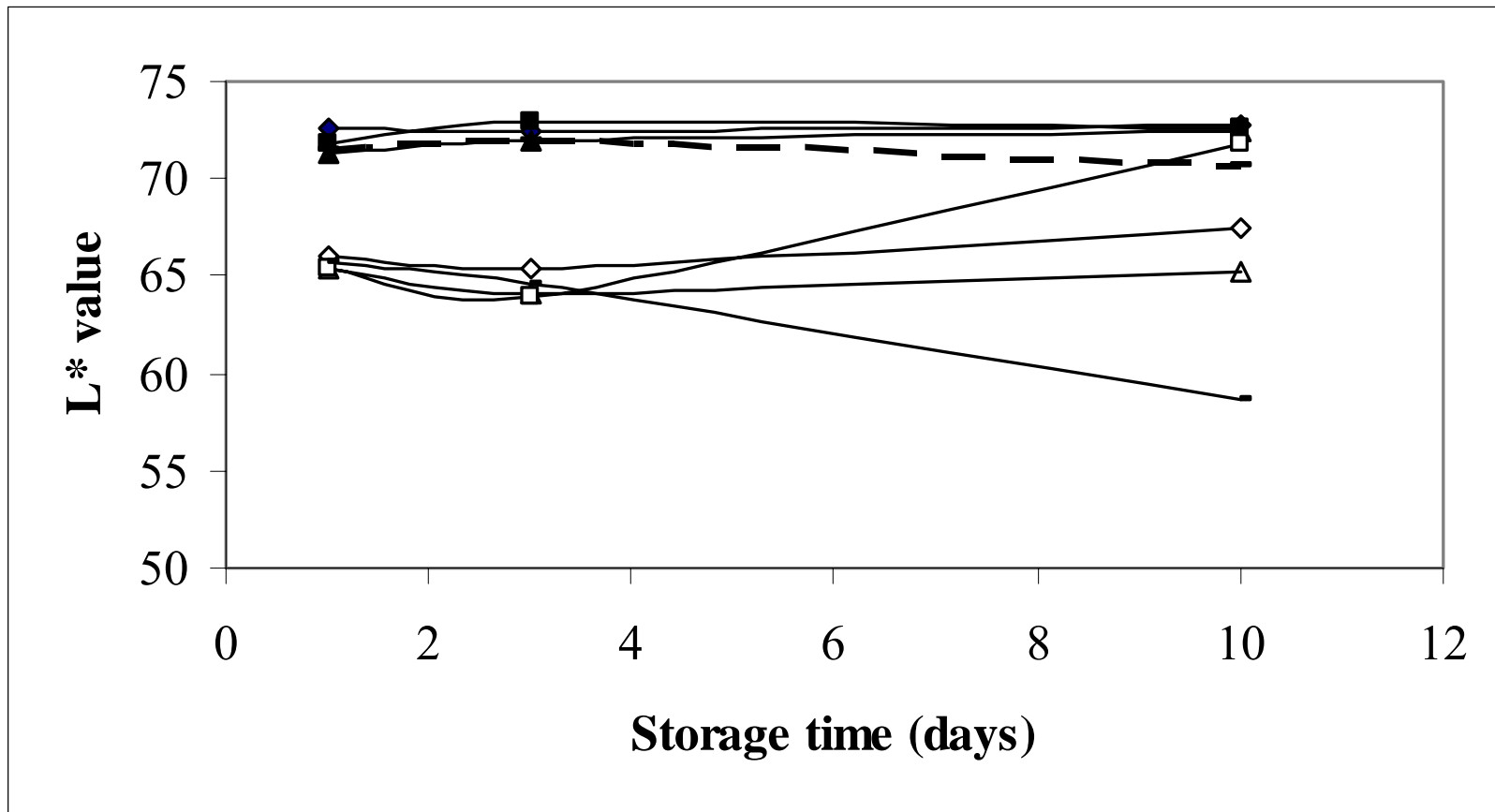


Figure 10. Color measurement of whey-banana beverages (L^* -value) as a function of storage temperature and time. (Refer to Figure 1 for legend)

Table 1. Summary of Anova data for factors influencing the physical properties of UHT and non-UHT whey-banana beverages

Factors	% Sed ⁴	n ⁵	K ⁶	⁷ D ₄₃	⁸ D ₃₂	a* ⁹	b* ¹⁰	c* ¹¹	H* ¹²	L* ¹³
<i>Main effects</i>										
Process ¹	S*****	NS	S*****	S*****	S*****	S*****	S*****	S*****	S*****	S*****
Time ²	NS	S*****	S*****	NS	S*	S*****	S*****	S*****	S*****	NS
Temp ³	S*****	S*****	S*****	S*****	NS	S*****	S*****	S*****	S*****	S*****
<i>Inter-actions</i>										
Process/ Time	S*****	NS	S*	NS	NS	S*****	S*****	S*****	S*****	S**
Process/ Temp	S*****	S****	S*	S*****	NS	S*****	S*****	S*****	S*****	S*****
Time/ Temp	S*	S*****	S*****	S*	S**	S*****	S*****	S*****	S*****	S*****
Process/ Time/ Temp	S**	NS	S****	S*****	NS	S*****	S*****	S*****	S*****	S*****

S***** represents a significant effect at $p < 0.0001$, S**** represents a significant effect at $p < 0.001$, S** represents a significant effect at $p < 0.01$, S* represents a significant effect at $p < 0.05$, NS indicates no significant effect ($p \geq 0.05$).

¹The processing mode (UHT or not UHT), ²Storage time, ³Storage temperature, ⁴Sedimentation (% w/w), ⁵Flow behavior index, ⁶Consistency coefficient, ⁷Particle volume mean diameter, ⁸Particle surface area mean diameter, ⁹redness, ¹⁰yellowness, ¹¹Chroma, ¹²Hue, ¹³Whiteness.

CHAPTER 7

SUMMARY AND CONCLUSIONS

In this study, whey-banana beverages were formulated as a way to use bananas that would otherwise be discarded for being overripe. A major challenge in tropical fruit beverages is the prevention of sedimentation. This work is a step in that direction by studying the cloud components of other tropical fruits including orange, mango, and banana pulps. Identification and characterization of the compounds present in the fruit pulps helped to get a better grasp of the nature of the compounds contributing to the cloud properties of the whey-banana beverage. Low molecular weight polypeptides as well as similar ratio of amino acids were observed in the selected tropical fruits. The total phenolic content of banana pulp was higher than that of mango and orange. Banana pectin, unlike mango or orange pectin was non-branched.

Model systems were formulated and revealed a strong interaction between whey proteins and added pectin for the rheological properties of the whey-banana beverages. Sucrose increased the particle sizes in the whey-banana beverages.

Particle size analysis indicated the resistance of the whey-banana beverages to particle growth during storage. Serum separation increased with temperature and did not mirror sedimentation. This was evidence that serum separation and sedimentation occurred for different reasons. High viscosity may help prevent serum separation at low temperature while the large particles in the whey-banana beverages may not resist sedimentation upon centrifugation. The color dimensions were the best indicators for changes during storage.

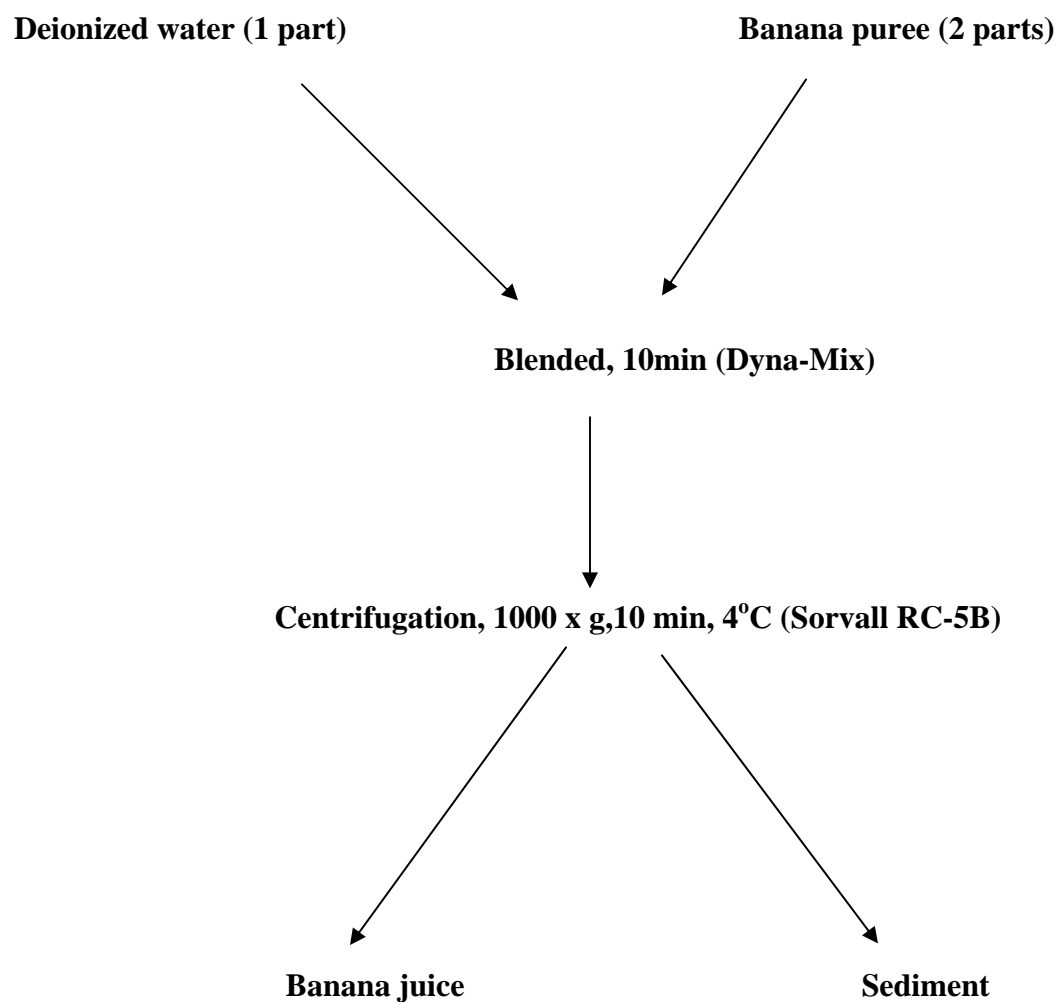
Investigation of the sediments and supernatants collected after centrifugation of the whey-banana beverages revealed that the pectin content was similar for the supernatant and the sediments. However proteins and phenolics were found at higher levels in the sediments when compared to the supernatants. This result is likely due to preferential binding of proteins to phenolics.

Sensory descriptive panel assessment of the whey-banana beverage indicated that attributes like banana flavor, smoothness, sweetness and acidity were maintained up to 60 days. Consumer evaluation indicated a split decision over the acceptability of the whey-banana beverage. Two segments of the consumer population were obtained which liked or disliked the beverage for flavor, sourness, and sweetness.

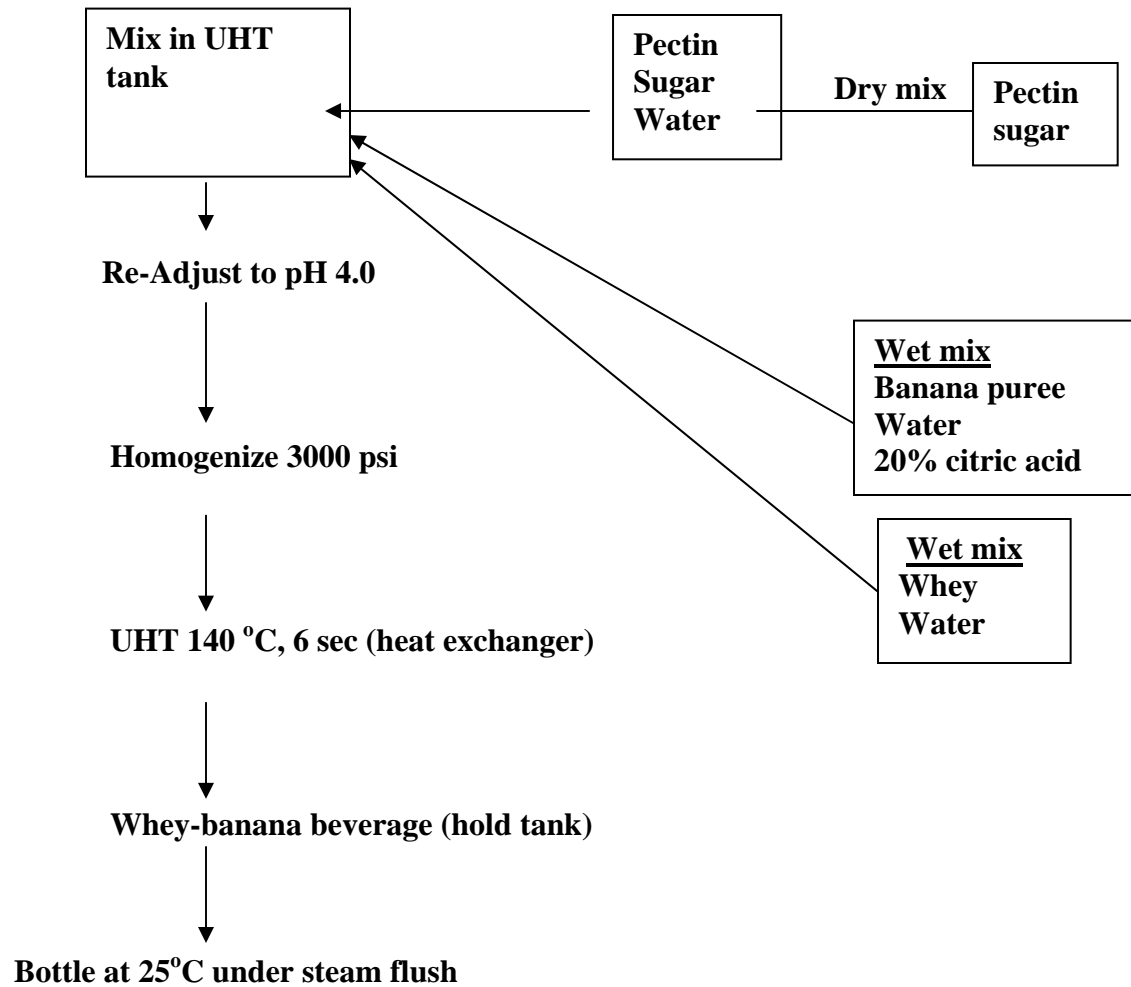
The effect of heating of whey-banana beverages during the UHT process reduced particle size and the amount of sediment produced in the beverage, whereas color was enhanced for the UHT treated beverage. The resistance of the particles to growth for the whey-banana beverages during storage was also observed with the non-UHT treated beverage.

The sour taste and acidity of the whey-banana beverage could be improved. Other acidulant such as malic acid (main organic acid in banana) (Shimokawa et al. 1972) could be used instead to see how perceived sourness is affected. The sugar level can be reduced to a level that satisfies the consumer. However, the sugar acid ratio should be carefully balanced to make a product of superior quality. A slight increase in pH of the product and the effects of combination of acid and concentration needs further research. The segment of the population that found the beverage acceptable, liked its smooth texture and sweet taste. According to Shewfelt (2000) the acceptability expressed as a percentage distribution of the segment that likes the product can be modeled as a function of the critical quality characteristic that drives acceptability.

Finally, the potential to develop a nutritious product from banana and whey is real and could help improve the nutritional status of populations in developing countries.

APPENDIX

Flow diagram of banana-juice preparation
(Refer to page 101, chapter 4)



**Flow sheet for the production of whey-banana beverages
(refer to page 118, chapter 5)**

Averages of % Sediment of centrifuged (3000 x g, 20 minutes), homogenized (3000 psi) whey dispersions (pH 4.0) with varying pectin levels.

