THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Food Science)
DEGREE

Food Science
FIELD

Food Science and Technology
DEPARTMENT

TITLE: The Effect of Okra Cell Wall and Okra Polysaccharide on Physical Properties and Stability of Ice Cream

NAME: Miss Pilapa Yuennan

THIS THESIS HAS BEEN ACCEPTED BY

______________________ **THESIS ADVISOR**

(Assistant Professor Tanaboon Sajjaanantakul, Ph.D.)

______________________ **THESIS CO-ADVISOR**

(Associate Professor Parichat Hongsprabhas, Ph.D.)

______________________ **THESIS CO-ADVISOR**

(Professor H. Douglas Goff, Ph.D.)

______________________ **DEPARTMENT HEAD**

(Assistant Professor Wannee Jirapakkul, Ph.D.)

APPROVED BY THE GRADUATE SCHOOL ON ______________________

______________________ **DEAN**

(Associate Professor Gunjana Theeragool, D.Agr.)

THESIS

THE EFFECT OF OKRA CELL WALL AND OKRA POLYSACCHARIDE ON PHYSICAL PROPERTIES AND STABILITY OF ICE CREAM

PILAPA YUENNAN

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Food Science)
Graduate School, Kasetsart University
2014

Pilapa Yuennan 2014: The Effect of Okra Cell Wall and Okra Polysaccharide on Physical Properties and Stability of Ice Cream. Doctor of Philosophy (Food Science), Major Field: Food Science, Department of Food Science and Technology. Thesis Advisor: Assistant Professor Tanaboon Sajjaanantakul, Ph.D. 149 pages.

Texture quality of ice cream is strongly affected by ice recrystallization. In this study, water soluble polysaccharide from the okra plant (OKP) and its corresponding cell wall extract (OKW) were prepared, and their physico-chemical properties were determined. OKW showed the better water holding capacity, foaming capacity than OKP. OKP had the high water solubility, foaming stability, apparent viscosity, freeze-thaw stability, and showed synergistic effect with guar gum. The effects of varying concentrations (0.00, 0.15, 0.30, and 0.45% (w/w)) of OKP and OKW on physical characteristics and stability of ice cream were investigated. The ice cream mix viscosity was determined as well as ice cream overrun, ice cream meltdown, and consumer perceptibility. The ice recrystalization was determined after ice cream was subjected to fluctuated temperature in the range of -10 to -20^{o}C storage for 20 days. The content of OKP and OKW increased significantly the ice cream mix viscosity, due to water binding of the polysaccharide. The addition of OKP significantly increased overrun of ice cream and improved the melting characteristic. Moreover, OKP and OKW could slow down ice crystal growth. Thus, our results suggest the potential use of OKP and OKW as stabilizer to control ice recrystallization and ice cream quality. A high relationship (R^2 = 0.94) between rheological parameters of ice cream mix and ice crystal growth of ice cream was found. Hence, the measurement of ice cream mix rheological properties (yield stress and storage modulus (G´)) can be used to predict ice crystal growth in ice cream.

______________________________ ______________________________ ___ / ____ / ____

Student's signature Thesis Advisor's signature

ACKNOWLEDGEMENTS

I would like to express my great appreciation to my advisor, Dr. Tanaboon Sajjanatakul for your guidance and support. I would also like to thank the members of my committee, Dr. Parichat Hongsprabhas and Dr. Douglas Goff, for their great addition advice and support, as well as the financial support from Thailand Research Fund through the Royal Golden Jubilee Ph.D. program (Grant No. IUG/0020/2550).

I would like to acknowledge all my friends, lab members, faculty members and staff of the department of Food Science and Technology, Kasetsart University and department of Food Science, University of Guelph for their support, advice and friendship.

I would like to thank Dr. Lisa Duizer for her kind help to perform the ice cream sensory test. Special thanks to Carlos Mendez-Velasco for the friendship and technical support.

A very special acknowledgment to my parents, my sisters for their love and support me all my life.

Pilapa Yuennan
February 2014

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF TABLES (Continued)

Appendix Table **Page**

LIST OF FIGURES

LIST OF FIGURES (Continued)

Figure **Page**

LIST OF FIGURES (Continued)

Figure **Page**

LIST OF FIGURES (Continued)

THE EFFECT OF OKRA CELL WALL AND OKRA POLYSACCHARIDE ON PHYSICAL PROPERTIES AND STABILITY OF ICE CREAM

INTRODUCTION

Okra, *Abelmoschus esculentus* (L.) Moench, a native plant of Africa, is now cultivated throughout the tropical and warm temperature regions around the world (Rudrappa, 2013; BeMiller *et al.*, 1993). Okra pod is usually harvested while it is green, tender, and immature, which takes only about 45-60 days after seeding to get ready to harvest. The immature okra pod, is normally used as a vegetable in soup and stews (Rudrappa, 2013), contains thick and slimy mucilage which is mainly polysaccharide (Ndjouenkeu *et al.*,1997), the polysaccharide can be obtained by aqueous extraction of fresh or dried and ground pod (Ramsden, 2004). Previously, it has been reported that okra polysaccharide is viscous, pseudoplastic and viscoelastic aqueous dispersions (BeMiller *et al.*, 1993). In year 1977, Woolfe *et al*. pointed out that the okra polysaccharide dispersion rapidly increased viscosity with concentration and formed gel at high concentration. In addition, okra polysaccharide also shows having unusual lubricity properties and to be able forming a tenacious coating on the skin which is difficult to remove by washing (BeMiller *et al*, 1993). The interesting unusual properties of okra polysaccharide are possible offering an alternative stabilizer to food industry.

Ice cream is a complex food colloid consists of small air bubbles and ice crystals disperse in continuous unfrozen serum phase (Marshall *et al.*, 2003). The quality of ice cream is highly dependent on the physical structures. However, ice crystal perhaps plays the most important role in texture quality. Ice cream often present a coarse texture after storage, a defect commonly attributed to the presence of large ice crystals. This deteriorative effect is a result of ice recrystallization and is more prevalent in products stored at higher temperature or that undergoes temperature fluctuation during storage and distribution (Marshall and Arbuckle, 1996).

Minimizing ice recrystallization is thus one of the greatest concerns to the manufacturer in order to maintain ice cream smooth texture. In ice cream making, stabilizers have been used to prevent ice recrystallization, produce smoothness in body and texture, and provide uniformity of product and resistance to melting (Marshall and Arbuckle, 1996). Normally, stabilizers such as guar gum and locust bean gum are used in ice cream to retard the growth of ice crystal (Caldwell *et al*., 1992). However, those traditional used polysaccharides can protect the ice cream texture quality for short time. It should be better if ice cream is more resistant to temperature fluctuation, because it will be easy to handle even during storage and distribution in a warm climate country zone. The searching for a novel polysaccharide that provides the better function, more powerful, higher efficiency for ice recrystallzation retardation is a challenge.

A water soluble okra polysaccharide (OKP) as well as its corresponding cell wall (OKW); the sample with a simple preparation method, were interested for this challenge. The developing ingredient formulation of ice cream that slow down the growth of ice crystal require the understanding of the ingredient physic-chemical properties, the ice cream product characteristics and the relationship between ingredient properties and stability of ice cream. The overall objective of this research was to investigate the effect of OKP and OKW on physical properties and stability of ice cream. The understanding the relationship between rheological properties of ice cream mix and ice crystal growth of ice cream may help to predict the stability of ice cream. This contributes to lower ice recrystallization as well as retention of ice cream texture quality which significantly affects the consumer acceptance. The stabilization of ice cream by rheological modification benefits the promotion of shelf life of ice cream, consumer, as well as ice cream industry.

OBJECTIVES

1. To prepare the okra cell wall (OKW) and water soluble okra polysaccharide (OKP) and investigate their physico-chemical properties.

2. To determine the rheological properties of OKW and OKP in ice cream mix model.

3. To investigate the effect of OKW and OKP concentration on physical properties and stability of ice cream.

4. To study the relationship between rheological properties of ice cream mix and ice crystal growth in ice cream as a function of OKP addition.

LITERATURE REVIEW

Okra

1. Okra plant

Okra, *Abelmoschus esculentus* (L.) Moench, family Malvaceae, is an African origin plant that is introduced into other warm climate areas such as Middle East, the Southern states of the USA and numerous countries of Asia such as India, Malaysia, Philippines and Thailand (Gilles, 2002). The okra plant is easy growing and prolific. Its pod is, 8 to 15 inches long, ready for harvest in about 45 days after seeding. The pod, is harvested while still tender and immature (K center, 2013), is used as fresh as vegetable in soups and stews. Okra canning, freezing or drying are also processed (Center for New Crops and Plant Products, 2007). Moreover, it is also used as a traditional medicine for many different purposes such as diuretic agent, for treatment of dental diseases, and to reduce and prevent gastric irritation (Ndjouenkeu *et al*., 1996). This medicinal characteristic is suggested to originate from the high polysaccharide content of immature okra pod (Ndjouenkeu *et al*., 1996). Okra polysaccharide composition, functional and nutritional properties have been the subject of studies for many years (Woolfe *et al*., 1977). However, it was reviewed in next part of the thesis.

World production of okra is around 6.9 million tons per year (FAO, 2012). India is the world leader in okra production (4.8 million tons). Other major okra producing countries are Nigeria (0.95 million tons), Sudan (0.26 million tons) (Boone, 2006). Thailand is 17th in world okra production (9.0 thousand tons). The major okra producing areas in Thailand are Nakornnayok, Patumthani, Nonthaburi, and Ayutthaya provinces (Department of Agriculture Extension (Thailand), 2013). Okra pod is not only used as fresh vegetable or can be used to thicken soups and stews (BeMiller *et al*., 1993) but it is also applied for many food and nonfood products. Okra flour was added to bread production to extend dough resistance (Acquistucci and Francisci, 2002). Okra gum was used in chocolate bar cookies as fat replacer

(Romanchik-Cerpovicz *et al*., 2002). Okra polysaccharide was used for whey protein-okra polysaccharide composite edible film (Prommakool *et al*., 2010). Moreover, okra flour was found to posse antioxidant activity which increases by roasting. It has been reported to rich in phenolic compounds (Arapitsas, 2008). Okra seed is a high-protein-oilseed, considered to be used to complement with other protein sources (Bryant *et al*., 1988).

2. Plant cell wall

The plant cell is enclosed by a cell wall which consists of different macromolecules to form a complex network. Generally, cell wall of higher plant consists predominantly of polysaccharides (cellulose, hemicelluloses and pectin) with a relatively small proportion of proteins, phenolic compound and lipid (Aman and Graham, 1990). The schematic of plant cell wall is shown in Figure 1. The hemicellulose is linked to two or more cellulose microfibrils via hydrogen bonding (Carpita and Gibeaut, 1993). Pectin and structural protein are suggested being as a co-extensive network which is physically entangled with the cellulose-hemicellulose network (Cosgrove, 2001).

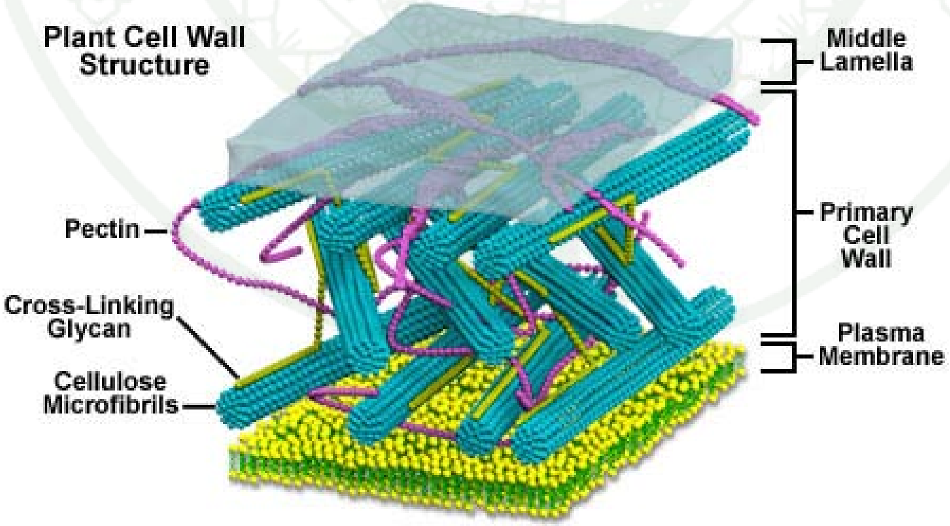


Figure 1 Schematic view of the plant cell wall complex network

Source: Davidson (2013)

2.1 Cellulose

Cellulose is the major component which determines the strength properties of wood pulp fiber (Akerholm *et al*., 2004). It is a homo-polysaccharide consisting of a backbone of β (1, 4)-linked-D-glucosyl residues with wide molecular weight distribution (Aman and Westerlund, 1996). A cellulose molecule in a higher plant cell wall may contain 2000-6000 glucose building blocks (Delmer, 1987).

2.2 Hemicelluloses

Hemicelluloses are defined as polysaccharide in plant cell walls which are solubilized by aqueous alkali. Xyloglucans, ylans, mannans and arabinogalactans are the most abundant representatives of hemicellulose (O’Neill and York, 2003).

2.3 Pectins

Pectins are an important cell wall component of plants and probably the most complex macromolecule in nature (Vincken *et al*., 2003). The highest concentrations of pectin are found in the middle lamella of cell wall, with a gradual decrease as one passes through the primary wall toward the plasma membrane (Thakur *et al*., 1997). The pectin backbone has been found to be composed of two main structural elements, homogalacturonans or smooth regions and rhamnogalacturonans (RGs) or harried regions (Schols *et al*., 1990). Schematic representation of the basic structure of pectin is shown in Figure 2.

Homogalacturonan (HG) is linear polymer, can be called smooth regions of pectin (Jayani *et al*., 2005). It is composed of a backbone of α-(1,4)-linked-D-galacturonosyl residues in which a variable part of the galacturonic acid is methyl esterified (Talmadge *et al*., 1973). Rhamnogalacturonan (RG) I contains a backbone of alternating α-(1,2)-linked-L-rhamnosyl and α-(1,4)-linked-D-galacturonosyl residues with the ratio of rhamnose to galacturonic acid of 1:1 (Schol *et al.*, 1990). The galacturonic residues can be acetylated and both residues can carry side chains of neutral sugars as galactose, arabinose and xylase (Willats *et al*., 2006). And rhamnogalacturonan II is a homogalacturonan chain with complex side chains attached to the galacturonic residues (Willats *et al*., 2006).

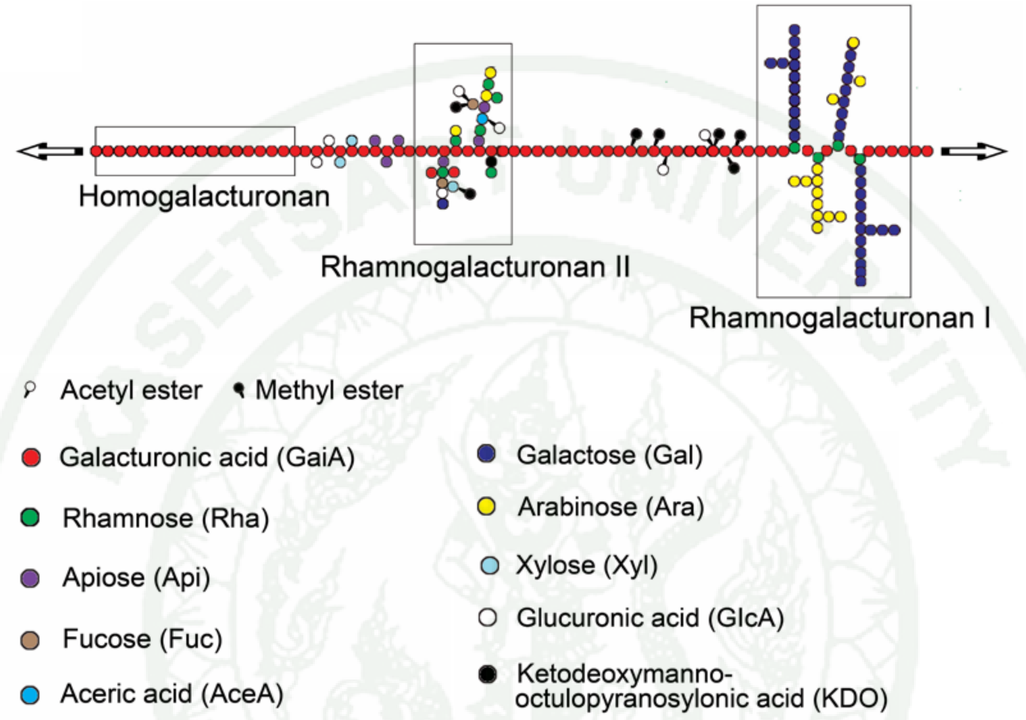


Figure 2 Schematic representations of the basic structure of pectin

Source: Willats *et al*. (2006)

3. Okra cell wall and water soluble okra polysaccharide structures

3.1 Okra cell wall structure

The cell wall structure of okra consists mainly glucose (44 mol %), galactose (17 mol %) and galacturonic acid (16 mol %). In addition to polysaccharides, the okra cell wall also contained 15.8% of protein. The sugar composition suggests that okra cell wall consisted of different types of polysaccharides including pectin, hemicellulose such as xylan and xyloglycan, and cellulose (Sengkhamparn *et al*., 2009). Okra cell wall composted of both water insoluble and water soluble polysaccharides.

3.2 Water soluble okra polysaccharide structure

The water soluble okra polysaccharide consists of mainly pectin (Woolfe *et al*., 1977). The structure of water soluble okra polysaccharide has been studied by many researchers. It was found firstly as an acidic polysaccharide consisting of galactose, rhamnose and galacturonic acid (Whistler and Conrad, 1954). According to the studied of Tomada *et al.* (1980), they concluded that the major polysaccharides component of okra which extracted with cold water and isolated with 10% cetyltrimethyl ammonium bromide contained repeating unit of alternating α-(1, 2)-linked rhamnosyl and α-(1, 4)-linked galactosyl moieties attached to O-4 of about half the l-rhamnosyl residues. The infrared spectrum of the okra polysaccharide had absorption bands at 1250 and 1730 cm^{-1}, suggested that the okra polysaccharide had ester linkages. Besides that, acetyl content of the okra polysaccharide was determined to be 5.5% and protein content was about 12%. Molecular weight of okra polysaccharide was estimated to be about $1.7 x 10^6$ daltons (Tomada *et al*., 1980).

Recently, okra polysaccharide has been studied by Sengkhamparn *et al*. (2009, 2010). Structurally, polysaccharide that was extracted with hot buffer composes of galactose (34 mol %), rhamnose (26 mol %), and galacturonic acid (35 mol %) in the ratio 1.3:1.0:1.3. The polysaccharide mainly contain rhamnogalacturonan type I (RG I) next to homogalacturonan (HG) (Sengkhamparn *et al*., 2010). The degree of acetylation is relatively high (DA = 58) while the degree of methyl esterification is relatively low (DM = 24) (Sengkhamparn *et al*., 2009). Okra polysaccharide comprised of charged (zeta potential -21.5mV), polysaccharides size was between 5 kDa (d ~ 3 nm) and 50 kDa (d ~ 200 nm) (Sengkhamparn *et al*., 2009). The hydrodynamic diameter was 30 ± 5 nm measured by dynamic light scattering. The polysaccharide dispersions demonstrate an elastic behavior (G´ > G´´) over a wide range of 10^{-1}-10 Hz frequencies, at strain of 10% (Sengkhamparn *et al*., 2010), which G´ (storage modulus) and G´´ (loss modulus) are the rheological parameters that represent the elastic behavior and viscous behavior of material, respectively (Mezger, 2002).

4. Okra polysaccharide functional properties

Okra polysaccharide is mainly constituted by galactose, rhamnose, and galacturonic acid. The polysaccharide can be extracted as a viscous gum (Ndjouenkeu *et al.*, 1996). Some researchers have studied okra polysaccharide extraction and their functional properties. The detail of these properties is described below.

4.1 Thickening

As it was mentioned earlier that the immature okra pod is normally used as a vegetable for thickening soup and stews (Rudrappa, 2013) since it contains thick and slimy mucilage which is mainly polysaccharide (Ndjouenkeu *et al.*,1997). And it has been reported that okra polysaccharide is viscous, pseudoplastic and viscoelastic aqueous dispersions (BeMiller *et al*., 1993). Thickening is the process of increasing viscosity of liquid, information about viscosity of okra polysaccharide is reviewed here.

According to the studying of Baht and Tharathan (1987), a viscosity of borohydride-soluble okra polysaccharide (BSP) was maximized at a pH range of 4-6. The BSP dispersion exhibited a pseudoplastic behaviour and its viscosity decreased when adding water-soluble agent. In contrast, its viscosity slightly increased with the addition of maltodextrins, which may be the result of hydrogen bonding interactions.

The viscosity of okra polysaccharide dispersion (0.5% w/w) decreased two times when heated to 70^{o}C as compared to the sample at room temperature. Upon cooling the dispersion back to room temperature, there was loss of viscosity (Monpien, 2005). Moreover, okra polysaccharides in solution with a fixed ionic concentration (0.1 M NaCl) gave intrinsic viscosity of 7.6 dl g^{-1}, showed shear thinning behavior, and strong tendency to self-association (Ndjouenkeu *et al*., 1996).

Ramsden (2004) mentioned that okra polysaccharide can act as a very good thickener but was only a weak emulsification.

4.2 Substituting

Okra polysaccharide also showed to have unusual lubricity properties (BeMiller *et al*., 1993) which was an important for a good food fat mimetic (Glicksman, 1991) and it could be used as fat substitute in many products like chocolate bars and cookies. Many quality characteristics of such low fat cookies were comparable with those of full fat cookies (Romanchik-Cerpovicz *et al*., 2002). Likewise, okra polysaccharide has also been used as a milk-fat substitute in chocolate frozen dairy desserts where it could replace the milk-fat up to 70% with the melting points of the products did not change, although the melting rate decreased slightly (Constantino and Romanchik-Cerpovicz, 2004).

Moreover, the polysaccharide also exhibited foam (Baht and Tharathan, 1987) and emulsion stabilizing properties (BeMiller *et al*., 1993), behaved like egg white at higher concentrations which could stabilize foams, therefore, okra polysaccharides was also used as a dried egg white substitute (Woolfe *et al*., 1977).

4.3 Gelling

Okra polysaccharide (borohydride soluble fraction, BSP) exhibited gelling properties. The aqueous dispersion of okra polysaccharide, when kept at $60^{o}C$ for 30 min followed by cooling at $4^{o}C$ for 24 h could form a stable gel. And when blend okra polysaccharide with xanthan gum (ionic polysaccharides) gave a more stable gel, while blend with locust bean gum did not form a gel. This phenomenon of gelling may be attributed to a synergistic effect, which caused by polymer-polymer interactions between the two polysaccharides, which compete for water (Baht and Tharathan, 1987).

Sengkhamparn *et al*. (2010) pointed out that the crossover between elastic (G´) and viscous (G´´) behavior of okra polysaccharide (hot buffer soluble solid) was at a frequency of 0.1 s^{-1} (Sengkhamparn *et al*., 2010). The crossover of G´ and G´´ values provides a good indication of viscoelastic behavior of the material (Iagher *et al*., 2002) and defied the beginning of the elastic behavior or approaching gel state (Norziah *et al*., 2001).

4.4 Film forming

Okra polysaccharide showed a film forming properties, which okra polysaccharide-whey protein isolate composite edible film had a good film property for preserving the live biomaterial quality. The film had a higher percentage of elongation, lower water vapor and oxygen permeability than the film without okra polysaccharide (Prommakul *et. al*., 2010).

4.5 Medical using

Okra polysaccharide is not only good for use in food application but its unusual lubricity property is also interested for medicinal use.

In year 2009, Ogbonnaya *et al.* patented a process for the production of okra polysaccharide and the use in an ophthalmic surgery, dermatology and orthopedics (Ogbonnaya *et al*, 2009). Okra polysaccharide showed to act as a promoter for hyaluronic acid synthesis when applied in cosmetic. Moreover, the pharmaceutical polysaccharide was used as an anti-wrinkle injectable gel, was injected under the skin of the patient’s face in an effective amount to decrease the visible wrinkles (Ogbonnaya *et al*, 2009). Remarkably, okra polysaccharide has many interesting functional properties.

Ice cream

Ice cream is a frozen mixture of a combination of milk, sweeteners, stabilizers, emulsifiers and flavoring. This mixture is called an ice cream mix. It is pasteurized, homogenized and frozen (Marshall *et al*., 2003). The quality of ice cream is characterized in part by a smooth creamy texture (Marshall and Arbuckle, 1996). A coarse or icy defect caused by large ice crystals develops at constant and especially fluctuating temperatures (Flores and Goff, 1999) through a process well known as recrystallization. It depends on storage time, temperature and the solutes present in ice cream, small ice crystal disappear as larger ice crystals grow, which is undesirable quality attribute. However, the desired quality is achieved by both proper ice cream processing and proper ice cream formulation.

1. Ice cream ingredients

Ice cream has the following composition of milk fat greater than 10 % and usually between 10-16% fat in some premium ice creams, between 9-12% milk solids non fat and sweetener between 12-16% and 0.2-0.5% added stabilizers and emulsifiers (Table 1). The balance, usually 55-64% is water, which normally comes from the milk (Goff, 2008). Moreover, stabilizers used in ice cream formulations are added in relatively small amounts (0.0-0.5%).It increases the viscosity but have no significant effect on freezing point or freezing properties of the ice cream mix. It does not affect the nucleation process or thermo-mechanical properties of the mix (Hagiwara and Hartel, 1996). Stabilizers have a high water holding capacity that is effective in smoothing the texture and giving body of the product, but their major role is to prevent crystal growth as temperature fluctuates during storage. In summary, stabilizers improve smoothness of body, retard recrystallization of ice during storage, slow moisture migration from the product to the package or the air, give uniformity of product and hold flavoring compounds in dispersion and give desired resistance to melting (Flores and Goff 1999).

Table 1 Typical compositional range of ice cream mixes

Component	Range (%wt./wt)
Milkfat	10-16
MSNF	9-12
Sucrose	9-12
Corn syrup solid	4-6
Stabilizer/Emulsifier	0.0-0.5
Water	55-64

Source: Goff and Sahagian (1996a)

2. Ice cream processing

Ice cream manufacturing process is shown in Figure 3. Briefly, all ice cream ingredients are blended and form the premix, which is pasteurized to destroy pathogenic microorganism and also comprises further dissolving of most solids (Marshall *et al*., 2003), homogenized to reduce the fat globule sizes from initially up to 15 µm in the premix to below 2 µm in order to obtain an emulsion stable during the long aging period (Barger, 1990) and cooled before aging for 24 hours. During aging at 4^{o}C, fat crystallized, hydrocolloids and protein are hydrated and the fat globule membrane rearranges in order to form the final membrane. Freezing step, involves rapid removal of heat while agitating to incorporate air (Marshall *et al*., 2003), additionally around 40% to 50% by volume air are whipped into the ice cream, thus imparting the desirable smoothness and softness of the frozen product (Marshall *et al*., 2003), 33% to 67% of water is frozen in this step, depending on drawing temperature and composition of the mixture (Marshall and Arbuckle, 1996) and the intermediate structure are from before hardening. Final step, hardening and storage, frozen ice cream is filled and cooled down to below -25^{o}C to -30^{o}C, no new ice nucleation occurs anymore (Sutton and Bracey, 1996), around 70% to 80% of water are frozen and ice crystal growth (Hartel, 1996).

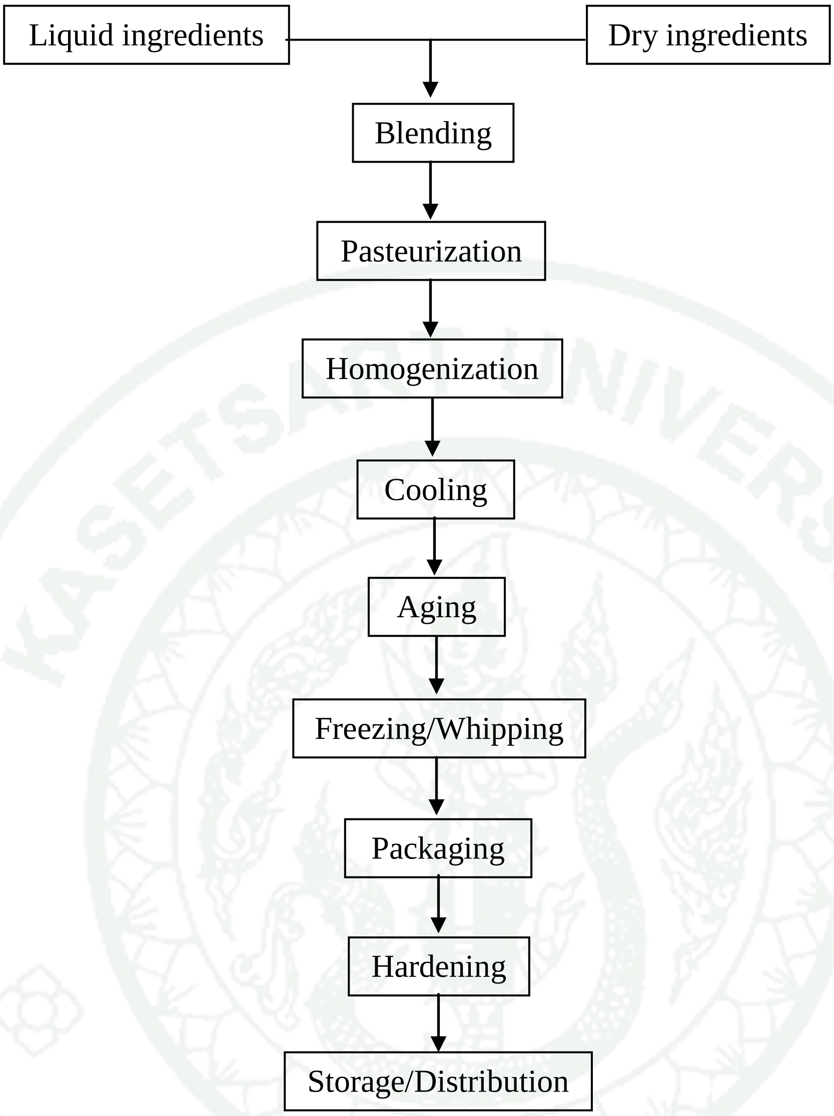


Figure 3 Flow sheet of the manufacturing process of ice cream with the major unit operation

Source: Adapted from Goff (1997a)

The final structure of ice cream product (Figure 4) can be summarized as a complex colloid consisting of a discontinuous foam, a network of partially coalesced fat surrounding air bubbles, ice crystals and a continuous freeze-concentrated unfrozen aqueous solution (Goff, 1997a). Ice cream texture quality is strongly depended on their physical structure, especially, ice crystals. Small ice crystals provide smooth texture, large crystal cause coarse texture. Spontaneously, ice crystals are temperature unstable and will undergo recrystallization, so one of the most important goals of an ice cream manufacturer is to produce a product with an ice crystal size distribution that results in a smooth texture and to preserve that ice crystal size until consumption (Flores and Goff, 1999). However, several compositional and processing factors affect the ice recrystallization in ice cream.

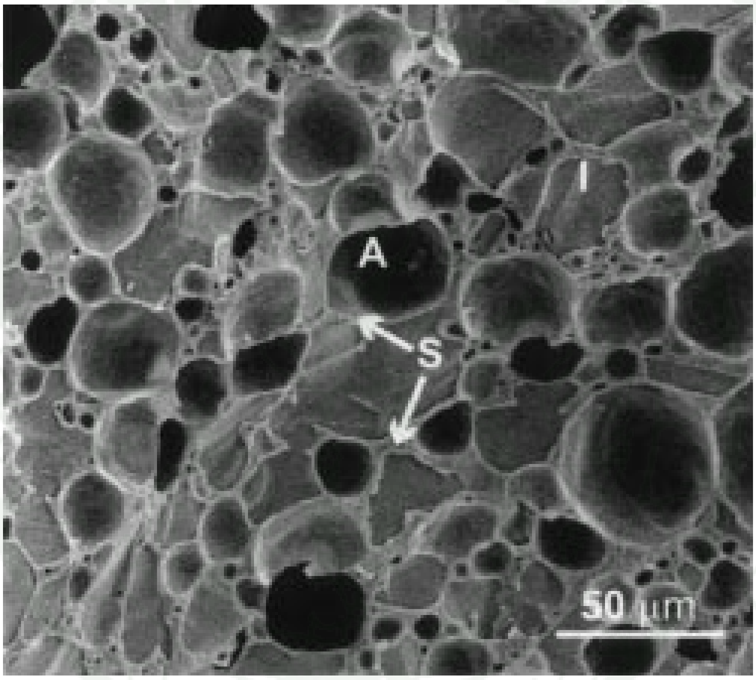


Figure 4 Typical microstructure of ice cream showing the air bubbles (A), serum phase (S) and ice crystals (I)

Source: Flores and Goff (1999)

3. Ice recrystallization in ice cream

As mention before, ice crystal sizes control smoothness during consumption (Hartel, 1996). The size of ice crystals that are formed during recrystallization stage has a strong influence on the texture of ice cream (Regand and Goff, 2002). For example, although ice crystals in the range of 15-20 µm give a desirable smooth texture to the ice cream, those that are larger than 40 µm give an unacceptable coarse and grainy texture to the ice cream (Hagiwara and Hartel, 1996). In addition, temperature fluctuations during storage and handling of ice cream promote ice crystal growth. The growth rate of ice crystal is slow at lower storage temperatures, especially when the product is stored below its glass transition temperature, is typically in the range of -30 to -40°C (Levine and Slade, 1989). Thus, one of the major challenges faced by frozen foods manufacturers is developing appropriate technological conditions or ingredient formulations that can inhibit ice crystal growth during storage and distribution.

Ice cream storage ideally occurs under low temperature and static condition resulting in constant ice content (Russell *et al*., 1999). In reality, temperature fluctuation, during storage and distribution cannot be completely controlled. At that time, ice recrystallization, the process of changes in numbers, size and shape of ice crystals during frozen storage occurs (Hartel, 1998). It basically involves small crystals disappearing, large crystals growing, and crystals fusing together (Goff, 1992). Mechanisms of ice recrystallization are reviewed here as well as factors affecting ice recrystallization.

3.1 Mechanism of ice recrystallization

Fenema (1973) pointed out several mechanisms of ice recrystallization, which may occur in ice cream and other frozen foods. These include ostwald ripening, isomass rounding and accretion, which all of them can occur at constant temperature. In addition, the last mechanism, is melt-refreeze recrystallization, occurs specifically in fluctuating temperature condition, cause significant changes in the ice crystal size. The details of each mechanism are described as below.

3.1.1 Ostwald ripening

Ostwald ripening is a processing that many small crystals slowly disappear but a few crystals grow larger. This is a spontaneous process that occurs because larger crystals are more energetically favored than smaller crystals. Ostwald ripening is important for crystals smaller than 1 to 2 µm in diameter (Hartel, 2001).

3.1.2 Isomass rounding

Isomass rounding, the total volume of the crystal does not change, but the crystal becomes more spherical to minimize a surface-to-mass ratio. This process does not cause a change in the number of ice crystals that present in a system (Hartel, 2001).

3.1.3 Accretion recrystallization

Accretion is process that two crystals which are in direct contact or in very close proximity and a bridge is formed to connect the two crystals to grow into a larger single crystal (Hartel, 2001).

3.1.4 Melt/refreeze recrystallization

Melt/refreeze recrystallization is due to thermal fluctuations that naturally occur during storage and distribution. A change in the phase volume of ice crystal occurs as temperatures fluctuate. As temperature increases, crystals melt. When temperature cycles back down, ice crystal grows back onto the remaining crystals. The smallest crystals are most sensitive to temperature increases and may eventually melt away (Hartel, 2001).

3.2 Factors affecting ice recrystallization

Many factors influence recrystallization rate in ice cream. Manufacturing conditions have an impact on the rate of recrystallization. It is resulting in formation of many small ice crystals provide maximum stability that against recrystallization. And rapid hardening processes maintain this number of ice crystals and promote stability against recrystallization. Moreover, storage conditions, such as temperature and extent of fluctuation, influence recrystallization rate. As the temperature increase, the amount of ice phase volume decrease and recrystallization rates increase rapidly. (Hartel, 2001). The details of each factor affecting ice recrystallization are provided as follow.

3.2.1 Initial freezing process

Conditions during the initial freezing process affect the shape and size of ice crystal in the ice cream that proceeds into the hardening, storage and distribution step. The highest freezing rate, the more ice crystals are formed initially, the smaller all the ice crystals remain for a given ice phase volume (Hartel, 1998).

3.2.2 Hardening rate

Once the initial stage of ice crystal production has been completed in the continuous freezer, ice cream is hardened in a low temperature storage freezer. The purpose of hardening is to lower the temperature of ice cream from draw temperature to about -20^{o}C to -30^{o}C (Berger, 1990). Anyway, no new ice crystals are formed in this step. Thus, existing crystals increase in size to accommodate the increase ice phase volume as the temperature is lowed in the hardening process. However, the longer ice cream remains at draw temperature, the more rapidly recrystallization occurs. Thus, it is critical to provide rapid hardening of ice cream to maintain as high a number as possible of ice crystals (Hartel, 1998).

3.2.3 Storage temperature and temperature fluctuations

Storage ice cream at high temperatures results is more rapid recrystallizaton. Donhowe and Hartel (1996) documented the effects of storage temperature and temperature fluctuations on recrystallization in ice cream. Ice crystals stored at -20 ±1.0^{o}C fluctuations did not show appreciable increase in ice crystal size, whereas an increase in storage temperature resulted in dramatic increase in mean size.

Mean size increased even when the temperature was constant, although the rates of size increase were significantly less than fluctuation temperature.

3.2.4 Product formulation

The composition of the ice cream plays an important role in recrystallization rates. The other ingredients in a food, besides the crystallizing species, have several effects on recrystallization (Hartel, 2001). Hydrocolloid stabilizers are widely used in ice cream to hinder ice crystal growth as temperature fluctuation during storage (Regand and Goff, 2002). However, hydrocolloid stabilized not only affects ice recrystallization in ice cream but also affects other physical properties of its.

4. Effect of stabilizer on physical properties and ice recrystallization of ice cream

One of the most important goals of an ice cream manufacturer is to produce a product with an ice crystal size that results in a smooth texture and to preserve that ice crystal size until consumption (Flores and Goff, 1999). As it was mentioned above that many factors, both ice cream manufacturing process and ice cream formulation, affect ice recrystallization in ice cream. Stabilizer has been reported to be used in ice cream for preventing ice recrystallization and improve ice cream characteristics.

4.1 Stabilizer used in ice cream

The stabilizers are added in relatively small amounts (0.1-0.5%). They increase the viscosity but have no significant effect on the freezing point (Goff *et al*., 1993; Hagiwara and Hartel, 1996). They have high water holding capacity which is effective in smoothing texture and giving body to the ice cream. The major function of stabilizer is to prevent ice crystal growth in temperature fluctuation during storage. In summary, stabilizers (1) improve smoothness of body, (2) retard ice recrystallization during storage, (3) slow down lactose crystallization, (4) slow down moisture migration from the product to the package, (5) give uniformity of product and hold flavouring compounds in dispersion and (6) give desired resistance to melting (Goff and Caldwell, 1991). There are some of stabilizers that normally use in ice cream to maintain ice cream smooth texture.

Guar gum (GG): it is extracted from the seed of a tropical legume (*Cyamoposis tetragonolba*). It has been grown in India, Pakistan and United States (Goff and Hartel, 2004). Guar gum consists of a β, 1, 4-linked chain of D-mannopyranose units, with side chain of a single α, 1, 6-linked D-galactopyranose units in the ratio of one galactose per every two mannose units. Due to this high degree of substitution, it is soluble in cold water (Sanderson, 1996). Moreover, it readily disperses and does not cause excessive viscosity in the ice cream mix. Generally, 0.1-0.2% is required in a mix (Kilara and Chandan, 2008).

Locust bean gum (LBG): it is obtained from the bean of tree *Ceratonia siliqua*. It has been grown mostly in Mediterranean area (Goff and Hartel, 2004). It is a neutral polysaccharide, like guar gum, is made up of a mannose backbone and a single unit galactose side chain. However, it presents a lower level of substitution (manose:galactose, 4:1) (Sanderson, 1996). This strong stabilizer is used at 0.1-0.2% and causes phase separation in ice cream mix (Kilara and Chandan, 2008).

Some other stabilizers are minor used in ice cream such as 1) sodium carboxymethyl cellulose (CMC), is chemical modified cellulose, is normally use to thicken, suspend, stabilize solutions and can be used as a bulking agent because of its high water holding capacity (Sanderson, 1996). This polysaccharide cans form weak gel by itself but gel well in the combination with carageenan, locust bean gum or guar gum (Marshall *et al*., 2003). It is a strong stabilizer and only 0.1-0.2% is need in a mix. It imparts body and chewiness to ice cream (Kilara and Chandan, 2008). 2) carrageenan, is derived from red seaweed, has been produced in coastal regions of Chile, the Phillipines, Canada, the United States, France, Spain, Denmark and Ireland (Marshall *et al*., 2003). Carrageenan is used in many stabilizer blends at levels of 0.01-0.02% to prevent phase separation through its interaction with milk protein (Kilara and Chandan, 2008). 3) xanthan, is a high molecular weight extracellular polysaccharide obtained by the growth of organism *Xanthomonas campestris* in culture (Goff and Hartel, 2004), is comprised of a cellulosic backbone with charge side chains (Launay *et al*, 1997), is readily dispersed in water due to its cellulosic nature so high viscosity can be obtained rapidly in hot or cold systems (Sanderson, 1996). 4) alginate, is extracted from brown algae and kelp, dissolves in cold water and gel in the presence of calcium and acid. However, because of its price, it is not widely used (Marshall *et al*., 2003). And 5) gelatin, is a protein derived from collagen of animal origin (Marshall *et al*., 2003). This relative expensive stabilizer is effective at concentrations of 0.3-0.5%. However, it may not very well prevent the effect of heat shock (Kilara and Chandan, 2008) and it is also not acceptable to certain religious and vegetarian population.

4.2 Effect of stabilizer on ice cream mix viscosity

Ice cream mix is a mixture of water, milk fat, milk solid non fat, sweeteners, stabilizers, and emulsifier, which is an unfrozen blend of all ingredients (Marshall *et al*., 2003). Viscosity, which is one of the most important rheological properties of ice cream mix and the unfrozen portion of ice cream, is influence by mix composition (mainly stabilizer), type and quality of ingredients, processing and handling of the mix, concentration (total solid content), and temperature (Marshall *et al*., 2003). It is understood that mix viscosity is important to impart desirable quality of ice cream, as the viscosity increases, the resistance to melting and the smoothness of texture increases, but the rate of whipping decreases (Marshall *et al*., 2003).

Numerous studies have investigated the viscosity and flow behavior of ice cream mix (Kaya and Tekin, 2001; Kus *et al*., 2005; Dogan *et al*., 2007). Kaya and Tekin (2001) showed that salep concentration had a greater effect on viscosity than temperature. Previous studies have shown that an increase in stabilizer concentration increases pseudoplasticity of ice cream mix (BahramParvar *et al*., 2010).

4.3 Effect of stabilizer on ice cream overrun

Ice cream is generally characterized as frozen foam (Adapa *et al*., 2000 (b)). Increasing ice cream volume is one role of stabilizers, brought about through increasing viscosity and maintaining the air bubbles (Marshall *et al*., 2003). Chang and Hartel (2002 (b)) studied the effect of operation conditions (freezing, not freezing, and partial freezing) and formulation (fat, emulsifier, and stabilizer contents) on development of air cells. Change in stabilizer level (0.0, 0.3, and 0.5% C-196 stabilizer which contained 12% carageenan, 33% guar gum (GG), and 55% CMC) had no effect on overrun. Addition of stabilizer, however, reduced air cell size compared to a similar ice cream mix made without stabilizer. Changes in air cell size could be directly attributed to changes in rheological properties of the ice cream during freezing (Chang and Hartel, 2002(b)).

4.4 Effect of stabilizer on thermodynamic properties

Differential scanning calorimetry (DSC) indicated that thermodynamic properties such as glass transition, heat capacity, and ice content determined by the melting endotherm are similar in systems with and without the presence of a stabilizer (Hagiwara and Hartel, 1996). Herrera *et al.* (2007) investigated the thermal properties of fructose or sucrose frozen solution solutions containing hydrocolloids, found that melting onset was not affected by the addition of hydrocolloids.

4.5 Effect of stabilizer on ice cream melting rate

When ice cream is in the form of cone or stick, melting rate is most importance to the consumer. The slow meltdown, slow serum drainage, and good shape retention are some of the desired important quality parameters of ice cream (Wildmoser *et al*., 2005). If the product melts too fast, a messy situation can occur. A fast-melting product is undesirable also because it tends to become heat shocked readily (Marshall *et al*., 2003). One function of stabilizers in ice cream is to increase the melting resistance (BahramParvar *et al*., 2009). Hydrocolloids, due to their water holding and microviscosity enhancement ability, significant affect melting quality of ice cream (Marshall *et al*., 2003).

4.6 Effect of stabilizer on sensory characteristic

Hydrocolloid stabilizers influence the sensory properties of ice cream (Soukolis *et al*., 2008). Viscosity of the serum phase affects the mouth feel of the ice cream; better body and texture further improve the overall acceptability of the product (Minhas *et al*., 2002). Minhas *et al*. (2002) investigated the relationship between concentration of stabilizers, viscosity, body and texture, and overall acceptability of ice cream. The stabilizer concentration was highly correlated with the viscosity of ice cream mixes body and texture of ice cream.

4.7 Effect of stabilizer on ice recrystallization in ice cream

Many researchers have been studied the effect of stabilizer on ice recrystallization in ice cream. Patmore *et al.* (2003) documented that the locust bean gum (LBG) help maintain smooth texture in ice cream by slowing down ice crystal growth during constant and fluctuating temperatures. The experiment was performed; briefly, LBG and guar gum (GG) were dissolved in sucrose solutions without or with milk solid-not-fat (MSNF), fat, and/or emulsifier. Solutions were temperature cycled at subzero temperatures. LBG solutions developed weak gel structures with temperature cycling, especially in the presence of MSNF, but GG solutions did not. Fat droplets interfered with the formation of LBG weak gel networks. They concluded that the ability of a polysaccharide to cryo-gel with temperature cycling and protein/stabilizer incompatibility leading to phase separation both helped create elastic structures, with this mechanism may be the factor affecting the slowing down of ice crystal growth.

Flores and Goff (1999) studied effect of stabilizers (carboxymethyl cellulose (CMC) or locust bean gum (LBG)) on ice recrystallization of ice cream under storage conditions of constant and fluctuating temperatures. They found that storage under low constant temperature (-30^{o}C) had no effect on the overall ice crystal size of stabilized or unstabilized ice cream samples, whereas storage at a higher temperature (-16^{o}C) showed clear evidence. Temperature cycles (-15 ± 5^{o}C) of samples after hardening (-30^{o}C) had an even greater effect than did storage at a high constant temperature (-16^{o}C). Also, increase of the number or time length of cycles had greater impact than did an increase in amplitude of temperature range. After extended temperature fluctuation, smaller crystals disappeared. The predominant recrystallization mechanism at this stage would have most likely involved partial melting and refreezing of ice crystals.

In year 2003, Regand and Goff studied structure and ice recrystallization in frozen stabilized ice cream model systems. They determined the existence of a relationship between the capability of the stabilizer to form a gel-like network, ether by itself or with milk protein, and the ability of the stabilizer to retard ice recrystallization in sucrose and sucrose/MSNF solutions. Hydrocolloid stabilizers (carrageenan, carboxymethyl cellulose, xanthan gum, sodium alginate, LBG and gelatin) were incorporated into solutions of sucrose with or without MSNF. Resultant solutions were quenched frozen and cycled between -3.5 and -6^{o}C for five times. They found that, significant retardation of recrystallization was observed in alginate and xanthan sucrose solutions without MSNF. In the presence of proteins (MSNF), all stabilizers were effective retarding recrystallization except for gelatin with MSNF.

Hagiwara and Hartel (1996) studied the influence of sweetener (sucrose, 20 dextrose equivalent corn syrup, 42 dextrose equivalent corn syrup and 42 high fructose corn syrup), with and without stabilizer, on ice recrystallization in ice cream at three storage temperatures. They found that ice recrystallization rate increased as temperature increased for all ice cream and was highest for ice cream made with high fructose corn syrup and lowest for ice cream made with 20 dextrose equivalent corn syrup.

Goff *et al*. (1995) simulated freeze-concentration by formulating a series of ice cream mixes containing diminishing quantities of water. A change in slope occurred in the viscosity-concentration curve when 60% water was frozen. The equivalent freezing temperature was -7^{o}C. It was suggested that helical coil overlap or entanglement of the stabilizers may occur as a result of freeze-concentration of the stabilizer, which would reduce the rates of diffusion of polysaccharides, other solutes and water, thus inhibiting ice crystal growth.

Conclusively, the mechanisms by which stabilizers limits ice recrystallization have been extensively studied but are still not fully understood. The cryoprotective effects of hydrocolloids on ice cream were proposed by three mechanisms.

The first mechanism, viscosity and molecular mobility, the enhancement of viscosity was correlated to the control of ice crystal growth (Hagiwara and Hartel, 1996). Miller-Livney and Hartel (1997) suggested that the macroviscosity changes caused by hydrocolloids addition were possibly associated with the microviscosity so the diffusion of unfrozen water to the ice crystals may be retarded. Bolliger *et al*. (2000) reported that the rheological changes achieved by freeze concentration of hydrocolloids in the serum phase may contribute to the efficient control of water diffusion on the ice crystal surface.

The second mechanism, cryo-gel formation, correlated the cryoprotectivity hydrocolloids with their ability to form cryo-gel as a result of temperature fluctuations during storage. Goff *et al*. (1999) demonstrated that LBG could form cryo-gel in sucrose-protein systems and thus was able to control ice recrystallization more efficiently than GG. GG could not hinder recrystallization as did not form cryo-gel during the freeze-thaw cycles.

The third mechanism, hydrocolloid phase separation, the incomparibility of hydrocolloids with proteins provoking phase separation may contribute to the recrystallization retardation (Regand and Goff, 2002, 2003).

5. Relationship between ice cream mix rheology and ice crystal growth in ice cream

Understanding the mechanism by which effects occur is desirable, so the modifications can be made to ice cream mix formulations, with predicted effects on ice cream structure and quality. The determining of ice recrystallization results could be predicted base on the rheological properties of the polysaccharide in the unfrozen phase were interested. Some experiments about this are reviewed.

Rheology is the science of deformation and flow (Mezger, 2002). The rheology of ice cream mix has been studied by numerous researchers. Goff *et al* (1995) measured the rheological properties (G´ and G´´) of ice cream mixes made with and without stabilizers and found that stabilized mixes exhibited significantly greater storage (G´) and loss moduli (G´´) at temperatures of less than -8^{o}C, which G´ and G´´ describes the solid behavior and viscous behavior, respectively. The unstabilized sample also had greater tanδ (tan delta; G´´/G´) values at all temperatures than stabilized samples. The tanδ reveals the ration of the viscous to the elastic portion of the deformation behavior.

In year 2000, Bolliger *et al.* found a linear relationship (r^2 = 0.97) between a normalized ‘breakpoint’ apparent viscosity (break point apparent viscosity divided by initial mix apparent viscosity) and recrystallization rate. They suggested that some aspects of stabilizer functionality with respect to recystallization protection could come from structure, as measured by rheological properties that result from freeze-concentration of the polysaccharide in the unfrozen phase of ice cream. This structure from stabilizer would affect the rate at which water can diffuse to the surface of a growing crystal during temperature fluctuation or the rate at which solutes and macromolecules can diffuse away from the surface of growing ice crystal.

MATERIALS AND METHODS

Materials

1. Okra pods, were purchased from a local vegetable supplier (Tim Food Co, Ltd., Bangkok, Thailand), were growth in Nakornnayok province, Thailand
2. Ethanol (commercial grade, SAC Science-Eng Inc., Bangkok, Thailand)
3. Sodium carbonate (Ajax Finechem, Seven Hills, Australia)
4. Sodium bicarbonate (Ajax Finechem, Seven Hills, Australia)
5. Citric acid anhydrous (Merk, Darmstadt, Germany)
6. Sodium phosphate dibasic anhydrous (Ajax Finechem, Seven Hills, Australia)
7. Calcium chloride (Ajax Finechem, Seven Hills, Australia)
8. Magnesium chloride (Ajax Finechem, Seven Hills, Australia)
9. Potassium chloride (Ajax Finechem, Seven Hills, Australia)
10. Sodium chloride (Ajax Finechem, Seven Hills, Australia)
11. Iso-amyl-butanol (Sigma-Aldrich Corp., St. Loius, MO., U.S.A.)
12. Milk-solid-not-fat (MSNF) (Parmalat, Toronto, ON, Canada)
13. Milk fat (unsalted butter, 80% fat) (Gay Lea, Guelph, ON, Canada)
14. Sucrose (Lantic Sugar Ltd., Toronto, ON, Canada)
15. Corn syrup solid (CSS) (42DE, Casco, Inc., Toronto, ON, Canada)
16. Mono-diglycerides (MDG) (Danisco Inc., Toronto, ON, Canada)
17. Guar gum (GG) (Danisco Inc., Toronto, ON, Canada)
18. Xanthan gum (Danisco Inc., Toronto, ON, Canada)
19. Vial with screw cap (50 ml glass vial)
20. Sieve (Retsch®, Retsch GmbH, Haan, Germany)
21. Waring blender (32 BL80, New Hartford, Connecticut, U.S.A.)
22. Hand homogenizer (IKA® Werke GmbH & Co. KG, Staufen, Breisgau, Germany)
23. Overhead stirrer (IKA® RW20D digital, IKA® Werke GmbH & Co. KG, Staufen, Breisgau, Germany)
24. High speed mixer (Silverson L4RT, Silverson Machines Inc., Chesham, Buckinghamshire, UK)

25. Two-stage homogenizer (APV Gaulin Inc., Delavan, WI, USA)
26. HunterLab UltraScan XE Spectrocolorimeter equipped with Universal 4.10 software (Hunter Associates laboratory Inc., Virginia, USA)
27. Acrylic 2^o cone (60 mm, diameter) and plate geometry
28. Rheometer (AR 2000, TA Instruments, New Castle, DE, USA)
29. Rheometer (Physica MCR, Anton Paar® GmbH, Graz, Austria-Europe)
30. Light microscope (Olympus, Tokyo, Japan).
31. Sony XC-75 camera (Sony Corporation, Tokyo, Japan)
32. Cold stage (Linkam Scientific Instruments Ltd., Surrey, UK)
33. Water bath (WB22, memmert, Germany)
34. Refrigerated centrifuge (HITACHI Himac CR 20B2, Hitachi, Tokyo, Japan)
35. Freeze-dryer (FTS systems Dura-Top MP, ON Canada)
36. Freeze-dryer (Industrial freeze-dryer, 400 kg capacity, Sun Shine Co, Ltd., Junthaburi, Thailand)
37. Ice cream batch freezer (Taylor Batch Freezer model 104, Taylor company, Rockton, IL, USA)
38. Blast freezer (Foster Refrigeration, Drummondville, QC, Canada)
39. Cabinet freezer (Scientemp, model 34-23, Chicago, IL, USA)
40. Rheoplus V3.40 software (Anton Paar GmbH, Graz, Austria-Europe).
41. Image-Pro Plus 6.0 software (Media Cybernetics, Inc., Silver Spring, MD, USA)
42. Compusense® *five* software (Compusense, Inc., Guelph, Ontario, Canada)

Methods

Experiment 1: Preparation of okra cell wall (OKW) and okra polysaccharide (OKP), and investigation of their physico-chemical properties

This experiment was done at Kasetsart University, Bangkok, Thailand

1. Preparations of OKW and OKP

1.1 Preparation of OKW

The okra pod, was purchased from Timfood Co., Ltd., Thailand, was grown in Nakornnayok province, was harvested at 7 days after blooming. It size was around 9-12 cm length and 2 cm diameter. The okra pod was kept storage in a cold room (4^{o}C) until use. The process of OKW and OKP preparations is shown in Figure 5. Briefly, okra pod was removed seed and placenta. Then the pericarp was chopped in 95% ethanol at 1:2 (w/v, okra:ethanol) with Waring blender for 40 sec. The slurry was transferred to cylindrical stainless container which was placed in 60^{o}C water bath (memmert, Germany), continuously stirred with overhead stirrer (IKA$^{®}$ RW20D digital, IKA$^{®}$ Werke GmbH & Co. KG, Staufen, Breisgau, Germany), and the sample was held at this temperature for 30 min.

The slurry was pressed with hydraulic press in order to move out the liquid portion. The okra cell wall pellet was collected and then dispersed in 95% ethanol. The steps of stirring, heating and hydraulic pressing were repeated to ensure the green chlorophyll color was completely removed. The okra cell wall pellet was air-dried in fume hood, ground with Waring blender, and then passed through 200 mesh stainless steel sieve (Retsch$^{®}$, Retsch GmbH, Haan, Germany). The alcohol insoluble solid or okra cell wall (OKW) was obtained, packed in high-density polyethylene (HDPE) plastic bags and kept storage in desiccators until used.

Yield of OKW was calculated with the Eq. 1:

$$\text{Yield (\%)} = \frac{\text{Weight of OKW (g)}}{\text{Weight of okra pods (g)}} \times 100$$

1.2 Preparation of OKP

The air-dried okra cell wall pellet, from the above preparation, was used for polysaccharide preparation. The cell wall pellet was extracted with distilled water (1:50, w/v) at 70°C for 30 min in water bath while continuously stirred with overhead stirrer. The solubilized polysaccharide was separated by refrigerated centrifuge (HITACHI Himac CR 20B2, Hitachi, Tokyo, Japan) at 14,000xg for 30 min at 25°C. Supernatant was freeze-dried (FTS systems Dura-Top MP, ON Canada and Industrial freeze-dryer, 400 kg capacity, Sun Shine co, Ltd., Junthaburi, Thailand), then ground. The water soluble okra polysaccharide was obtained, packed in high-density polyethylene (HDPE) plastic bags and kept storage in desiccators until used. Yield of OKP was calculated with the Eq.2:

$$\text{Yield (\%)} = \frac{\text{Weight of OKP (g)}}{\text{Weight of okra pods (g)}} \times 100$$

2. Physico-chemical characterization of OKW and OKP

2.1 Color measurement

Color of OKW and OKP in powder and dispersion (0.30%, w/w) forms were directly measured with HunterLab UltraScan XE Spectrocolorimeter equipped with Universal 4.10 software (Hunter Associates laboratory Inc., Virginia, USA). Calibration of the instrument was performed with white and black standard (Instrument standard, Hunter Associates laboratory Inc., Virginia, USA). L*, a*, and b* values of the samples were reported. The color of the samples was measured in three replications.

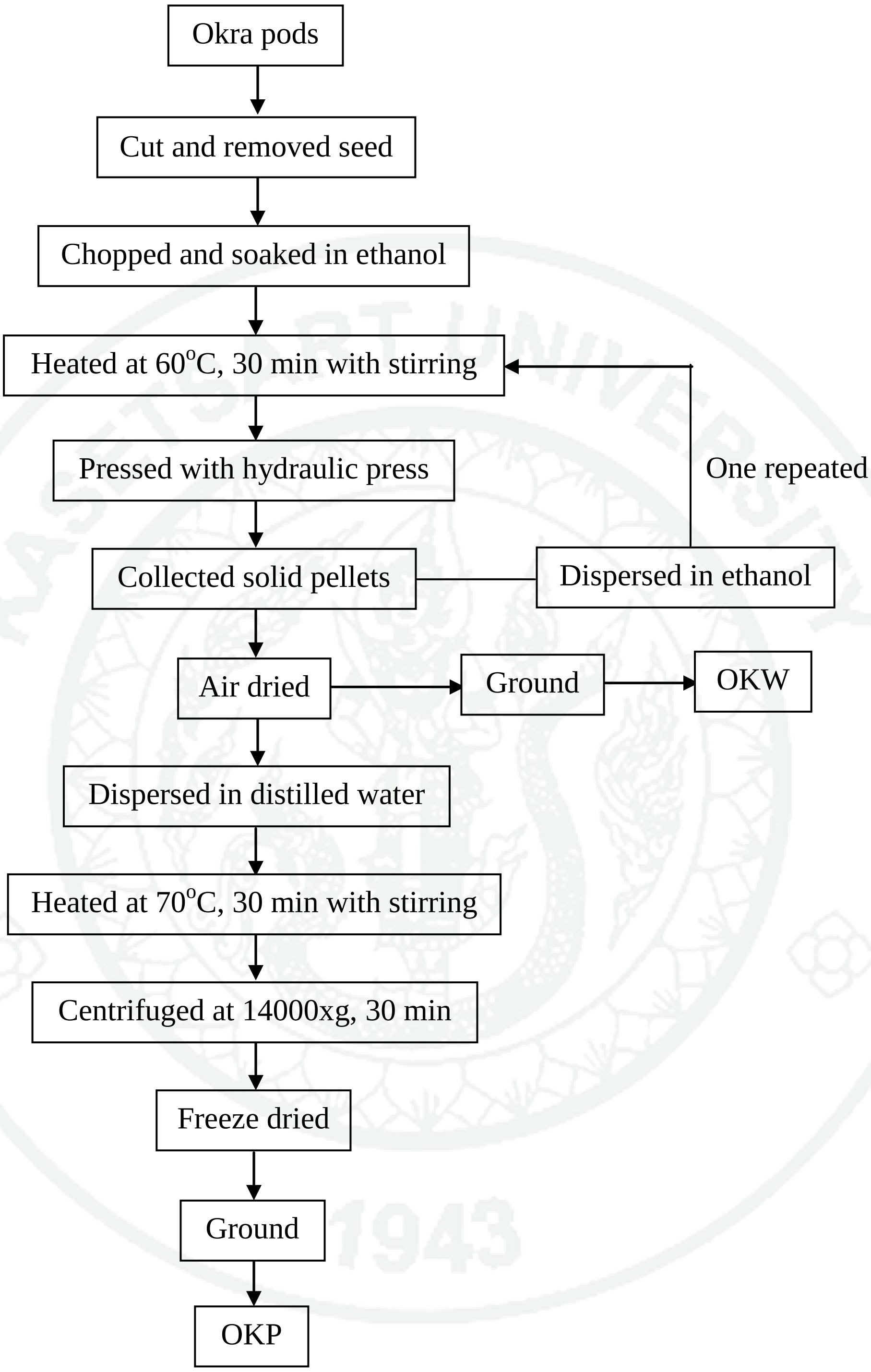


Figure 5 Schematic represented OKW and OKP preparations

Source: Modified from Sengkhamparn *et al*. (2009)

2.2 Bulk density measurement

The bulk density was determined by the method described by Wang and Kinsella (1976). A known amount of sample was weighed into 50 ml graduated measuring cylinder. The sample was packed by gently tapping the cylinder on the bench top from a height of 5 cm. The volume of the sample was recorded. The bulk density was calculated with the Eq. 3:

$$\text{Bulk density} = \frac{\text{Weight of sample (g)}}{\text{Vol. of the sample after tapping (cm}^3\text{)}}$$

2.3 Chemical composition analysis

Proximate analysis including moisture, protein, fat, ash, and crude fiber contents of OKW and OKP were performed according to AOAC (2006) procedures. Total carbohydrate was calculated by different. The details of each method were described in appendix section.

2.4 Functional properties characterization of OKW and OKP

The functional properties are intrinsic physico-chemical characteristics which affect the behavior of properties in food systems during processing, storage, and distribution (Aremu *et al*., 2007). Information about functional properties of the sample should provide the idea of using OKW and OKP properly.

2.4.1 Water solubility (WS) and water holding capacity (WHC) measurements

WS and WHC of sample were measured at four concentrations levels. OKW and OKP dispersions at concentration of 0.10-0.40% (w/w) were prepared with distilled water in a beaker. The dispersions were gentle stirred with a magnetic stirrer for 60 min at ambient temperature (~23^{o}C). The samples were thereafter centrifuged at 2500xg for 30 min, and the supernatant and sediment were separated to each weight trays, heated in oven at 105^{o}C for 3 h or until the weight was constant. The dried solid was weighted and the water content of the sample was calculated. The WS and WHC of the sample were calculated by the method described by Aremu *et al.* (2008). The WS was express as gram of dissolved solid per gram of sample. Whereas, the WHC was expressed as a gram of water was held per gram of sample. The experiments were done in three replications. The calculations were done with the Eq. 4, 5:

$$\text{WS (\%)} = \frac{\text{Weight of solid in supernatant (g)}}{\text{Weight of sample (g)}} \times 100$$

$$\text{WHC (\%)} = \frac{\text{Weight of water in sediment (g)}}{\text{Weight of sample (g)}} \times 100$$

2.4.2 Foaming capacity and stability analysis

Foaming capacity and stability are important for product quality and also play an important role in the analyses of the processing conditions in food production such as mixing and whipping operations. Foaming capacity and stability of OKW and OKP were studied at different concentration. OKW and OKP at 0.10 - 0.50 % (w/v) were prepared in distilled water and stirred with magnetic stirrer at room temperature for 60 min to ensure completely dispersion. The 50 ml of resulting solutions were transferred into a 250 ml measuring cylinder. The aeration was performed using a rotor/stator hand homogenizer (Ultra turrak T50, IKA® Werke GmbH & Co. KG, Staufen, Breisgau, Germany) at speed number 2 (ca. 13,000 rpm) for 2 min. The samples were immediately recorded the volume. The foaming capacity was expressed as the percentage of volume increase. Foaming stability was expressed as foam volume remaining from 0 to 15 min (modified from Lin *et al*., 1974). The foaming capacity and foaming stability were calculated with the Eq. 6, 7:

$$\text{Foaming capacity (\%)} = \frac{\text{Vol. after aerated} - \text{Vol. before aerated}}{\text{Vol. before aerated}} \times 100$$

$$\text{Foaming stability (\%)} = \frac{\text{Foam volume after time (t)}}{\text{Initial foam volume}} \times 100$$

2.4.3 Apparent viscosity measurement of OKW and OKP dispersions

The apparent viscosity of gums are important for product quality and also play an important role in the analyses of the flow conditions in food processes such as pasteurization and aseptic processing. Apparent viscosity measurements were performed on a AR2000 rheometer (TA Instruments, New Castle, DE, USA) equipped with a acrylic 2^{o} cone (60 mm, diameter) and plate geometry. Temperature was controlled at 20^{o}C using circulating water bath and peltier system. The steady shear test was performed. Shear stress (τ) involved 25 readings at a shear rate of 20 to 200 s^{-1}. Apparent viscosity of each sample was reported at 50 s^{-1}, the shear rate represented the sensing shear rate in the mouth of low viscosity food (Akhtar *et al*., 2006; Stanley and Taylor, 1993). Factors affecting apparent viscosity were studied.

1) Effect of shear rate on apparent viscosity

OKW and OKP dispersions at different concentrations were prepared by adding the OKW or OKP into distilled water then gentle stirred with magnetic stirrer at room temperature for 60 min to make sure the sample were completely dispersed. The apparent viscosity was measured with the method described above. The results were reported at shear rate of 20-200 s^{-1}

2) Effect of polysaccharide concentration on apparent viscosity

OKW and OKP dispersions at different concentrations were prepared by adding the OKW or OKP into distilled water then gentle stirred with magnetic stirrer at room temperature for 60 min to make sure the sample were completely dispersed. Apparent viscosity GG was also measured in order of comparison. The apparent viscosity was measured with the method described above.

3) Effect of freeze-thaw cycles on apparent viscosity

OKW and OKP dispersions at 0.30% (w/w) were prepared. The 20 ml of the dispersions were packed in vials with screw caps then subjected to the walk in freezer for freezing at -21°C for 20 h, then thawed at ambient temperature (~ 23°C) for 4 h, each cycle took 24h. The apparent viscosity of the samples was measured at every single freeze-thaw cycle, for 8 cycles. The apparent viscosity of the samples was measured with the method of apparent viscosity measurement as described in previous section. The result was reported at shear rate of 50 s^{-1} .

4) Effect of pH on apparent viscosity

OKP at 0.30% (w/w) with different pH (ranged from 3-9) were prepared. OKP was dispersed in buffer solution with gentle stirred with magnetic stirrer at room temperature for 60 min to ensure a completely dispersion. The apparent viscosity of the samples was measured with the method of apparent viscosity measurement as was described before. A detail of the pH buffer solutions (phosphate-citrate buffer (pH 3.0-8.0) and carbonate-bicarbonate buffer (pH 9.0)) preparations were shown in an appendix. The apparent viscosity result was reported at shear rate of 50 s^{-1} .

5) Effect of heating and cooling on apparent viscosity

OKP at 0.30% (w/w) dispersion was prepared and subject to plate geometry of the rheometer. The temperature of the sample was varied from 20°C-90°C-20°C. The heating and cooling rate was 1.67°C/min. The varying temperature was performed on the rheometer using circulating water bath and peltier system. The apparent viscosity of the samples was measured with the method of apparent viscosity measurement as was described before. The share rate was set constantly at 50 s^{-1} .

6) Effect of salt ion on apparent viscosity

OKP at 0.30% (w/w) dispersions were prepared in salt solution including potassium chloride, sodium chloride, magnesium chloride and calcium chloride solutions at the concentration of 50 to 200 mM. The apparent viscosity of the samples was measured with the method of apparent viscosity measurement as was described before. The result was reported at shear rate of 50 s^{-1} .

7) Effect of OKP-GG and OKP-xanthan gum mixing ratio on apparent viscosity

In order to study the synergism of OKP with other commercial polysaccharides, the effect of OKP-gum mixing ratio on apparent viscosity was measurement. The mixtures of OKP-GG and OKP-xanthan gum at different ratios were prepared, which polysaccharide blends were formed from 1.0% (w/w) dispersion of OKP and 1.0% (w/w) dispersion of guar gum and xanthan gum according to different ratios (0:10 to 10:0) then the apparent viscosity of the samples was measured with the method of apparent viscosity measurement as was described before. The result was reported at shear rate of 50 s^{-1} .

Experiment 2: Determination of the rheological properties of OKW and OKP in ice cream-mix-model

This experiment was done at University of Guelph, Guelph, Canada

In order to investigate the individual effect of stabilizers (GG, OKW, and OKP) addition on the rheological properties, ice cream mix model including stabilizer dispersion, sucrose/stabilizer, and sucrose/stabilizer/MSNF were prepared and their rheological characteristic was studied.

2.1 Preparation of ice cream mix model

Ice cream mix model was prepared in accordance with the typical ice cream mixture composition, which is 15% sucrose with and without 10% skim milk powder and varying concentration of stabilizer (GG, OKW and OKP) from 0.00% - 0.60%. Water was placed in 200 ml beaker and heated up to 40°C, then al dry ingredients were added into the warm water in the beaker. The sample was then stirred while heating at 75°C for 15 min. The prepared sample was cooled to 20°C. Ice cream mix model was prepared in three triplications.

2.2 Apparent viscosity measurement

The apparent viscosity of the ice cream mix model was determined at 20°C using the steady shear test which performed on a AR2000 rheometer (TA Instruments, New Castle, DE, USA) equipped with a acrylic 2° cone (60 mm, diameter) and plate geometry. Shear stress (τ) involved 25 reading at a shear rate range from 20 to 200 s^{-1}. In order to explain the ice cream mixes rheological behavior data were fitted to the Power law model: $\tau = K(\dot{\gamma})^n$, where τ = shear stress (Pa), $\dot{\gamma}$ = shear rate (s^{-1}), K = consistency coefficient (Pa.s) and n = flow behavior index (dimensionless) (Mezger, 2002). Apparent viscosity of each sample was calculated at 50 s^{-1} which represents the sensing shear rate in the mouth of low viscosity food (Akhtar *et al*., 2006; Stanley and Taylor, 1993).

Experiment 3: Investigation the effect of OKW and OKP on physical properties and stability of ice cream

This experiment was done at University of Guelph, Guelph, Canada

3.1 Ice cream preparation

In order to study the physical properties and stability of ice cream with different concentrations of OKW and OKP were prepared. Formulations for ice cream mix preparation are shown in Table 2. The basic ice cream mix formulation consisted of 11% milk solids-not-fat, 10% milkfat, 12% sucrose, 4% CSS, and 0.15% MDG. Two controls were studied: 0% stabilizer and 0.15% guar gum. OKW and OKP were used at concentrations of 0.15%, 0.30% and 0.45%. A flowchart of preparation is shown in Figure 3. Briefly, Sugar and stabilizer were dry-mixed and added into water. The rest of the dry ingredients were then blended into the water and pasteurized at 75^{o}C for15 min. The coarse mix was pre-homogenized with a high-speed mixer (Silverson L4RT, Silverson Machines Inc., Chesham, Buckinghamshire, UK) at 7500 rpm for 5 min to ensure complete dispersion of ingredients. The mixture was then homogenized with two-stage homogenizer (APV Gaulin Inc., Delavan, WI, USA) at 20 MPa and 7 MPa, cooled, and aged at 4^{o}Cfor 24 h. The aged ice cream mix was frozen to -5^{o}C in 12 min in an ice cream batch freezer (Taylor Batch Freezer model 104, Taylor company, Rockton, IL, USA) (Mendez-Velasco and Goff 2011). Ice cream was packed into 250 mL-cylindrical plastic containers with lids and hardened at -35^{o}C in a blast freezer for 24 h. Three replications were prepared of all treatments.

Table 2 Ice cream formulations used in the experiment

Ice cream	MSNF	Milk fat	GG	OKW	OKP	Sucrose	CSS	MDG	Water
1	11	10	0	0	0	12	4	0.15	62.85
2	11	10	0.15	0	0	12	4	0.15	62.70
3	11	10	0	0.15	0	12	4	0.15	62.70
4	11	10	0	0.30	0	12	4	0.15	62.55
5	11	10	0	0.45	0	12	4	0.15	62.40
6	11	10	0	0	0.15	12	4	0.15	62.70
7	11	10	0	0	0.30	12	4	0.15	62.55
8	11	10	0	0	0.45	12	4	0.15	62.40

*MSNF = milk solid not fat, GG = guar gum, CSS = corn syrup solid, MDG = mono and diglycerides

3.2 Apparent viscosity and flow behavior of ice cream mix

Ice cream mix was characterized for its rheological properties using the steady shear test. All rheological measurements were performed on a AR 2000 rheometer (TA Instruments, New Castle, DE, USA) equipped with an acrylic 2^{o} cone (60 mm, diameter) and plate geometry. Temperature was controlled at 20^{o}C using a circulating water bath and Peltier system. Shear stress (τ) was measured with25 readings at a shear rate between 20 and 200 s^{-1}.To explain the ice cream mix rheological behavior, data was fitted to the Power Law model (Eq. 8):

$$\tau = K(\dot{\gamma})^{n}$$

where τ = shear stress (Pa), $\dot{\gamma}$ = shear rate (s^{-1}), K = consistency coefficient (Pa.s^{n}), and n = flow behavior index (dimensionless) (Mezger 2002). Apparent viscosity of each sample was calculated at 50 s^{-1}, which represented the sensing shear rate in the mouth of low viscosity foods (Akhtar and others 2006; Stanley and Taylor 1993).

3.3 Physical properties of ice cream

3.3.1 Determination of overrun

Overrun was determined according to the method described by Goff and Hartel (2013). A known volume of ice cream mix and frozen ice cream were weighed, and overrun was calculated according to Eq. 9:

$$\text{Overrun (\%)} = \frac{\text{Weight of ice cream mix (g)} - \text{Weight of ice cream (g)}}{\text{Weight of ice cream (g)}} \times 100$$

3.3.2 Melting characteristics

To study the ice cream melting behavior, three objective measurements were used: (1) first dripping time, (2) melting rate and (3) mass retention. A day prior to melting characteristics determinations, samples were transferred from -35^{o}C to a -20^{o}C freezer and left overnight. Ice cream samples (110 g) were removed from the container, put on a10 mesh stainless steel wire screen, and allowed to melt at ambient temperature (~23^{o}C) (Mendez-Velasco and Goff 2012). The time required for the dripping of the first drop of melted ice cream was recorded. The weight of the dripped material passed through the screen was recorded at 10-min time intervals for 70 min duration. The melting rate was determined as the slope of the graphs of the dripped portion as function of the time, expressed in g.min^{-1}. The weight of the ice cream that remained on the screen after 70 min of melting time was calculated for percent mass retention.

3.4 Ice crystal growth of ice cream

3.4.1 Temperature cycling storage of ice cream

The temperature cycling of ice cream was carried out in a temperature-programmable cabinet freezer (Scientemp, model 34-23, Chicago, IL, USA) to promote ice crystal growth during storage as previously described by Regand and Goff (2006). Ice cream samples were transferred from -35^{o}C to the cabinet freezer at -20^{o}C. Each sample was subjected to a programmed heating and cooling cycle during which the freezer was kept at -20^{o}C for 12 h, then heated to -10^{o}C in 12 h, held there for another 12 h, and cooled back to -20^{o}C in 12 h. This heating/cooling cycle was repeated 10 times, each cycle took a length of 48 h (Regand and Goff 2006; Costa *et al*., 2008).

3.4.2 Ice crystals size analysis

Ice crystal size was measured from images obtained from the microscopic technique. Ice cream, either directly from -35^{o}C storage or after cycling, was tempered at -20^{o}C for 24 h then prepared for analyses by light microscopy. All equipment used for the sample preparation for microscopic analysis was pre-cooled to -20^{o}C. Approximately 1 cm^3 ice cream sample was taken from the core section at the center of the container using a sharp knife. A thin slice was subsequently cut with a sharp blade and placed onto a drop of iso-amyl-butanol on a standard glass microscope slide. The slide was covered with a cover slip, placed on stainless wire above liquid nitrogen in a cylindrical stainless container, and transported immediately to the cold stage (Linkam Scientific Instruments Ltd., Surrey, UK) that was previously set to -17^{o}C (Aleong and others2008; Regand and Goff 2006).

Images were acquired using a Sony XC-75 camera (Sony Corporation,Tokyo, Japan) mounted onto an Olympus BH light microscope (Olympus, Tokyo, Japan). Five different fields on the slide were photographed to obtain at least 300 crystals for each replication. Measurement of ice crystal size was performed using the analysis software Image-Pro Plus 6.0 (Media Cybernetics, Inc., Silver Spring, MD, USA). The diameter of each ice crystal was traced by the software. The cumulative distribution of equivalent diameters was determined by Microsoft Excel, which was then characterized by the Logistic Dose Response Model, as described by Flores and Goff (1999). The median equivalent diameter (X_{50}) was calculated at 50% of the cumulative distribution of the fitted data to the model. Ice crystal growth was calculated with the Eq. 10:

$$\text{Ice crystal growth (\%)} = \frac{X_{50}\text{ after cycling} - X_{50}\text{ before cycling}}{X_{50}\text{ before cycling}} \times 100$$

3.5 Sensory evaluation

Ice creams with 0.45% OKW and OKP were excluded from the sensory study because of their trace okra flavor. Ice cream samples with 0.15% GG, 0.00%, 0.15% and 0.30% OKW and OKP formulations were utilized for the test. All samples were flavored with vanilla (3 mL/kg). 25 g of ice cream were scooped into 50-mL polystyrene styrofoam containers with lids and kept in storage at -16°C for up to 3 h prior to testing. Consumer preference was determined by asking 75 untrained volunteers from the Department of Food Science, University of Guelph to indicate their degree of liking (flavor, texture and overall liking) on a 9-point hedonic scale (9 = like extremely, 5 = neither like nor dislike, and 1 = dislike extremely).Orders of serving were randomized, and Compusense® *five*software (Compusense, Inc., Guelph, Ontario, Canada) was used for data collection. Temperature of the sensory room was controlled at 21°C. Panelists were instructed to clean their palates with filtered drinking water and unsalted cracker before and between samples. Questionnaire for the sensory test is shown in an appendix.

3.6 Statistical analysis

Each experiment was independently replicated 3 times and all analysis and enumerations were done in duplicate. Significant differences between means were determined using Duncan’s multiple range tests. Statistical significance was given in terms of *P* values, with differences at the 95% confidence interval ($P < 0.05$) being considered statistically significant. The statistical analysis was performed by SPSS statistical software version 12.0.

Experiment 4: Investigation of relation between ice cream mix rheology and ice crystal growth in OKP-stabilized ice cream

This experiment was done at Kasetsart University, Bangkok, Thailand

The relation between ice cream mix rheology and ice crystal growth in OKP-stabilized ice cream were investigated in order to understand the correlation between ice cream mix rheological properties and the stability of ice cream containing OKP.

4.1 Rheological measurement of ice cream mix

Ice cream mix that was prepared in the previous experiment (Experiment 3) was also used in this study. Rheological properties of ice cream mix were measured by oscillatory rheology before and after heat shock. The heat shock of ice cream mix was performed by temperature cycling, ice cream mix was placed on a Peltier plate to give temperature cycles from -1°C to -10°C (1°C /min) and held at -10°C for 10 min, then warmed back to -1°C (1°C /min) and held at -1°C for 10 min. This temperature cycling was repeated 5 times. Physica MCR rheometer (Anton Paar GmbH, Graz, Austria-Europe) was used with a stainless steel 5 cm diameter, 2° angle cone. A gap of 90 µm was adjusted at -1°C. Strain sweeps from 0 to 100%, 1 s^{-1}, at -1°C were conducted.

The yield stress from dynamic shear data was analyzed by Rheoplus V3.40 software (Anton Paar GmbH, Graz, Austria-Europe). Conceptually, magnitudes of storage modulus G´ and loss modulus G´´ were determined for each sample. As the strain was increased, G´ and G´´ remained relatively constant until at a critical value of strain the magnitude of G´ decreased sharply. The magnitude of G´ at the critical strain value was recorded as the experimental yield stress (Yoo and Rao, 1995). Determination of the yield stress using amplitude sweeps, automatic analysis using a software analysis program. The bandwidth of the tolerated deviation was defined at 5%.

In separate experiments, ice cream mix was subjected to 5 temperature cycles as earlier. Frequency sweeps over the range 0.01 to 110 s^{-1} were conducted in the linear viscoelastic domain (0.1% strain), at -1°C. Complex viscosity was recorded. The differentiation of yield stress, G´, G´ and complex viscosity values between 0 and 5 temperature cycles were calculated as percentage of changing. The low percentage change represented the high stability of the ice cream mix. The rheological characteristics of the ice cream mix and percentage change were correlated to the ice crystal growth of the ice cream. Multiple linear regression analysis was applied for a prediction equation; the significant correlated variables for the equation were collected by the statistic software SPSS 12.0.

RESULTS AND DISCUSSION

1. Physico-chemical properties of okra cell wall (OKW) and okra polysaccharide (OKP)

Figure 6 Okra pod with 9-12 cm length and 2 cm diameter using in this study

1.1 Yields of OKW and OKP

The okra pod, was purchased from Timfood Co., Ltd., Thailand, was grown in Nakornnayok province, was harvested at 7 days after blooming. It size was around 9-12 cm length, 2 cm diameter (Figure 6). In order to have an impression on the proportion of individual parts of the fresh okra pod, the whole okra pod was taken part into three parts; pedicel, placenta and seed, and pericarp. A relative amount of different part of fresh okra pod, and yield of OKW and OKP is shown in Table 3. The pericarp was the major part (60.01%) of okra pod. Placenta and seed was 25.79%, and the minor part was pedicel (12.97%). Besides that, around 1.23% of the material was not recovered and this was probably due to some losses of the seed fraction. Since the okra pericarp is the part of interested, it was subjected to further studies preparing

OKW and OKP. As mentioned before, OKW was obtained after okra cell wall was ground and sieved, whereas, OKP was obtained after okra cell wall was extracted with distilled water. Resulting, OKW was compost of both water insoluble and water soluble polysaccharide, and OKP was a water soluble okra polysaccharide only.

Table 3 Relative amount of the different part of fresh okra pod and yield of OKW and OKP

Part of okra pod	% (base on fresh okra pod)
Pedicel	12.97 ± 0.11
Placenta and seed	25.79 ± 0.93
Pericarp	60.01 ± 0.70
Output of sample preparation	**Yield (%) (base on fresh okra pod)**
Okra cell wall	5.59 ± 0.52
OKW	4.11 ± 0.31
OKP	1.06 ± 0.22

Yield of okra cell wall and OKW were 5.59% and 4.11%, respectively, which compared to the fresh okra pod. The data showed that the operation of grinding and sieving of okra cell wall during OKW preparation loss 1.48% of okra cell wall.

Yield of OKP was 1.06% of fresh okra pod which comparatively higher than the yield of pectin that was extracted from spent guava (0.06%) and nopal cactus (0.6%) (Chakraborty and Ray, 2011; Cardenas *et al*., 2008) but lower than the yield of pectin extracted from apple pomace (6.13%), apple flour (9.73%) (Centeri-Schemin *et al*., 2005). Different source of pectin provide different pectin yield. Moreover, extraction conditions such as temperature, pH, extract medium, time length and extraction method also affected pectin yield, extracted components, and functional properties of its (Chakraborty and Ray, 2011; Cardenas *et al*., 2008; Centeri-Schemin *et al*., 2005; Khule *et al*., 2012). Ideally, the source of pectin that provides a high yield and the good functional properties has been looked for, but, in reality, the pectin with the good functional properties even with no high yield is also considered. It is interesting to search for the new sources of pectin because it is a challenge to find some novel functional properties of use.

1.2 Visual appearance of OKW and OKP

The visual appearance of OKW and OKP powders is shown in Figure 6. OKW was a fine powder with light-brown color. OKP was fluffy with light-yellow color. OKW smell greenly. OKP smell sweet.

OKW and OKP dispersions at 0.30% (w/w), is shown in Figure 7, was viscous liquid. Aqueous OKW was a translucent slimy liquid, showed invisible suspense particles when the sample was stirred, and no sedimentation was observed at 3 h of static settle. OKP at 0.30% displayed a clear transparent viscous liquid. Both OKW and OKP at 0.30% had no greeny and sweet smell that could be detected by smelling at room temperature like in powder forms.

Figure 7 Visual appearance of OKW (a) and OKP (b)

Figure 8 Visual appearance of OKW (a) and OKP (b) dispersions at 0.30% (w/w)

1.3 Color of OKW and OKP

The color of OKW and OKP was measured. The result is shown in CIE L*a*b* color scale (Table 4). Normally, the maximum value for L* is 100, which represents a perfect reflecting diffuser. The minimum for L* is zero, which represents black. The a* and b* have no specific numerical limits. Positive a* is red. Negative a* is green. Positive b* is yellow. Negative b* is blue (HunterLab, 2008).

L*, a*, and b* values of OKW powder were 78.75, -0.19, and 13.42, respectively, Whereas L*, a*, and b* values of OKP powder were 80.35, -0.86, and 14.52, respectively (Table 4). These indicated the colors of OKW and OKP powder were light green-yellow. However, OKP color was lighter than OKW. Rahimi *et al*. (2013) reported color of Persian gum which L*, a*, and b* values were 80.23, 5.60, and 18.68, respectively. Persian gum is a type of exudates gum which naturally secretes from the barks of mountain almond trees (*Amygdalus scoparia* Spach). (Rahimi *et al*., 2013). Color of stabilizer should be light because the color of stabilizer may affects color of food product. However, it depends on the concentration of use. Keshkaran *et al*. (2013) who studied the effect of gum tragacanth on rheological and physical properties of a flavored milk drink made with date syrup mentioned that color parameter (L*, a*, and b*) were significantly affected by the concentration of the gum tragacanth and also type of gum.

Dispersion of the sample changed the color of the sample which the L*, a*, and b* values of OKW dispersion were 63.72, -0.66, and 13.32, respectively, whereas, they were 92.97, -2.15, and 13.33, respectively, for OKP. The OKW dispersion was darker than the powder. In contrast, the dispersion caused OKP be lighter than its powder. The different color between powder and dispersion may was affected by different reflection of the samples. It may not be directly affected by the color pigment of the sample.

Table 4 Color values (L*, a* and b*) of OKW and OKP powders, and OKW and OKP dispersions at 0.3% (w/w)

Samples	L*	a*	b*
OKW powder	78.75 ± 0.20	-0.19 ± -0.01	13.42 ± 0.11
OKP powder	80.35 ± 0.29	-0.86 ± -0.74	14.52 ± 0.28
OKW dispersion	63.72 ± 0.29	-0.66 ± -0.02	13.32 ± 0.05
OKP dispersion	92.97 ± 0.56	-2.15 ± -0.05	13.33 ± 0.13

1.4 Bulk density

The bulk density was often referred to a derived property of powder which depend mainly on particle size, particle shape and tendency of the particles to adhere together (Kumar *et al*., 2011). This property is important with regard to its packaging (Aguilera *et al*., 2009). The bulk density of OKW and OKP were 0.32 ± 0.01 and 0.02 ± 0.00 g.cm^3, respectively. OKP had obviously 15 times lower bulk density than OKW. It had low bulk density because of its sponge structure. The freeze-drying process of sample left a very porous and low-density cake (Blaine *et al*., 2013).

The bulk density values indicated desirable packing characteristics which OKW powder had a better flow properties than OKP. Other polysaccharides had different bulk density such as the bulk density of tamarind seed polysaccharide was 0.74 g.cm^3 (Sravani *et al*., 2011), the bulk density of polysaccharide from microbial *Agaricus blazei* Murill was 0.33 g.cm^3 (Hong and Choi, 2007) and chickpea flour had 0.53 g.cm^3 of bulk density (Batistuti, 1998).

1.5 Chemical compositions of OKW and OKP

The result of proximate chemical composition was presented in Table 5. Data indicated that proximate compositions varied between OKW and OKP. Protein and crude fiber contents in OKW were higher than in OKP which the protein contents were 18.31 and 11.58% in OKW and OKP, respectively. The crude fiber contents in

OKW and OKP were 16.23 and 0.12%, respectively. These results indicated that the protein and crude fiber components were largely removed during OKP isolation preparation. On the other hand, moisture, lipid, ash and total carbohydrate were detected in OKP at higher level than that found in OKW. Data obtained proved that the chemical composition of OKW and OKP was affected by the different step of sample preparation. These results confirmed by statistical analysis, for which highly significant differences ($P < 0.05$) were observed between the contents in the two samples.

The OKW (18.31%) and OKP (11.58%) contained high protein contents compared to the results of previous studies of other research groups. Alba *et al*. (2014) reported okra polysaccharides that were extracted with buffer solution at pH 4 and 6 contained 2 and 3% protein, respectively. Okra fruit (seed removed) contain 9.6% protein (Ndjouenkeu *et al*., 1997). However, the protein content of OKP was slightly lower than the protein content of okra polysaccharide that was prepared by Prommakool (2011) (12.30%).

The protein in OKW and OKP possibly was co-extracted or conjugated with polysaccharide molecules. Conjugated protein is a protein that functions in interaction with other chemical group attached by covalent bond or by weak interaction (Nelson and Cox, 2005) and it may play an important role in physic-chemical properties of the samples. It has been report by many researchers that protein conjugated polysaccharide had superior emulsifying properties compared to non-conjugated mixtures (Dickinson, 1993; Shepherd *et al*., 2000). The attachment of proteins to polysaccharides can also lead to bigger improvements in solubility, thermal stability and emulsifying properties without loss of function and undesirable effects (Kato *et al*., 1990). Furthermore, polysaccharides may also mask the structure of the allergenic proteins (Arita *et al*., 2001).

Generally, the conjugation between protein and polysaccharide is prepared in specific control condition, for example, protein-polysaccharide conjugates suitable for stabilizing emulsions can be made in the laboratory by Maillard reaction of protein with polysaccharide under controlled dry heating conditions (Dickinson and Galazka, 1991). By optimizing the relative humidity and the heating time/temperature (e.g., 60°C for several days), it is possible to make soluble protein-polysaccharide conjugate with good emulsifying properties. Maillard reactions of several proteins with polysaccharides, leading to covalent bond formation under being reaction conditions, i.e., by gently heating of a dry biopolymer mixture over a period of few weeks in order to prepare hybrids with superior emulsifying properties compared to non-treated mixtures (Dickinson, 1993; Kato *et al*., 1990).

However, it seems protein in OKW and OKP sample was a co-extracted protein. The heating at 60°C for 30 min in the step of OKW preparation and heating at 70°C for another 30 min in the step of OKP extraction may not long enough heating time for generating chemical bond between protein and polysaccharide molecule in order to generate protein-polysaccharide conjugation. Nevertheless, the protein in OKW and OKP should be investigated by SDS-PAGE or FTIR techniques to determine whether the protein conjugated or non-conjugated with polysaccharide. If the protein has to be eliminated from OKW and OKP, precipitation at isoelectric point pH or hydrolyzation with protease enzyme will be the options.

Table 5 Chemical composition of OKW and OKP

Components (%)	OKW	OKP
Moisture	$10.31^{b} \pm 0.15$	$12.51^{a} \pm 0.22$
Protein	$18.31^{a} \pm 0.10$	$11.58^{b} \pm 0.19$
Fat	$0.10^{b} \pm 0.01$	$0.27^{a} \pm 0.05$
Ash	$8.91^{b} \pm 0.05$	$15.28^{a} \pm 0.06$
Crude fiber	$16.23^{a} \pm 0.45$	$0.12^{b} \pm 0.02$
Total soluble Carbohydrate*	44.33	60.35

Means with the same letters in the same row do not differ ($P > 0.05$).

(mean ± SD, n=3)

* Total soluble carbohydrate was calculated by different

1.6 Functional properties of OKW and OKP

1.6.1 Water solubility (WS) and water holding capacity (WHC)

The water solubility and water holding capacity were studied at concentration of 0.10-0.40% (w/w). The results are shown in Table 6. The water solubility of OKW, ranged from 39.57% to 33.05%, was concentration dependent, which the solubility of OKW decreased with increasing concentrations. On the other hand, OKP, had higher water solubility than OKW, dissolved completely at all concentrations study.

The water holding capacity of OKW ranged from 3.68% to 11.33%. It increased with increasing concentration. The water holding capacity of OKW was relatively higher than some other sample such as tropical breadfruit flour (8.27% at concentration 7.0% (w/w)) (Akanbi *et al*., 2009). The higher water holding capacity of OKW could be attributed to the presence of the greater amounts of protein and water insoluble polysaccharide in the sample. The inherent protein and water-insoluble polysaccharide may play the major role in the higher water holding capacity. Moreover, Lawal and Adebowale (2004) mentioned that the major chemical compositions that enhance the water holding capacity of the samples were protein and carbohydrate, since these constituents contain hydrophilic parts such as polar or charged side chains. Nonetheless, the water holding capacity of OKP could not be determined since OKP had no sediment that could be separated by centrifugation.

Remarkably, the results of water solubility and water holding capacity of OKW were concentration dependent. The results may be affected by the viscosity of the samples, since the separation ability of centrifugation method is generally viscosity dependent. The sample with higher viscosity had less separation ability. Viscosity affected movement ability of the particles inside the fluid (Momen-Heravi *et al*., 2012). The viscosity of OKW varied among the concentrations (Table 7). That was why both water solubility and water holding capacity of OKW showed concentration dependence.

Table 6 Water solubility and water holding capacity of OKW and OKP

Concentration (%)	Water solubility (%)		Water holding capacity (%)	
	OKW	OKP	OKW	OKP*
0.10	39.57^{a} ± 3.00	100^{a} ± 0.00	3.68^{b} ± 0.26	nd
0.20	37.10^{ab} ± 2.84	100^{a} ± 0.00	7.36^{ab} ± 0.60	nd
0.30	34.71^{ab} ± 2.24	100^{a} ± 0.00	9.63^{ab} ± 0.29	nd
0.40	33.05^{b} ± 0.94	100^{a} ± 0.00	11.33^{a} ± 0.38	nd

*nd = not detected

$^{a, b}$ Values with the same latter in the column do not differ

1.6.2 Foaming capacity and stability of PKW and OKP

Foaming capacity of OKW and OKP as affected by concentration, was expressed as volume increase due to whipping, is shown in Figure 9. The results showed that the maximum foam capacity of OKW and OKP were 132% and 58%, respectively. The maximum foaming capacity of the OKW and OKP was obtained at concentration of 0.30% and thereafter started to decrease. At high concentration which had high viscosity, the foam forming was obstructed. Foam volume decreases with increasing concentration/viscosity (Izydorczyk and Biliaderis, 1992). Although, foaming capacity and stability are important for product quality. Nevertheless, the analysis of processing conditions in food production such as mixing and whipping operations had to be concerned, the sample such 0.30% OKW may overflow with high speed mixing.

The foaming capacity of OKW could be attributed to the presence of greater amount of protein (Table 5) which had hydrophilic constituents in them. So the inherent proteins in the samples may play the major role in the foaming capacity. Observation made in this study indicated that protein content may affect foam formation. Soluble proteins can reduce surface tension at the interface between air bubbles and surrounded liquid. Thus, the coalescence of the bubbles was obstructed.

In addition, protein molecules can unfold and interact with one another to form multilayer protein film with an increased flexibility at the air liquid interface. As a result, it was more difficult for air bubbles to break, and foams were form and more stabilized (Adebowale and Lawal, 2003), whereas the viscosity of the samples may play the role on the foam stability.

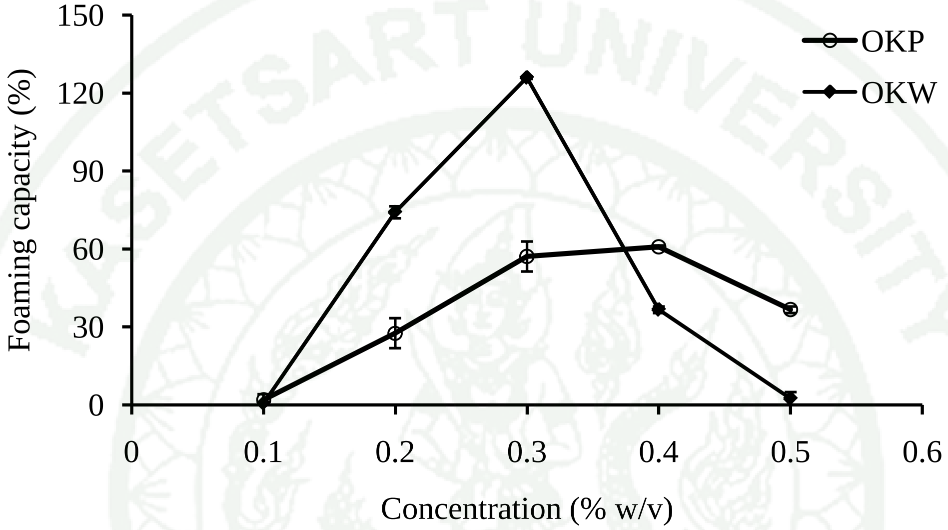


Figure 9 Foaming capacities of OKW and OKP at room temperature (~25^{o}C)

Foam stability of OKW and OKP are shown in Figure 10 and 11, respectively. Generally, when the foams are initially formed, the air bubbles are spherical and lamellas are thick containing large amounts of water. With time the liquid drains from the foam, the lamella thins, and air bubbles pack closer. Drainage of liquid from lamellae is the main destabilizing force as it allows the bubbles to become closer where, if the film is permeable, disproportionation occurs and large bubbles grow at the expense of small ones (Prins, 1988). Finally, rupture of the film at the air-foam interface leads to an increase of the foam collapse. The results of this study showed that OKW and OKP foam were more stable at higher concentrations. OKP at 0.40 and 0.50% obviously showed more foam stable than OKW. The highest foam stability, was observed in 0.50% OKP, was about 98% foam volume (%), was stabilized at all time study (15 min). In the high concentration of OKP, there was a significant increase in foam stability. This was due to their higher viscosity.

Obviously, the viscosity of 0.50% OKP was seven times higher than the viscosity of 0.50% OKW (Table 7). Similar results were reported for other polysaccharides (Izydorczyk and Biliaderis, 1992; Muralikrishna *et al*., 1987; Subba and Rao *et al*., 2004).

Rheological properties of sample have been shown to impact foam destabilization (Carp *et al*., 2001). Increased viscosity of the sample is known to retard foam drainage and the bubble disproportionation can be retarded by the presence of a viscous interface or viscous bulk, if the relevant viscosity is larger than a critical value. The presence of either a completely elastic interface or completely elastic bulk can stop bubble shrinkage (Kloek *et al*., 2001). Previous studies on the effect of polysaccharide (κ-carageenan) on foaming properties have shown that foam stability was strongly increased with increasing polysaccharide concentration (Carp *et al*., 2004). Xanthan at 0.25% strongly decreased the rate of liquid drainage of foams (Martinez *et al*., 2005). The lag time for foam collapse is influenced by the time before drainage leads to very thin films that rupture at the top of the foam. Therefore, the lower the rates of drainage are, the higher the lag times for collapse are expected to be. Nevertheless, the film structure and mechanical properties play a determinant role on the rupture of films (Martinez *et al*., 2005). The addition of polysaccharides at 0.25% to foam containing sunflower protein isolated increased the stability of the foams, against collapse (Martinez *et al*., 2005).

However, there was an inverse relation between foam capacity and foam stability observed in this study. OKW, had high foaming capacity, but had low foaming stability. Foam volume of 0.10% OKW decreased rapidly and foam completely was broken in 1 min, at higher concentration it required a few longer times. However, most of foams were broken in 5 min. This air bubbles might be easier to collapse and consequently lowered the foaming stability.

According to the foaming stability results, OKP may be more suitable than OKW using in ice cream. The high foaming stability of 0.40% and 0.50% OKP may be an advantage of OKP for improving an overrun of ice cream.

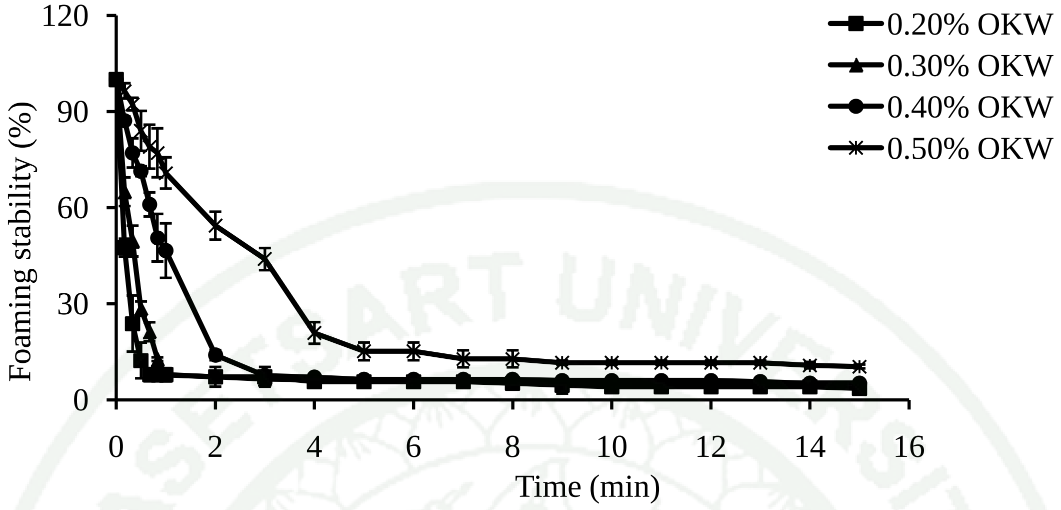


Figure 10 Foaming stability of OKW dispersion at room temperature (~25°C)

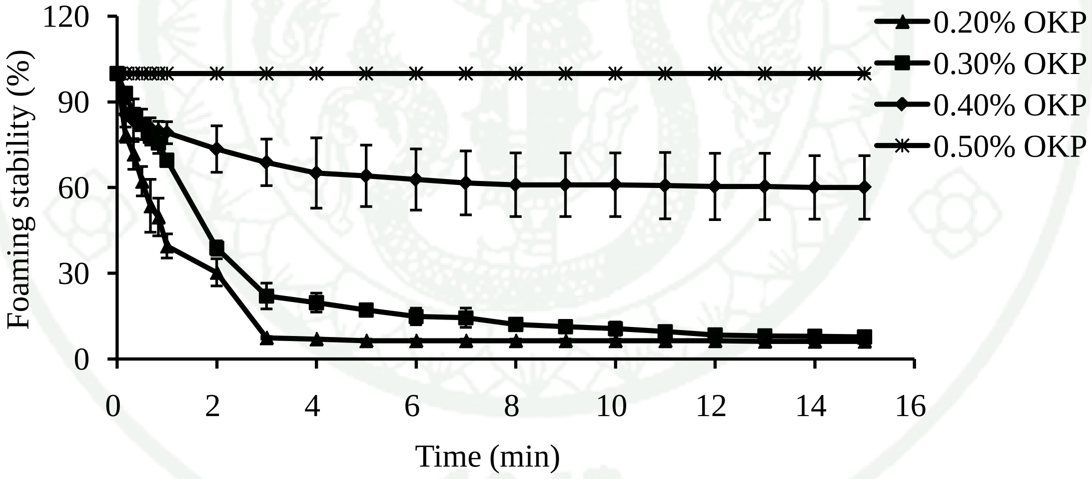


Figure 11 Foaming stability of OKP dispersion at room temperature (~25°C)

1.6.3 Apparent viscosity of OKW and OKP dispersions

In food systems, polysaccharides were used to provide a thickening and stabilizing effect. The corresponding enhancement of the viscosity, led to a better mouth feel of food products (Tuinier *et al.*, 1999), and also affected overrun, and texture quality of ice cream (Chang and Hartel, 2002) so the effects of food processing and food ingredients on the viscosity of polysaccharides are so important to be considered. An understanding of viscosity of OKW and OKP in different conditions which were simplified from a fundamental circumstance of ice cream ingredients and ice cream processing may help to decide the proper use of OKW and OKP in ice cream. The apparent viscosity results that were affected by the conditions of ice cream circumstance were shown and discussed as below.

1) Effect of shear rate on apparent viscosity of OKW and OKP

The apparent viscosity of the samples was measured with rheometer (AR 2000, TA Instruments, New Castle, DE, USA) equipped with acrylic 2^o cone and plate geometry. The results of shear rate on apparent viscosity of various concentrations of OKW and OKP were shown in Figure 12 and 13, respectively. As could be seen in these two figures, the apparent viscosity was observed decrease with the increasing shear rate, showing a typical non-Newtonian shear thinning behavior. GG and LBG also exhibited a shear thinning behavior (Cottrell *et al*., 1980). The reduction in the apparent viscosity with the shear rate has been related to the increased alignment of the constituent molecules (Rha, 1978) in the direction of flow as shear rate increases and therefore physical interaction between polymer chains decrease (Vardhanabhuti and Ikeda, 2006).

OKW and OKP concentrations in the range of the study (0.10-0.50%) were possible to be used in ice cream since its apparent viscosity was not too high comparing to other commercial polysaccharides that normally be used in ice cream such as guar gum (Table 7) (Clarke, 2004). The results helped to consider the apparent viscosity of OKW and OKP would be affected by the typical ice cream processing such as condition of pasteurization and homogenization of ice cream mix and freezing/whipping of ice cream.

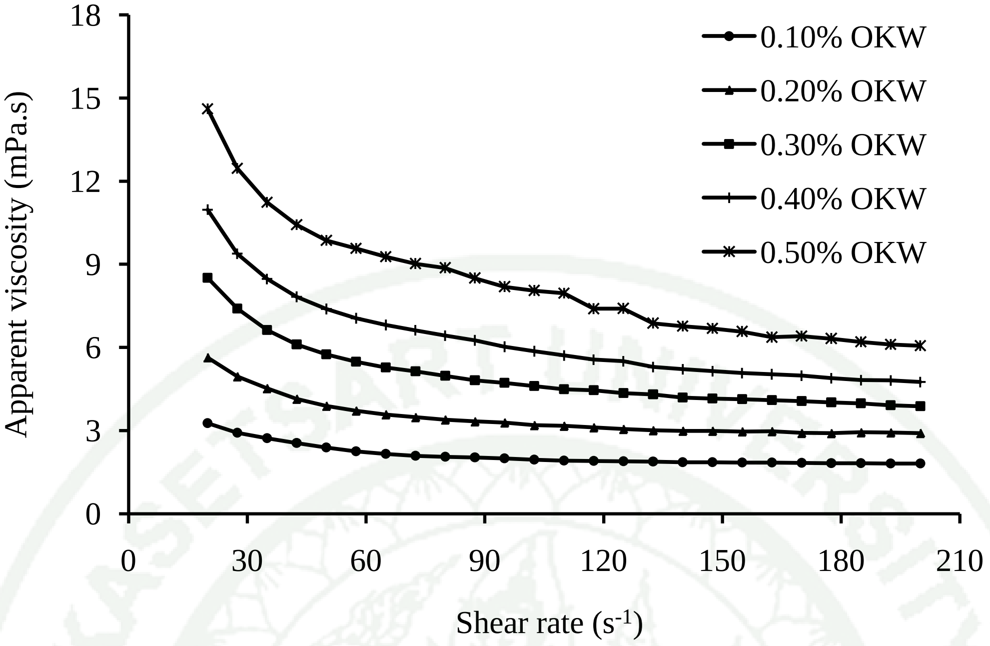


Figure 12 Viscosity curve of OKW dispersion at share rate 20 – 200 s^{-1}

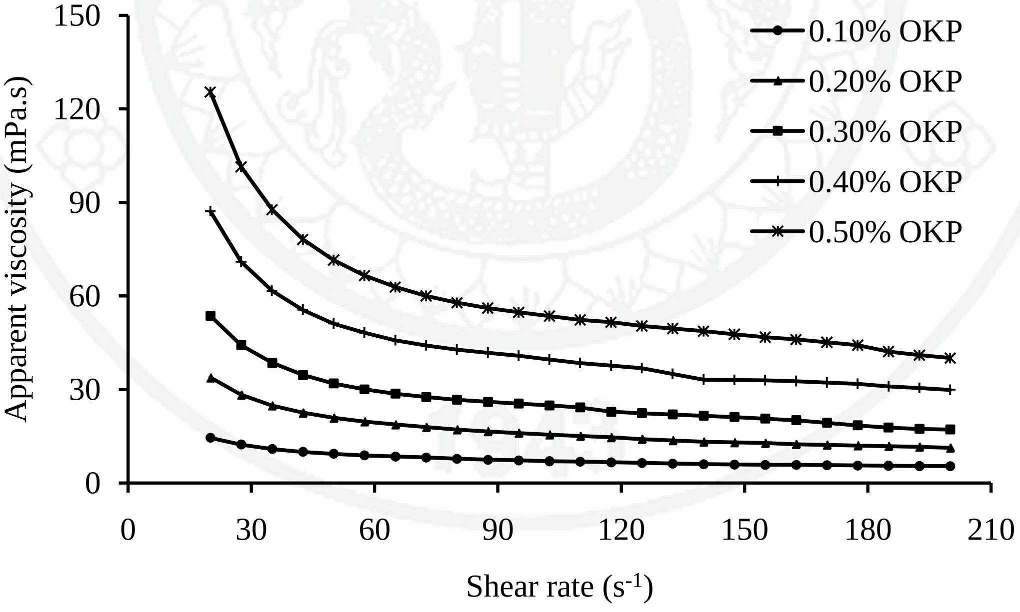


Figure 13 Viscosity curve of OKP dispersion at share rate 20 – 200 s^{-1}

2) Effect of polysaccharide concentration on apparent viscosity

The concentrations of OKW and OKP were varied from 0.10 to 1.6%. The apparent viscosity results of each concentration was reported at shear rate 50 s^{-1}, which represents the sensing shear rate in the mouth of low viscosity food (Akhtar *et al*., 2006; Stanley and Taylor, 1993). In this study, GG was included for comparative purposes. Apparent viscosities of OKW and OKP ranged from 2.40 to 40.00 mPas, and 8.82 to 566.85 mPas, respectively. OKP obviously had higher apparent viscosity than OKW (Figure 14). This was due to OKP was higher in water soluble polysaccharide content than OKW. The apparent viscosity of all samples increased with increasing concentrations. The increase in viscosity due to the increase in concentration was the result of greater chain interaction, which was evident in polysaccharides (Rao *et al*., 2004).

Apparent viscosities of OKP and GG increased sharply after concentration reached to 0.80% for OKP and 0.50% for GG. Whereas, OKW showed a linear viscosity increasing at all concentration studied. The apparent viscosity of polymers was dependent on their molecular weight and chain dimension (Flory, 1953). In dilute solutions, the polymer chains were separated, so apparent viscosity of a polymer in solution depends only on the dimension of the polymer chain (Rao, 1999). When concentration increased, the coils start to overlap and interpenetrate one another. The transition from dilute solutions to concentrate solution was usually accompanied by a produced change in the concentration dependence of solution viscosity (Morris *et al*., 1981; Morris, 1995).

OKP had high apparent viscosity which might be the good properties that can help maintain stability in ice cream, whereas, OKW showed low viscosity, it might be ideally used in high fiber ice cream, or fiber deprived health foods.

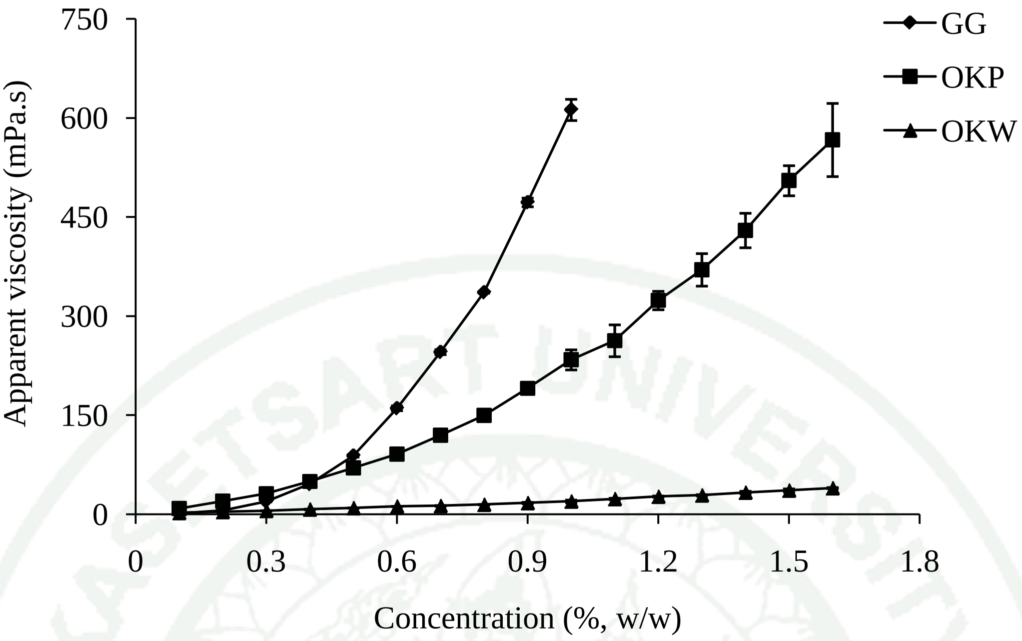


Figure 14 Apparent viscosity OKW, OKW and GG dispersions at shear rate 50 s^{-1}

.

3) Effect of freeze-thaw cycles on apparent viscosity

Temperature fluctuation during storage, distribution, and handling of ice cream and other frozen desserts strongly affects quality of the products, which result from ice recrystallization (Arbuckle, 1986). The information about changes of apparent viscosity affected by freeze-thaw cycle may help to estimate the changes of product quality occur during storage and distribution of ice cream. The results of this study, freeze-thaw cycles affecting apparent viscosity of the samples are shown in Figure 15. Changes in apparent viscosity between 0 and 1 cycle, apparent viscosity decreased considerably, the decreases of apparent viscosity were 19.87%, 23.15%, and 16.69% for GG, OKW, and OKP, respectively. Between 1-8 cycles, apparent viscosity of GG exhibited plateau zone, which the cycle numbers of GG did not significantly affect after their structures changes, possibly because it had already sustained considerable damage in the first cycle, whereas, OKW and OKP reached a plateau zone after second and third cycles, respectively.

The lower apparent viscosity of the samples subjected to freeze-thaw cycles with respect to the sample not subjected to cycles indicated that once subjected to cycles, dispersion never recover their original structure. Successive cycles could progressively lead to the creation of a aggregated structure of the polysaccharide (Eliasson and Kim, 1992) and lead to an increased degree of phase separation (Hermansson, 1997), as evidenced by the lower apparent viscosity obtained after cycle with respect to the samples not subjected to cycles.

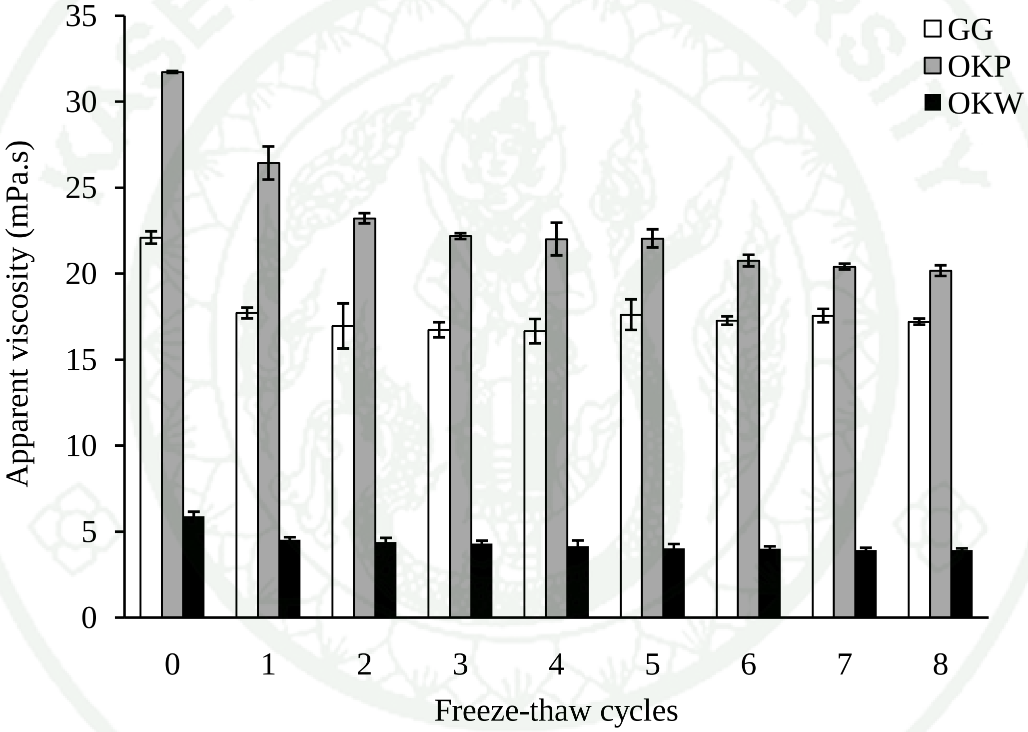


Figure 15 Apparent viscosity of 0.30% OKP after freeze-thawed cycle (20°C to -21°C to 20°C) the result was reported at shear rate 50 s^{-1}

4) Effect of pH on apparent viscosity

Application of OKP which is a charge polysaccharide has to be concerned about pH of food system. Normally, different types of ice cream have different pH such as typical ice cream, its pH is ~6.30 (Marshall *et al*., 2003), yogurt ice cream, its pH is 4.70-5.10 (Kim *et al*., 2009). So the study of effect of pH on apparent viscosity of OKP should not be neglected. The information about effect of pH on apparent viscosity of OKP helps to decide the suitable application of OKP.

The effect of pH on apparent viscosity of OKP was determined. However, since the previous studies showed that OKW did not much contribute the apparent viscosity so as the effect of pH on apparent viscosity, OKW was excluded from this study. The pH values of polysaccharide in dispersion were important because some functional properties such as viscosity were highly affected by pH changes. Apparent viscosity of OKP in different pH condition was showed in Figure 16. Apparent viscosity results showed that OKP was pH dependent in the pH ranges of 3-4.

The viscosity increased with increasing pH between 3-4. Apparent viscosity increased as the pH values increased until it reached minimum isoelectric point between pH 3.0 to 4.0 (pectin pKa ~ 3-4, Sriamornsak and Wattanakorn, 2008) or perhaps due to the repulsive effects of negatively charged carboxyl group that extends the chain and increases its water-binding capacity, typical of acidic polysaccharides (Rao *et al*., 2004). Moreover, OKP solution exhibited similar apparent viscosities between pH 4-9, increasing in pH was not significant on apparent viscosity. This stated that OKP was pH stable at basic pH. The study was in agreement with the study of Bath and Tharathan (1987), who found that the viscosity of aqueous extracts of okra polysaccharide was highest in the pH range of 4-5 and the apparent viscosity was not different in the pH range of 5-10.

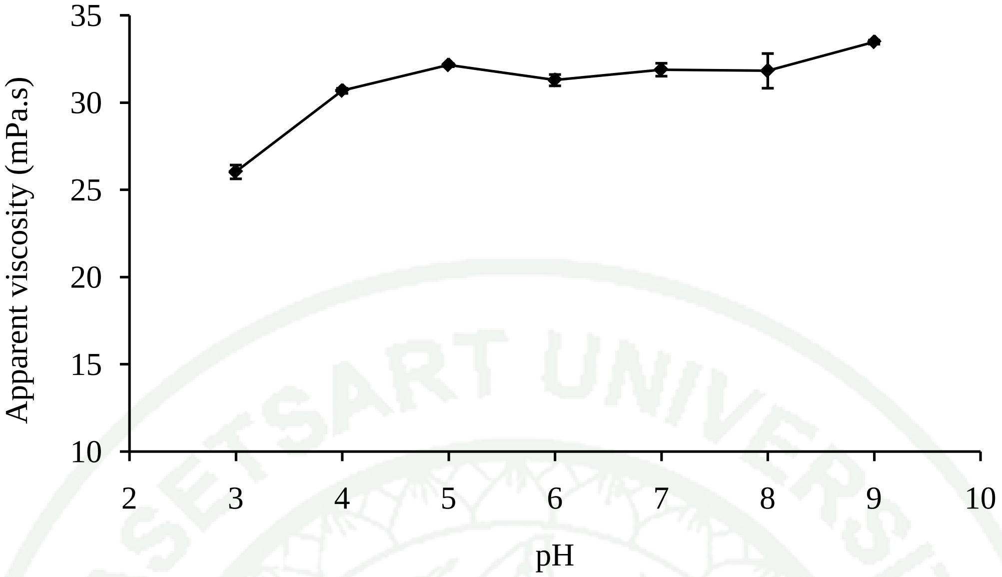


Figure 16 Apparent viscosity of 0.30% OKP in different pH conditions the viscosity was reported at 50 s^{-1}

5) Effect of heating and cooling on apparent viscosity

In food processing, pasteurization for destroying pathogenic microorganism is important. The study of effect of heating-cooling on apparent viscosity was simulated from the pasteurization of ice cream mix. The changes of apparent viscosity after heating and cooling have to be considered. In this study, the temperature of the sample was controlled by the peltier system on the plate geometry of rheometer during study. Changes in apparent viscosity during heating from 20 to 90^{o}C and cooling from 90 to 20^{o}C of 1.0% OKP are presented in Figure 17. Apparent viscosity decreased constantly as temperature increased and apparent viscosity increased with cooling. This result was in agreement with those reported for cowpea and linseed polysaccharides (Muralikrishna *et al*., 1987). The decreases in viscosity with increase in temperature could be explained by the as temperature increase, thermal energy of the molecules increases and molecular distances develop due to reduction of intermolecular forces hence viscosity of the fluid decreases (Togrul and Arslan 2004). The direction of changes in apparent viscosity was clockwise (start from the upper branch). Cooling after heating turned the apparent viscosity increased and highest at the final temperature (20^{o}C). Anyway, it was lower than the original viscosity. It was considerable loss of viscosity, which 17.16% of apparent viscosity could not recovery. However, OKP may be possible to recover its viscosity if it was allowed a longer rest.

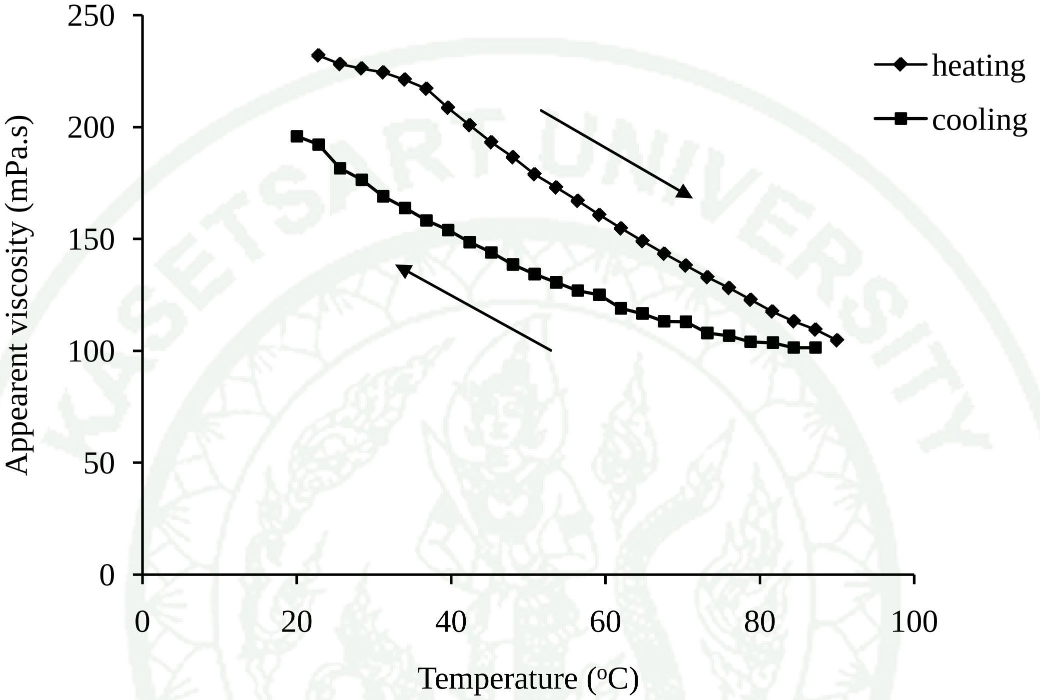


Figure 17 Apparent viscosity of 1.0% OKP during heating and cooling at 1.67°C/min at shear rate 50 s^{-1}

6) Effect of salt ion on apparent viscosity

Generally, in food formulation, salt was added for the purpose of processing or tasting. Moreover, salt ion also naturally presences in some foods such as milk. Milk is ice cream ingredient that naturally contains mineral salt such as calcium, magnesium, potassium, sodium, phosphorus (Fox and McSweeney, 1998). So the study of the effect of salt ion on apparent viscosity of OKP is important. The results of the effect of salt concentrations on apparent viscosity are shown in Figure 18. The apparent viscosity of OKP significantly decreased as the salt concentration increased. Mono- and divalent cations differed in their effects on the viscosity. Divalent cations caused more decreasing viscosity than monovalent cations. The decreased in apparent viscosity values in salt exhibit a typical electrolyte behavior. The decrease with the addition of salt was due to the charge screening of electrostatic repulsions of the polysaccharide (Muller *et al*., 1986; Rochefort and Middleman, 1987). The charge screening led to a more compact conformation and caused a reduction in hydrodynamic size of the polysaccharide molecule (Rochefort and Middleman, 1987), hence, lowering the viscosities. The result was in agreement with the study of the addition of NaCl into anionic xanthan solution (Khouryieh *et al*., 2007).

It was interesting to note that after reaching the 50 mM of salt, the higher concentration of salt in OKP dispersion did not cause much change in apparent viscosity. At 50 mM of monovalent cations salt, potassium and sodium caused apparent viscosity decreased 33% and 32%, respectively, whereas, divalent cation salt, magnesium and calcium, resulted decreasing 57% and 59% of apparent viscosity. It shod be noted that if food formula contains salt lower than critical zone (< 50 mM), the apparent viscosity of the system should be strongly considered. However, in typical ice cream formulation consists of potassium (47 mM) sodium (27 mM), magnesium (6 mM), and calcium (36 mM), and some other mineral such as phosphorus (37 mM) (Pearce, 2013; Fox and McSweeney, 1998). The information for estimation of minerals in ice cream was shown in appendix.

Moreover, the study of Monpien (2005) showed that salt at very low concentration (0-1,000 μM) of calcium ions did not significantly affect on okra polysaccharide dispersion. Beside that Sandford *et al*. (1978) reported that the trivalent metals, such as $Fe^{3+,}$ caused rapidly decreased viscosity and precipitate the polysaccharides, but it was not studied with OKP in this experiment.

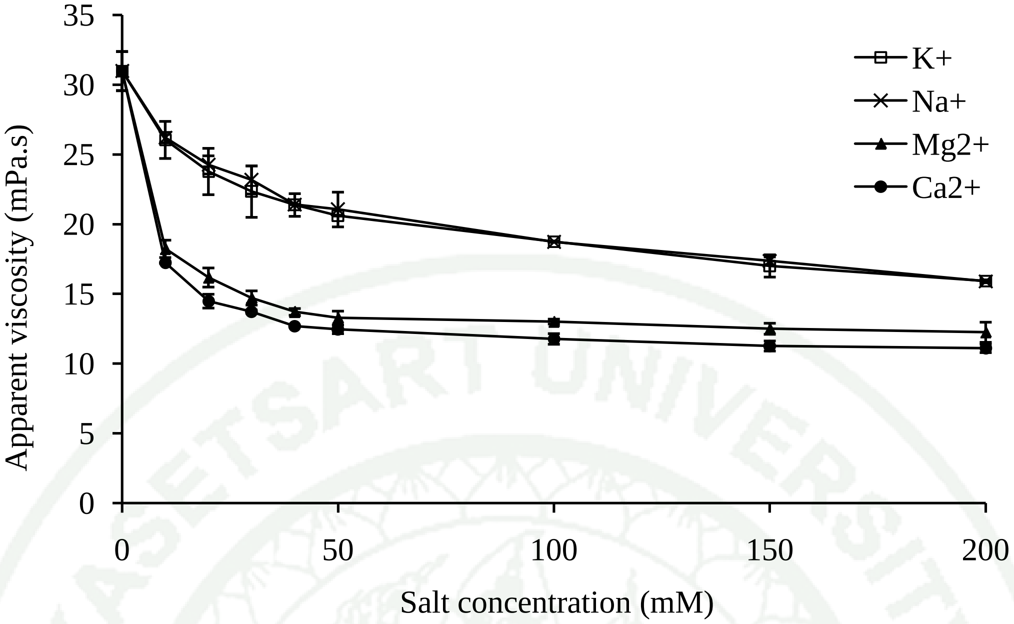


Figure 18 Apparent viscosity of 0.30% OKP in different salt concentration the result was report at shear rate 50 s^{-1}

7) Effect of OKP-GG and OKP-xanthan gum mixing ratio on apparent viscosity

When different polysaccharides are mixed together, they are expected to have specific behavior (Rodriguez-Hernandez *et al*., 2006). The synergistic behavior of aqueous polysaccharide blends is commercially valuable because it often lead to new desirable physicochemical properties or the development of new texture, which could not be results from aqueous dispersions of the component polysaccharides (Morris, 1990 and 1991).

The evaluation of the synergistic effect of the OKP-GG and OKP-xanthan gum mixtures, the apparent viscosities of the mixtures were plotted as a function of polysaccharide mass fractions (%). The results are shown in Figure 19. The apparent viscosity of OKP-GG mixtures were higher than the calculated values assuming no interaction, demonstrating that intermolecular interaction occurred between OKP and GG. If no interaction existed between the two polymers in the mixtures, a linear relationship between apparent viscosity and GG fraction would be observed, and the apparent viscosity of the mixtures would be only the weight average of the two polymers. Instead, the apparent viscosities of the mixture were out of linearity, suggesting that specific attractive forces were present between the OKP and GG molecules. When the OKP-GG with the ratio of 2:8 was mixed (80% GG), the apparent viscosity of the corresponding blend was found to be 805.60 mPa.s, while the apparent viscosity of component OKP and GG dispersions were only found to be 20.17 and 336.15 mPa.s, respectively (Appendix Figure 2). For the aqueous OKP-GG blends investigated, the resulting apparent viscosity was observed to be much greater than the combined apparent viscosity of the component polysaccharide dispersions, showing a synergistic viscosity property.

In the OKP-xanthan gum mixture, a synergistic interaction was not observed (Figure 19). The apparent viscosity of OKP-xanthan mixture was similar to the calculated values assuming no interaction. A linear relationship was found between the apparent viscosity of OKP-xanthan fractions, suggesting that the apparent viscosity of the mixture was only the weight average of the two polymers, and that no specific interaction forces were present between the two polymers that would encourage aggregation. This fact demonstrated that there was a synergistic effect when aqueous OKP solution was blended with aqueous GG solutions but it may not occurred in xanthan mixture.

This synergistic viscosity property was also found for other aqueous polysaccharide blends (Zhang, 1999). Chaisawang and Suphantharika (2005), investigated the effects of GG and xanthan gum addition on physical and rheological properties of cationic tapioca starch, and found that the GG was more effective than xanthan gum in term of increasing peak viscosity due to its ability to increase the swelling power and solubility index. Moreover, Yoo *et al*. (2005) studied the rheological properties of rice starch-galactomannan mixtures at different concentration of guar gum and locust bean gum in steady and dynamic shear, and found that the mixtures showed high shear-thinning flow behaviors with high yield stress (Yoo *et al*., 2005)

GG and xanthan affected on the apparent viscosity of OKP mixture differently. The different structures of GG and xanthan may play the important role on the synergistic effects. Since GG, is nonionic polysaccharide, is a galactomanan consisting of a linear mannose backbone substituted with galactose side chain (BeMiller and Whistler, 1996) whereas xanthan, is anionic polysaccharide, is a linear cellulosic backbone with trisaccharide side chain, attached to alternating backbone residues (Lapasin and Pricl, 1995).

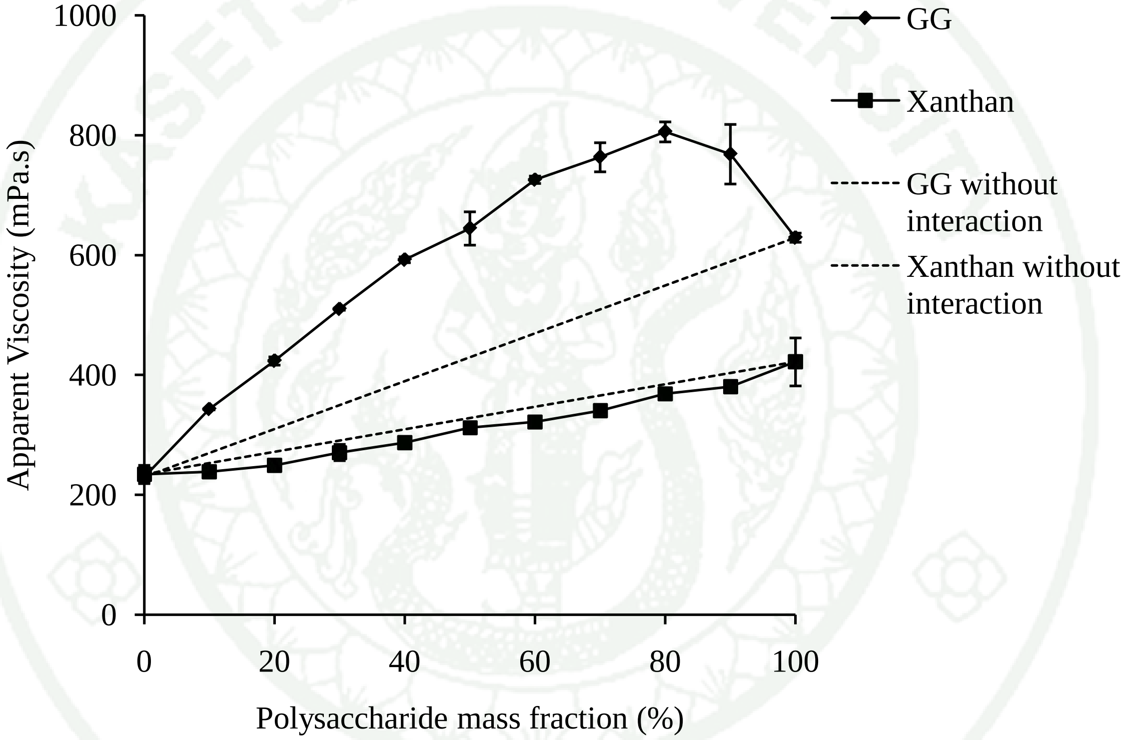


Figure 19 Apparent viscosity of OKP and polysaccharides mixture at difference mass fractions of GG or xanthan (%), shear rate of 50 s^{-1}

2. Rheological properties of ice cream mix model containing OKW and OKP

In order to understand the effect of each ice cream mix ingredient on rheological properties of ice cream mix model containing OKW and OKP, flow behavior and apparent viscosity of ice cream mix models were studied. The ice cream mix models were prepared from simple model solution to complex model solution. It had three levels of complication, which were 1) polysaccharide dispersion, 2) polysaccharide/sugar solution, and 3) polysaccharide/sugar/milk solid nod fat (MSNF) solution.

Viscosity curves and flow curve of three ice cream mix models with 0.30% polysaccharides are shown in Figure 20, 21, and 22. GG was included in the experiment for comparison purpose. Experimental results were found to exhibit a clearly pseudoplastic (shear thinning) behavior of all samples. Apparent viscosity of the samples decreased with increasing share rate (Figure 20a, 21a, and 22a) whereas shear stress increased with increasing shear rate (Figure 20b, 21b, and 22b). Such behavior has been observed for many of hydrocolloid solutions (Marcotte *et al*., 2001; Togrul and Arslan, 2003). The decreased in viscosity as the shear rate was increased has been related to the increased alignment of constituent polymer molecules of the model (Rha, 1975). It could explain that shearing caused the particles to orient in the flow direction. Shearing also caused agglomerates to disintegrate or particles to change their form. During this process the interaction forces between the particles usually decrease and this also lowers the viscosity (Mezger, 2002).

Both viscosity curve and flow curve of all samples showed that the samples were shear-thinning fluid. The viscosity of all sample increased with increasing concentrations of OKW and OKP. The higher solid contents generally cause an increase in the viscosity resulting from mainly molecular movement and interfacial film formation (Maskan and Gogus, 2000). However, in the polysaccharide dispersion, the viscosity of the samples were OKP > GG > OKW (Figure 20a) whereas in polysaccharide/sugar solution (Figure 21a) and polysaccharide/sugar/MSNF solution (Figure 21a) the viscosity of the samples were

GG > OKP > OKW. This implied that sugar competed binding water caused the viscosity of OKP decreased. The finding was similar to the study of Baht and Tharathan (1987) who reported that viscosity of the okra polysaccharide exhibited a pseudoplastic behaviour and its viscosity decreased when adding water-soluble agent such as sugar. Moreover, in polysaccharide/sugar/MSNF solution, the viscosity of OKP may was affected by both sugar and milk proteins. Due to sample complexity, rheological characteristic is affected by many factors including the presence of components and their concentration, hydration phenomena, protein aggregation (Goff *et al*., 1994; Nor Hayati *et al*., 2007).

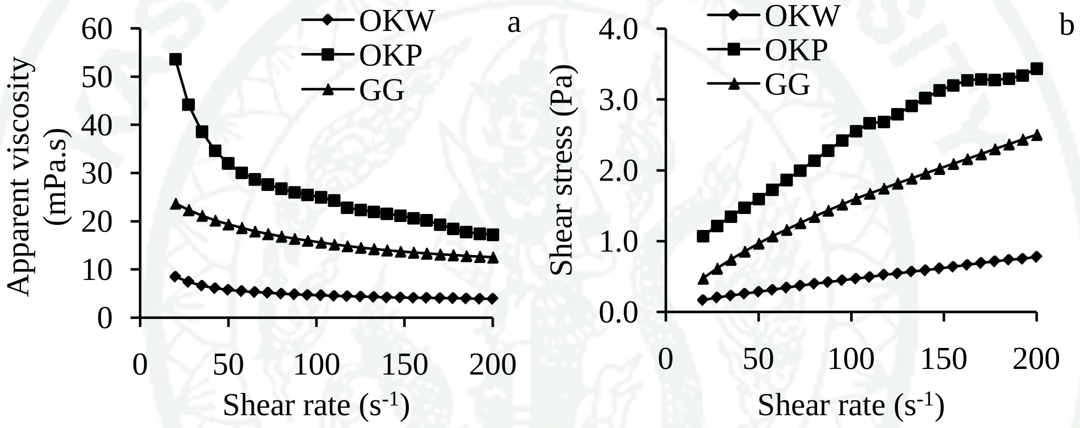


Figure 20 Viscosity curve (a) and flow curve (b) of 0.30 % OKW, OKP and GG in water at shear rate 20 to 200 s^{-1}

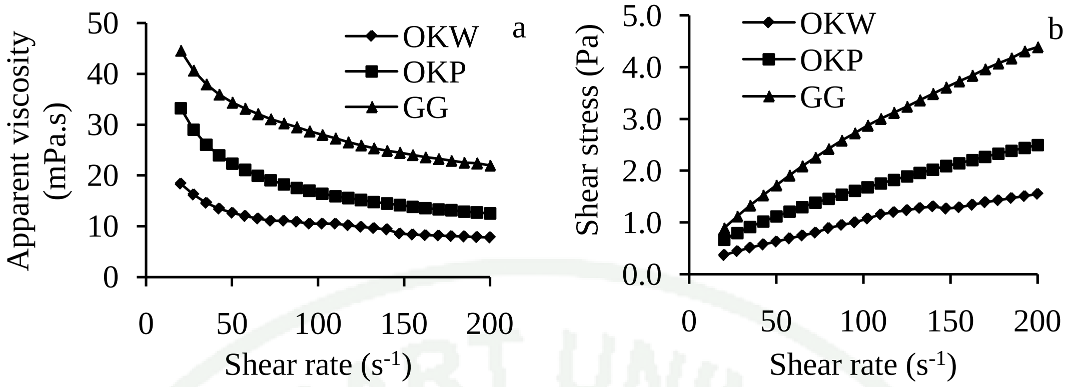


Figure 21 Viscosity curve (a) and flow curve (b) of 0.30 % OKP, OKW and GG in 15% sugar solution at shear rate 20 to 200 s^{-1}

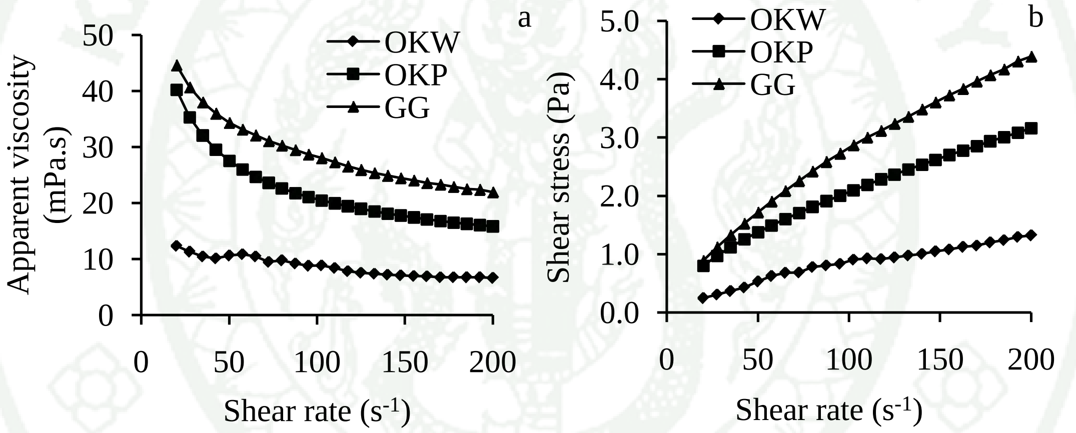


Figure 22 Viscosity curve (a) and flow curve (b) of 0.30 % OKP, OKW and GG in 15% sugar with 10% skim milk powder solutions at shear rate 20 - 200 s^{-1}

Steady shear flow behaviors of all ice cream mix models containing OKW and OKP concentrations of 0.10% to 0.60% were investigated. The power law model was used with concentration. The parameters obtained for the power law model are summarized in Table 7, 8, and 9 for 1) polysaccharide dispersion, 2) polysaccharide/sugar solution, and 3) polysaccharide/sugar/MSNF solution, respectively. The model appeared to be suitable for describing the flow behavior of the samples with high determination coefficients (R^2 = 0.98).

Table 7 Power law parameters of OKW, OKP and GG in water

Sample	Concentration (%)	Apparent viscosity (mPa.s)	*K* (mPa.s^n)	n	R^2
GG	0.1	1.655	0.001	0.993	0.999
	0.3	19.40	0.060	0.705	0.998
	0.5	87.51	0.682	0.470	0.999
OKW	0.1	2.393	0.006	0.752	0.994
	0.2	3.892	0.011	0.725	0.993
	0.3	5.752	0.020	0.677	0.996
	0.4	7.391	0.029	0.650	0.998
	0.5	9.864	0.043	0.628	0.996
	0.6	12.22	0.056	0.625	0.993
OKP	0.1	9.353	0.049	0.577	0.998
	0.2	20.89	0.128	0.569	0.998
	0.3	31.96	0.196	0.559	0.992
	0.4	51.13	0.288	0.547	0.993
	0.5	71.52	0.418	0.542	0.995
	0.6	92.53	0.548	0.535	0.993

Table 8 Power law parameters of OKW, OKP and GG in 15% sugar solutions

Sample	Concentration (%)	Apparent viscosity (mPa.s)	*K* (mPa.s^n)	n	R^2
GG	0.0	1.686	0.0271	0.7149	0.9909
	0.1	2.893	0.0487	0.7212	0.9878
	0.3	29.89	0.6779	0.5164	0.9937
	0.5	134.6	4.1231	0.3210	0.9938
OKW	0.1	3.725	0.0807	0.5876	0.9679
	0.2	6.994	0.1530	0.5647	0.9777
	0.3	12.64	0.3122	0.4893	0.9813
	0.4	14.22	0.3460	0.5096	0.9746
	0.5	17.37	0.4212	0.5100	0.9748
	0.6	22.47	0.5400	0.5153	0.9756
OKP	0.1	7.988	0.1865	0.5201	0.9763
	0.2	15.87	0.3945	0.4716	0.9827
	0.3	22.37	0.5790	0.4391	0.9864
	0.4	40.99	1.0877	0.4210	0.9860
	0.5	48.28	1.3362	0.3888	0.9886
	0.6	76.57	2.2107	0.3604	0.9893

Table 9 Power law parameters of solution of polysaccharide in 15% sugar and 10% milk solid not fat (MSNF) solution

Sample	Concentration (%)	Apparent viscosity (mPa.s)	*K* (mPa.s^n)	n	R^2
GG	0.0	1.611	0.0251	0.7578	0.9872
	0.1	6.073	0.1092	0.6798	0.9874
	0.3	34.36	0.7751	0.5288	0.9921
	0.5	125.8	3.4508	0.3973	0.9870
OKW	0.1	3.722	0.0767	0.6186	0.9882
	0.2	7.219	0.1588	0.5403	0.9893
	0.3	10.62	0.2240	0.5430	0.9893
	0.4	14.64	0.3066	0.5599	0.9870
	0.5	19.25	0.4000	0.5835	0.9843
	0.6	23.54	0.4964	0.6090	0.9789
OKP	0.1	6.610	0.1346	0.5961	0.9884
	0.2	17.32	0.3958	0.5210	0.9860
	0.3	27.56	0.6982	0.4529	0.9864
	0.4	56.32	1.5525	0.3965	0.9863
	0.5	87.36	2.4948	0.3728	0.9867
	0.6	155.5	4.6293	0.3438	0.9848

It can be seen that the addition of OKW and OKP affected the rheological behavior of ice cream mix models, enhancing viscosity development and strengthening shear thinning behavior as depicted by the increase of apparent viscosity and consistency coefficient (*K* value), and the decrease of flow behavior index (n value). The n values, *K* values, and apparent viscosity (shear rate 50 s^{-1}) of all samples containing OKP ranged from 0.577 to 0.344, 0.049 to 4.63 $mPa.s^n$, and 9.353 to 155.5 mPa.s, respectively. Whereas the n values, *K* values, and apparent viscosity of all samples containing OKW ranged from 0.752 to 0.609, 0.006 to 0.496 $mPa.s^n$, and 2.393 to 23.54 mPa.s, respectively (Table 7, 8, and 9). This confirmed that OKP had higher potential to increase pseudoplasticity and apparent viscosity of the samples than OKW. Since OKP had higher water soluble polysaccharide amount than OKW. Soukoulis *et al*. (2009) suggested that the increased viscosity of the water soluble polysaccharide enriched ice cream mix seem to be caused both by the contribution of the soluble matter to the composition of the aqueous phase and by the contribution of polysaccharide to the increase of total solids, affecting the three dimensional conformation of the hydrated biopolymers (Soukoulis *et al*., 2009).

The finding of n values and apparent viscosity of this study had similar trend to the results of the former studies. The flow behavior index (n) was reported to be around 0.7 for the ice cream mixes (Goff and Davidson, 1994). Cottrell *et al*. (1980) showed the n value of the ice cream mix to be in the range 0.98-0.68 and 0.88-0.48 for the GG and LBG used in the range of 0.05-0.40% concentrations, respectively. Kaya and Tekin (2001) obtained the n values of 0.96-0.77 for the ice cream mixes containing 0.4-1.0% salep. Minhas *et al*. (2002) mentioned that the flow behavior index of ice cream mixes made with different stabilizers was between 0.74 and 0.93. An increase in the polysaccharide concentration was accompanied with a decrease in the flow behavior index (n value). The results of this study agreed with former researches (Cotrell *et al*., 1980; Kaya and Tekin, 2001). It has been reported that for a polysaccharide type, the values of the flow behavior index and its change with concentration were highly dependent on the molecular size (Marcotte *et al*., 2001).

Aime *et al*. (2001) reported the consistency coefficient (*K* value) to their ice cream mixes between 0.0733 and 1.260 Pa.s^n. In Muse and Hartel's research (2004), the *K* values varied from 0.145 to 0.211 Pa.s^n. Minhas *et al*. (2002) concluded that the *K* values of the ice cream mixes ranged between 0.29 and 1.19 Pa.s^n. Moreover, Aime *et al*. (2001) published data, the apparent viscosity of ice cream mixes ranged from 18-149 mPa.s. Hagiwara and Hartel (1996) showed that the range of apparent viscosity of ice cream mixes at shear rate 115 s^{-1} and temperature of 5^{o}C was approximately 23-58 mPa.s for unstabilized mixes and 579-687 mPa.s for stabilized ice cream mixes.

For all samples, an increase in concentrations of polysaccharide were accompanied with an increase in pseudoplasticity, show by a decrease in values of the flow behavior index (n), a measure of the departure from Newtonian flow. The smaller the n values the greater the departure from Newtonian behavior (Chinnan *et al*., 1985). Comparatively, the magnitude of the flow behavior index was the lowest for the ice cream mixes containing OKP and the highest for the mixes with the OKW within in the range of concentration studied. As it was described above, OKP was more pseudoplasticity than OKW. It has also been shown that polysaccharide dispersion with a high of n value tends to feel slimy in the mouth. While high viscosity and a good mouth feel characteristics were desirable, the choice should be a polysaccharide system having a low n value (Szczesniak and Farkas, 1962). Since the samples with OKP had low n values and high apparent viscosity, OKP may provide a good mouth feel characteristic to the product.

3. Effect of Okra Cell Wall and Polysaccharide on Physical Properties and Stability of Ice Cream

3.1 Rheological properties of ice cream mix

Knowledge of the rheological characteristics of foodstuffs is important for quality control, texture, processing, and the selection of the proper equipment (Kus *et al.*, 2005). Smooth texture, which is the most commonly desired attributes of ice cream during consumption, could be provided by an ice cream mix with optimum rheological properties (Dogan and Kayacier, 2007). In this study, the rheological characteristics of the ice cream mix were evaluated using the steady shear test which performed on a AR2000 rheometer, shear rate range from 20 to 200 s^{-1}, temperature 20^{o}C.

Rheological parameters including flow behavior index (n value) and consistency coefficient (*K* value), obtained using the Power Law model of various OKW and OKP concentrations (0.15-0.45%) at 20^{o}C showed that the concentrations of okra cell wall and okra polysaccharides affected consistency coefficient (*K*) and flow behavior index (n) (Table 10) of ice cream mix. The *K* value indicated the relative thickness of a solution. In ice cream mix, consistency coefficients were positively correlated with the apparent viscosity of ice cream mix ($R^2 = 0.95$), similar to the finding of Aime and others (2001). The *K* value of the mixes with OKW and OKP ranged from 129 – 358 mPa.s and 280 – 1622 mPa.s, respectively. The *K* value of ice cream mix with OKP increased dramatically when the OKP concentration was higher than 0.15%, whereas the *K* value of ice cream mix with OKW increased linearly with the OKW concentration. This different response of *K* values may be due to higher content of water-soluble polysaccharide in OKP, resulting in greater polysaccharide interchain interaction as compared to OKW. Kontogiorgos and others (2012) indicated that the critical concentration of okra polysaccharide solution, exhibiting interchain interaction behavior, was 0.83% w/v. With 60% water content in our ice cream formulation, the 0.45% (w/w) OKP would correspond to 0.75% solution concentration resulting in rapid increase of the ice cream mix viscosity. Thus, the drastic increase in *K* values of the ice cream mix with OKP was observed. The increasing of OKW and OKP concentration 3 times (0.15-0.45%) caused the *K* values of ice cream mix with OKW and OKP increased 2.77 and 5.79 times, respectively (Table 10). Goff *et al*. (1994) also mentioned that the consistency coefficient strongly depends upon the kind and amount of stabilizer added to the mix (0.015 Pa.s^n to 0.25 Pa.s^n) as does the flow behavior index (0.98 to 0.38) (Goff *et al*., 1994).

The flow behavior index (n) of ice cream mix with OKW and OKP were in the range from 0.71-0.62, and 0.63-0.52, respectively (Table 10). The n value for commercial ice cream mix has been reported to average 0.7(Goff and Davidson 1994), although other investigators have found the n values of ice cream mix containing 0.1 and 0.2% stabilizers to vary from 0.95 to 0.45 (Soukoulis and others 2008). All flow behavior indexes (n) were less than 1 (Table 10), indicating the pseudoplastic behavior of the ice cream mix. The smaller the n value the greater the departure from Newtonian behavior, hence greater pseudoplasticity (BahramParvar and others 2010). As OKW and OKP concentration increased the pseudoplasticity of the ice cream mix increased, similar to the finding of Farhoosh and Riazi (2007) and BahramParvarand *et al*. (2010).The OKP-containing ice cream mix exhibited a greater viscosity, consistency coefficient, and degree of pseudoplasticity than OKW-containing mix. Moreover, it has been reported that a high pseudoplasticity (low n value) of polysaccharides allows liquid foods to be pumped easily and imparts a thinner consistency during swallowing (Vardhanabhuti and Ikeda, 2006). Early studies have been correlated a higher degree of pseudoplasticity with a lower degree of sliminess in the mouth produced by hydrocolloid (Szczesniak and Farkas, 1962). Consequently, the mouthfeel characteristics of ice cream provided by OKP may be better than OKW based on the higher shear thinning properties.

Table 10 Effect of polysaccharide concentration on rheological parameters of ice cream mixes

Ice cream mix	*K* (mPa.s^n)	n	R^2
0.00% gum	144.93^g	0.718^a	0.99
0.15% GG	330.80^d	0.609^d	0.98
0.15% OKW	129.25^g	0.705^a	0.98
0.30% OKW	234.03^f	0.662^b	0.98
0.45% OKW	358.57^c	0.620^{cd}	0.98
0.15% OKP	280.13^e	0.626^c	0.98
0.30% OKP	642.30^b	0.566^e	0.98
0.45% OKP	1622.00^a	0.517^f	0.98

Mean values with the same letters in a column do not differ

The apparent viscosity at shear rate 50 s^{-1} was calculated because it corresponded to the approximate shear rate at swallowing in the mouth (Bourne 2002). At this shear rate, apparent viscosity values ranged from 7.52 mPa.s (0.15%) to 17.50 mPa.s (0.45%) for ice cream mixes with OKW and viscosity values ranged from 13.50 mPa.s (0.15%) to 72.96 mPa.s (0.45%) for ice cream mix with OKP (Figure 23). At 0.15%, OKP increased the apparent viscosity of ice cream mix to nearly the same as 0.15% guar gum. Apparent viscosity values increased as okra cell wall or polysaccharide content increased. OKP provided higher apparent viscosity than OKW at the same concentration used in the ice cream formulation. This was likely due to higher amount of water-soluble polysaccharides in OKP as compared to OKW.

As it was mentioned that polysaccharide dispersions with a high of n value tends to feel slimy in the mouth. While high viscosity and a good mouth feel characteristics were desirable, the choice should be a polysaccharide system having a low n value (Szczesniak and Farkas, 1962). Since the samples with OKP had low n values and high apparent viscosity, OKP may provide a good mouthfeel characteristic to the ice cream product.

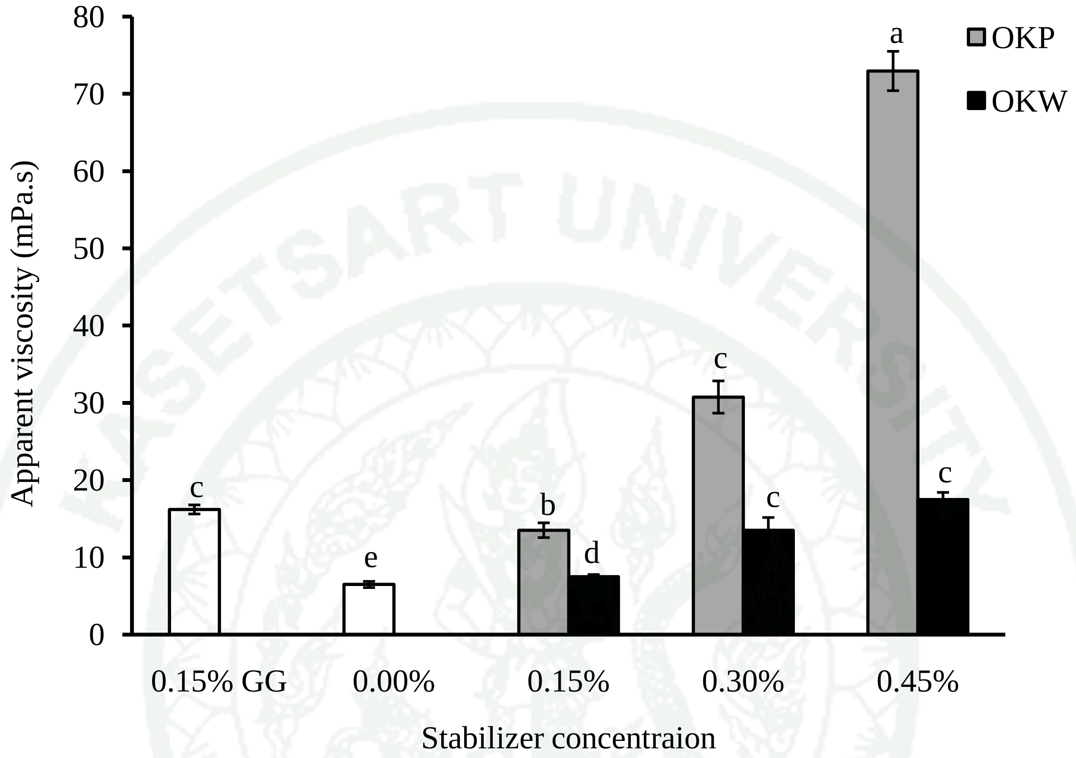


Figure 23 Apparent viscosity of ice cream mix at shear rate of 50 s^{-1}. The error bars represent the standard deviation (n = 3). Samples with the same letters are not different ($P > 0.05$).

3.2 Ice cream overrun

Overrun, which is directly related amount of air in ice cream, is important because it influences product quality, profit and is involved in meeting legal standards (Marshall *et al*., 2003). Overrun influences foam stability, meltdown and sensory perception of ice cream. It was mentioned that overrun values ranging from 70-120% are able to retard ice recrystallization, enhance melting resistance and improve foam stability under storage conditions (Flores and Goff 1999; Sofjan and Hartel, 2004). Proteins, fat and hydrocolloids have been reported to be important for air incorporation into ice cream mix and control of the thermodynamically unstable air cells (Chang and Hartel 2002(a); Marshall *et al*., 2003).

Overrun, the relative increase of volume by the inclusion of air which are injected into the ice cream mix and dispersed by the freezer in the step of freezing/whipping of ice cream processing (Goff, 1997) in order to obtain a smooth and soft product (Marshall *et al*., 2003). Whipping causes fat agglomeration by partial coalescence of fat globule (Goff *et al*., 1999). The air is trapped inside the fat crystal network structure. The overrun of ice cream, a measure of the amount of air in the product, is important because it influences product quality and profit, and is involved in meeting legal standards (Goff and Hartel, 2013). Moreover, overrun also influences foam stability, meltdown, and sensory perception of ice cream. The overrun of ice cream is shown in Figure 24. From the batch freezer, it ranged between 72 and 82% for all samples. Generally, ice cream with guar gum or OKW had lower overrun than the unstabilized sample, which is in agreement with the study of Adapa *et al*. (2000). They suggested that ice cream with polysaccharide exhibited higher viscosity, which hindered foaming capacity. The water-insoluble component of OKW might also reduce the foaming ability of ice cream, so ice cream containing 0.45% OKW showed the lowest overrun. However, ice cream containing OKP at 0.3 or 0.45% was not significantly different from the unstabilized sample and higher than ice cream with OKW. This may have resulted from other amphiphilic components of the water-soluble extract.

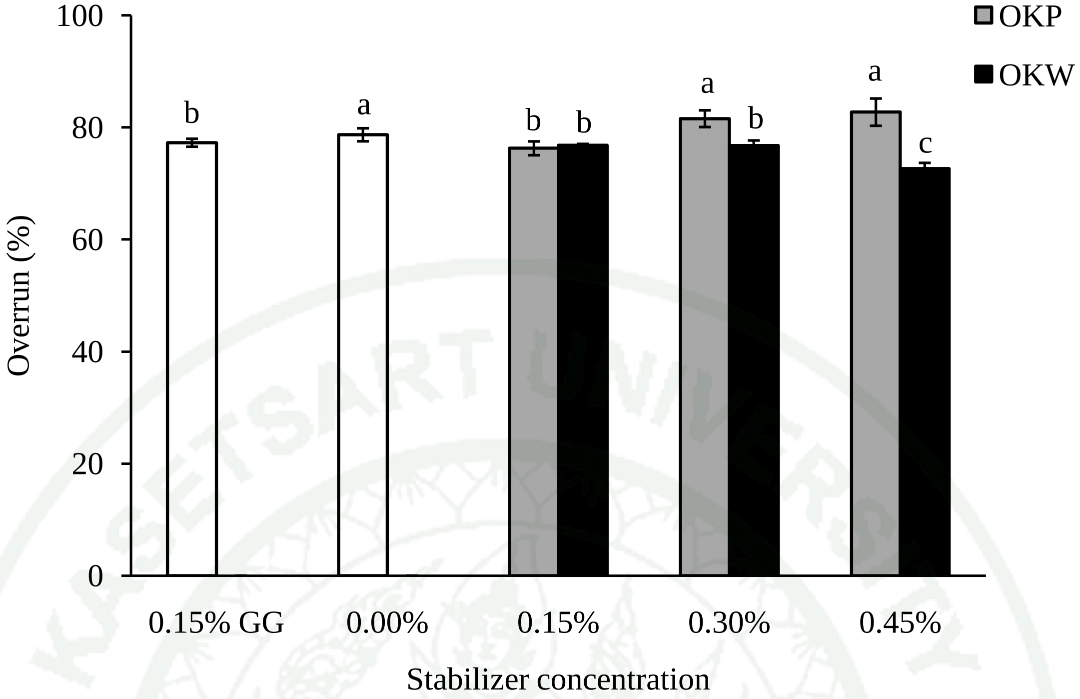


Figure 24 Mean values for overruns of ice creams. The error bars represent the standard deviation (n = 3). Samples with the same letters are not different (*P* > 0.05)

3.3 Ice cream melting characteristic

Slow meltdown, slow serum drainage, good shape retention, and slow foam collapse are some of the desired quality parameters of ice cream (Wildmoser and others 2005). It is undesirable for ice cream to lose its shape before it is consumed. A short first drop time indicates how rapidly the ice cream may lose its shape. The melting characteristics (first dripping time, melting rate and mass retention) of ice cream are shown in Figure 25. The results show that OKW and OKP improved the melting characteristics of ice cream. However, OKP showed higher potential than OKW.

OKP at 0.30% and 0.45% extended the first dripping time of ice cream compared to OKW, control, and GG samples (Figure 25a), indicating that OKP increased the melting resistance of ice cream. Ice cream with 0.45% OKP required the longest time for the first dripping (21.56 min) and ice cream with 0.30% OKP required a shorter minutes (16.91 min). Whereas, the other samples got the first dripping quickly (control (12.80 min), 0.15% GG (11.77 min), 0.15% OKW (11.21 min), 0.30% OKW (12.18 min), 0.45% OKW (12.88 min), and 0.15% OKP (12.40 min)) (Figure 25(a)). This study revealed that OKP improved the melting characteristic of ice cream. Hydrocolloids due to their water holding and microviscosity enhancement ability significantly improve melting quality of ice cream (Goff and Hartel 2013).

In agreement, ice cream with 0.30% and 0.45% also had low melting rate (Figure 25(b)). The lowest melting rate was obtained in the ice cream with OKP at 0.45% (Figure 25b). At 0.15% OKP, 0.15 - 0.30% OKW, and control, there were no significant differences between samples ($P > 0.05$). The melting profiles of ice cream are shown in Figure 26, which the melting rate was calculated from the slope of the graph in linear range (30 min to 70 min of melting time). Ice cream with 0.45% OKP had the lowest melting rate (1.01 $g.min^{-1}$), and the melting rate of 0.30% OKP ice cream was 1.39 $g.min^{-1}$. The melting rate of ice cream with OKP decreased with increasing OKP concentrations, this may be affected by its viscosity. Marshall *et al.* (2003) pointed out that hydrocolloids due to their water holding and microviscosity enhancement ability significantly improve melting quality of ice cream (Marshall *et al.*, 2003).

Moreover, Goff and Spagnuolo (2001) suggested that the melting rate of ice cream was also affected by amount of air incorporated, the nature of ice crystals, level of destabilized fat (Goff and Spagnuolo, 2001), the network of fat globules formed during freezing (Muse and Hartel, 2004), and overrun. The ice cream with higher overrun melted slower. This slower melting in the ice creams with high overruns was attributed to a reduced rate of heat transfer due to larger volume of air (Alamprese *et al*., 2002; Sofjan and Hartel, 2004). However, this study was in agreement with the previous study that was concluded that the addition of hydrocolloid stabilizer or polysaccharides to ice cream not only increased the viscosity, but also improve melting characteristic (Goff and Sahagian, 1996; Segall and Goff, 2002).

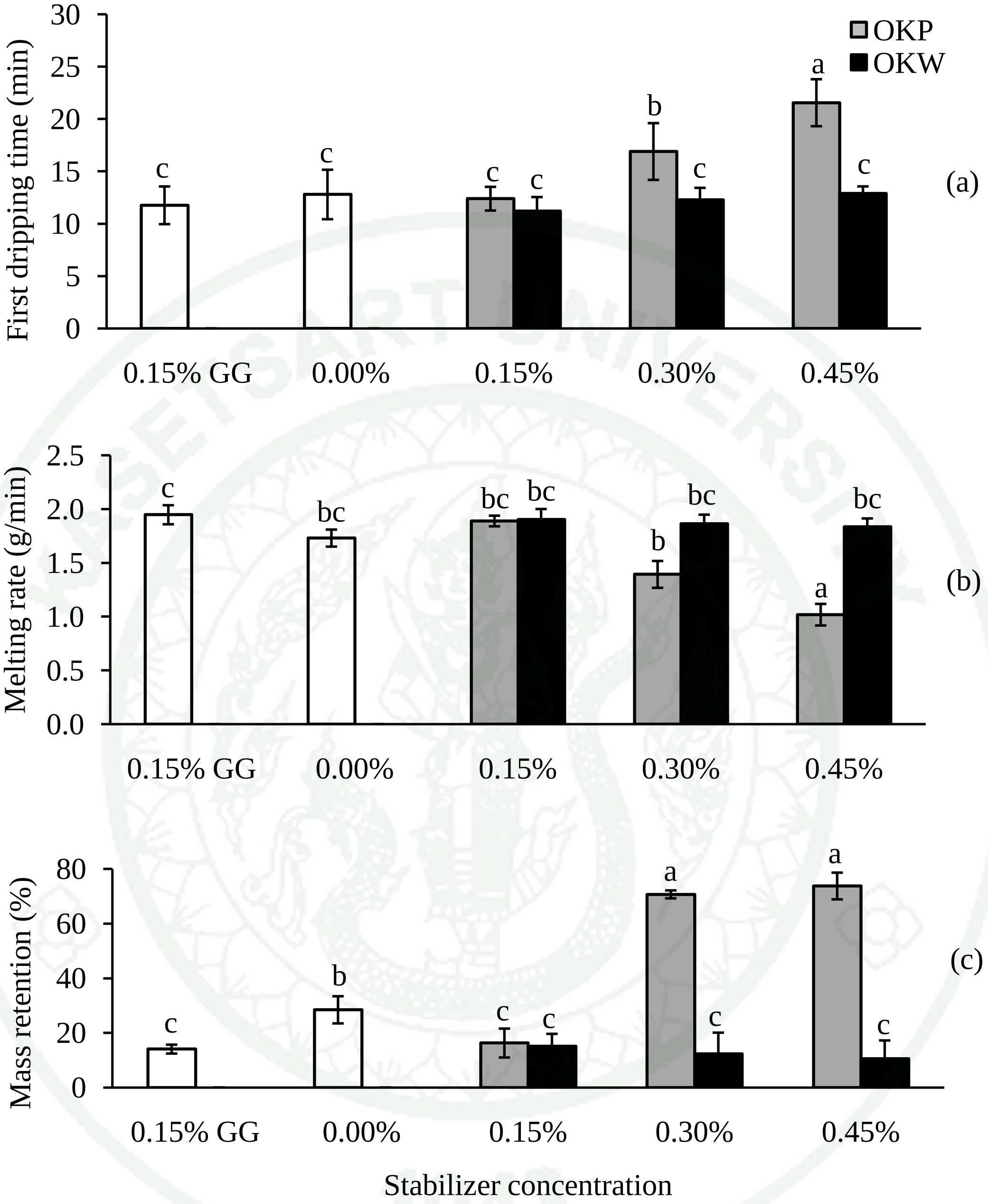


Figure 25 Mean values for first dripping time (a), melting rate (b), and mass retention (c) of ice creams. The error bars represent the standard deviation (n = 3). Samples with the same letters are not different (*P* > 0.05).

The mass retentions of ice cream are shown in Figure 25(c). The ice creams with 0.30% and 0.45% OKP also had the highest mass retentions. Ice cream with 0.45% OKP and 0.30% OKP, could retain shape very well, which 73.75 % and 70.69 %, respectively, were retained on the sieve after melting for 70 min (Figure 27). Whereas other samples were control (28.44 %), 0.15% GG (14.06 %), 0.15% OKP (16.27 %), 0.15% OKW (15.05 %), 0.30% OKW (12.28 %), and 0.45% OKW (10.51 %) could not retain shape well. That could also be explained by the viscosity (Figure 23). Ice cream with high viscosity had a greater resistance to flow. As ice crystal melt, it hardly diffused into the serum phase. Ice cream that did not flow as well as others would not drip through the screen as fast and thus, melted slower, had higher mass retention (Muse and Hartel, 2004).

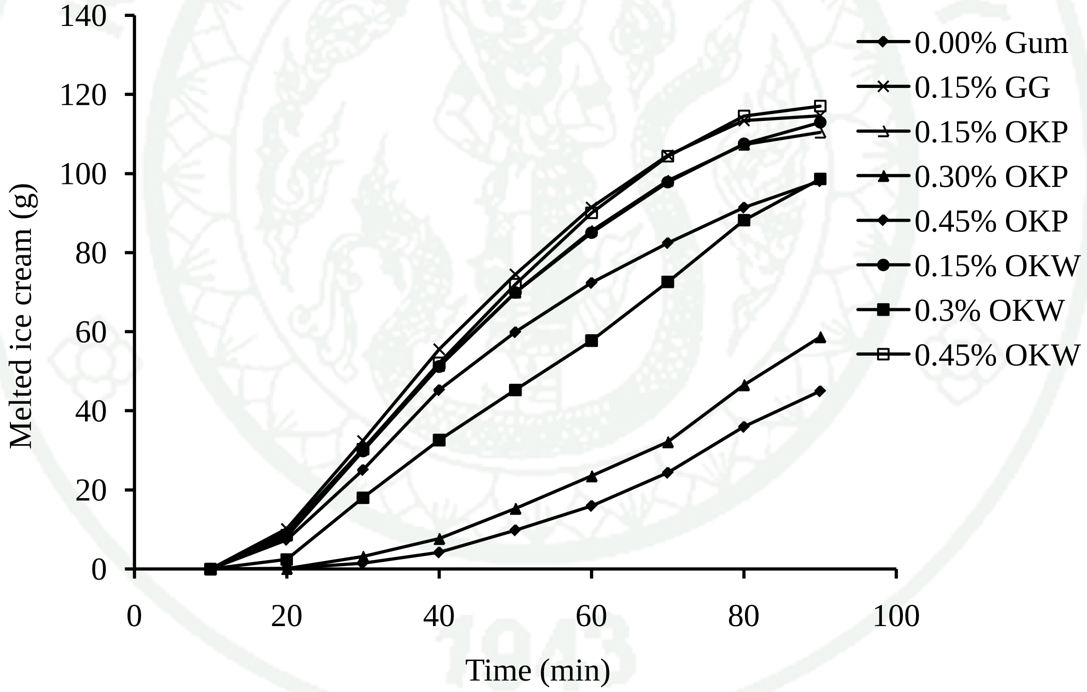


Figure 26 Melting profile of ice cream with different polysaccharide concentration

Figure 27 showed the pictures of ice creams during melting at different period of time. Generally, during the first stage of melting the temperature of the sample rises fast until get the freezing point (Fennema, 1973). As the ice crystals melt, the matrix phase is locally diluted and its viscosity lowered, which allows serum to drain. It flows through the fat network in the lamellae between the air cells and finally drips through a sieve. Usually the resulting loss in weight is recorded over time. A network of fat agglomerates forms a steric hindrance for serum drainage (Koxholt *et al*., 2001). Higher matrix phase viscosities caused by stabilizers slow down drainage as well (Cottrell *et al*., 1979). Figure 27 obviously showed that ice cream with 0.30% and 0.45% could retain shape very well.

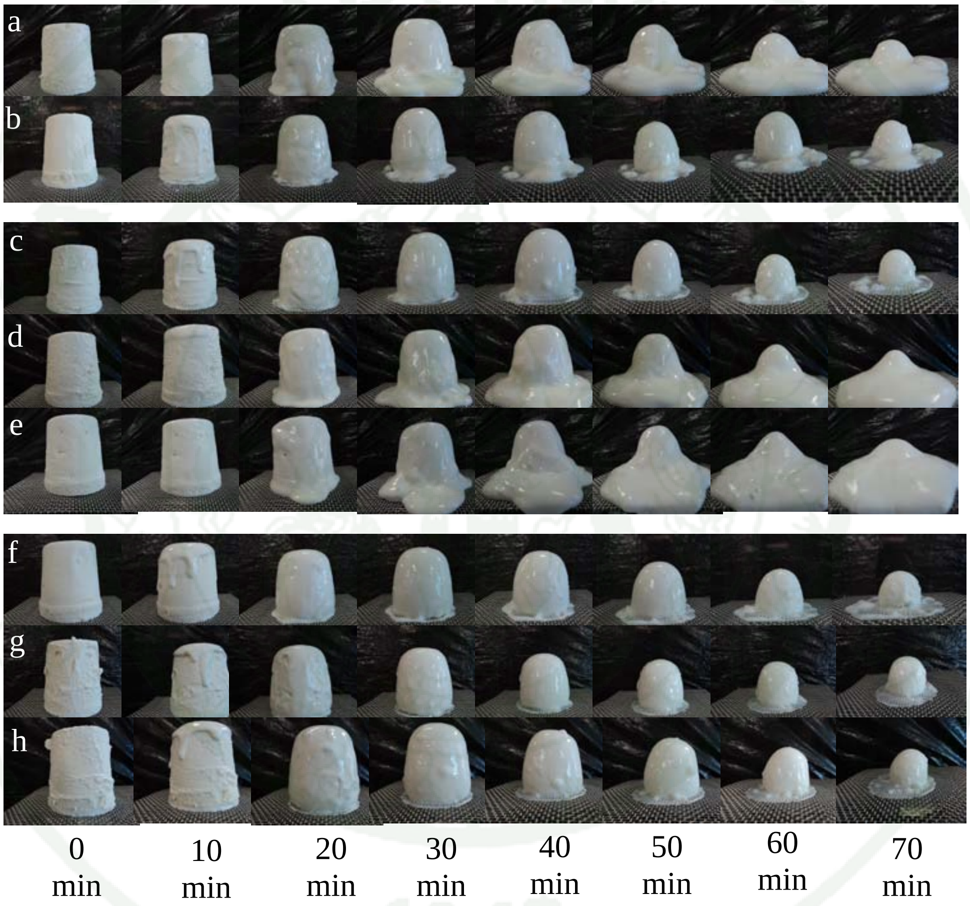


Figure 27 Melting characteristics of ice cream with polysaccharide (a = 0.00% gum, b = 0.15% GG, c = 0.15% OKP, d = 0.30% OKP, e = 0.45% OKP, f = 0.15% OKW, g= 0.30% OKW, h = 0.45% OKW) at melting time 0 to 70 min, 25^{o}C

3.4 Ice crystal growth of ice cream

Ice crystal size plays a major role in influencing the texture of ice cream (Hartel 1996). In this study, the microstructure pictures of ice cream were taken before and after temperature cycling (Figure 28), ice crystal size obtained from image analysis was characterized by the ice crystal equivalent diameter at 50% of the cumulative distribution (X_{50}). Median circular diameter (X_{50}) of ice crystal before, after temperature cycling and ice crystal growth are shown in Table 11. Before temperature cycling, the ice crystal sizes were not significantly affected by either absence or presence of OKW or OKP ($P > 0.05$). The overall diameter of ice crystal was ~20 µm which was similar to previous studies (Aleong *et al*., 2008). Thus, the presence of okra cell wall and okra polysaccharide did not directly impact on the ice crystal size after hardening (before temperature cycling), as has been reported by other research groups (Sutton and Wilcox 1998; Flores and Goff 1999). Normally, ice crystals sizes in the range of 15-20 µm give a desirable smooth texture to the ice cream (Hagiwara and Hartel, 1996).

The growth rate of ice crystal is slow at constant and lower storage temperatures, especially when the product is stored below its glass transition temperature, is typically in the range of -30 to -40^{o}C. However, temperature fluctuations during storage and handling of ice cream promote ice crystal growth. (Levine and Slade, 1989). In this study, temperature cycling significantly increased ice crystal sizes in all cases. In the temperature-cycled ice cream, the presence of OKW at 0.30-0.45% and OKP at 0.15-0.45% led to a significantly smaller ($P < 0.05$) ice crystal size. The percentages of ice crystal growth of ice cream with OKW and OKP at all concentrations were significantly (P<0.05) lower than ice crystal growth observed in ice cream without stabilizer (Table 11). The ice crystal growth was more retarded with increased stabilizer concentration. The study agreed with the results of Sutton and Wilcox (1998). They used guar gum (GG) and locust bean gum (LBG) in ice cream systems. Recrystallization of GG and LBG ice cream system decreased with increased concentration. A feasible mechanism for the preventive effect of polysaccharide against recrystallization during temperature cycling maybe related to the effect of polysaccharide on the bulk diffusion properties of the unfrozen phase of ice cream. Ice cream stability, in term of ice recrystallization, through heat shock cycle could be seen from the ice crystal growth. The lower ice crystal growth indicated higher ice cream stability through storage temperature fluctuation treatment.

Normally, the migratory recrystallization was the predominant mechanism in temperature fluctuations condition (Harper and Shoemaker, 1983). OKP exhibited ice crystal growth retardation effect in the ice cream. As OKP concentration increased the percentage of ice crystal growth was lower significantly. Higher OKP concentration increased the rigidity (yield stress, G´) behavior of continuous unfrozen serum phase of ice cream (Table 13 and 14), which correlated to ice crystal growth in ice cream (Appendix Table 5). This suggested that the rigidity behavior of continuous unfrozen serum phase of ice cream may associate with the retardation of the migratory of water in ice cream resulted in ice crystal growth from migratory recrystallization was slowed down in this case. As some stabilizers also were concluded that they promote redeposition of water onto the same ice crystal, rather than diffusion into the largest crystal which would be thermodynamic favored (Flores and Goff, 1999). It should be noted that 0.45% OKP sample which exhibited strongest ice cream mix structure (highest yield stress and G´, Table 13 and 14), had the lowest percentage of ice crystal growth thus implying the highest ice cream stability in this case.

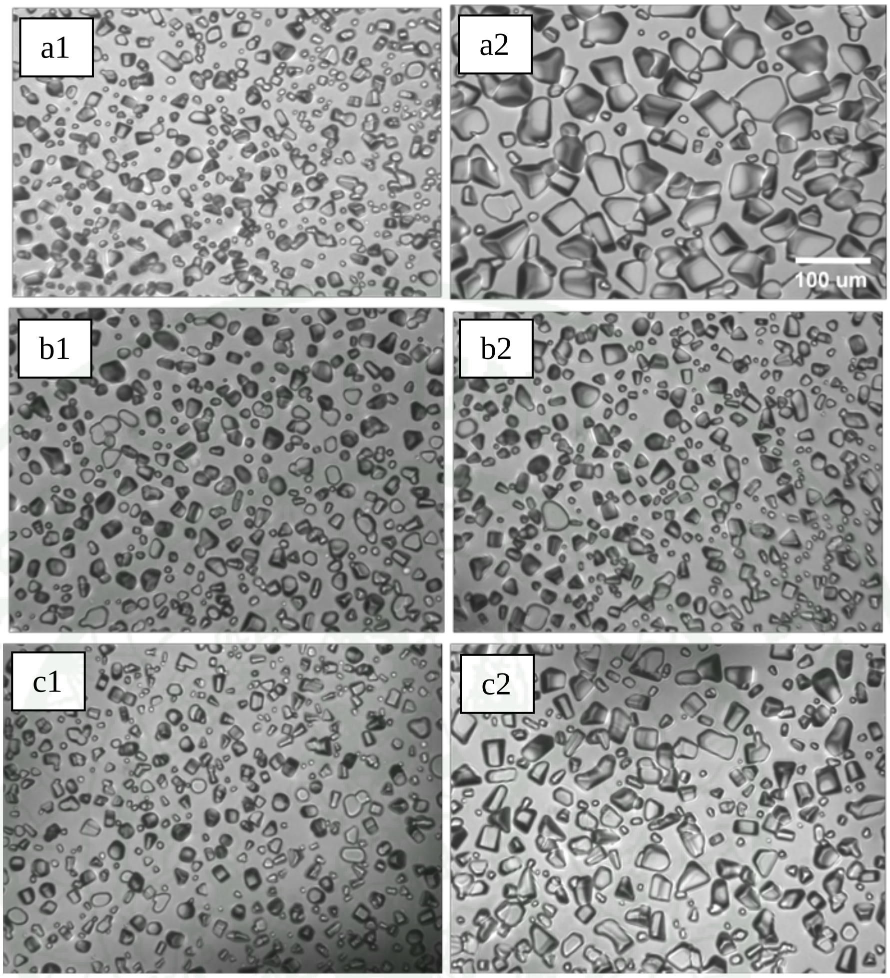


Figure 28 The microstructure of ice cream at 1) 0 cycle and 2) 10 cycles of temperature cycling (Bar = 100 µm): a) No stabilizer, b) 0.45% OKP and c) 0.45% OKW

Table 11 Ice crystal median equivalent diameters (X_{50}) of ice creams before and after temperature cycling and ice crystal growth

Ice cream sample	Ice crystal size (μm)		Ice crystal growth (%)
	Before	After	
0.15% GG	$22.86 \pm 1.69^{a, A}$	$42.02 \pm 2.10^{b,B}$	84.01 ± 4.59^{c}
0.00% gum	$22.64 \pm 1.02^{a, A}$	$52.65 \pm 4.73^{a, B}$	132.32 ± 13.44^{a}
0.15% OKP	$23.35 \pm 0.61^{a, A}$	$43.80 \pm 1.57^{b, B}$	87.64 ± 7.60^{c}
0.30% OKP	$22.96 \pm 3.27^{a, A}$	$36.14 \pm 1.96^{c, B}$	58.82 ± 14.76^{d}
0.45% OKP	$22.20 \pm 1.58^{a, A}$	$29.29 \pm 1.80^{d, B}$	32.03 ± 2.29^{e}
0.15% OKW	$23.44 \pm 0.75^{a, A}$	$48.74 \pm 2.22^{a, B}$	107.90 ± 5.62^{b}
0.30% OKW	$21.33 \pm 0.94^{a, A}$	$39.65 \pm 0.49^{bc, B}$	86.11 ± 7.22^{c}
0.45% OKW	$22.78 \pm 2.43^{a, A}$	$37.72 \pm 1.60^{c, B}$	66.39 ± 11.99^{d}

$^{a, b, c, d, e}$ Values with the same letter in a column do not differ ($P > 0.05$)

$^{A, B}$ Values with the same letter in a row do not differ ($P > 0.05$)

3.5 Ice cream perception

The sensory tests were performed on six ice cream samples including ice cream with 0.15% to 0.30% OKW and OKP, control, and 0.15% GG. However, ice cream with 0.45% OKW and OKP were excluded from this study because of their trace okra flavor. Using a nine-point hedonic scale (9 = like extremely, 5 = neither like nor dislike, and 1 = dislike extremely) to investigate the effect of stabilizer on the liking of the ice cream, the consumer preferences scores are shown in Table 12. Result showed that ice cream with 0.15% OKP and 0.15% GG had the same flavor-liking scores. The addition of more OKP into ice cream (0.30% OKP) significantly ($P < 0.05$) lowered flavor-liking. The high viscosity of 0.30% OKP ice cream may block more taste receptors than the less viscous one and may also decrease the diffusion rate of vanilla flavor to the taste receptors. The highest texture-liking score was found in ice cream with 0.15% OKP ($P > 0.05$). The higher stabilizer concentrations (0.30%) of both OKW and OKP showed lower texture-liking scores but the results were not significantly different between samples (*P* > 0.05). The result was in contrast with the finding of Sokoulis *et al*. (2008). They showed that an increase in hydrocolloid content improved the texture. Ice cream containing 0.2% sodium alginate or xanthan gum, which had the highest viscosity, provided the best texture. Moreover, ice cream with 0.15% OKP and 0.15% OKW showed higher overall-liking scores than ice cream with 0.30% OKP and 0.30% OKW (*P* > 0.05). These results indicate that OKP performed better than OKW at providing good characteristics for consumers. OKP and OKW at 0.15% were more suitable than 0.30% concentrations. By the way, the results were not significant between samples (*P* > 0.05). It can be noted that OKW and OKP can be used to stabilize ice cream with no undesirable effect on consumer perception.

Table 12 Sensory test liking and attributing of ice cream of 73 panelists

Ice cream characteristics	Scores					
	0.15% GG	0.00% gum	0.15% OKP	0.30% OKP	0.15% OKW	0.30% OKW
Flavour liking	6.32^{a}	5.93^{ab}	6.32^{a}	5.63^{b}	6.23^{ab}	5.84^{ab}
Sweetness	6.03^{a}	6.20^{a}	6.21^{a}	5.71^{a}	5.88^{a}	5.67^{a}
Vanilla Flavor	5.41^{a}	5.31^{a}	5.80^{a}	5.25^{a}	5.55^{a}	5.36^{a}
Dairy (cream) flavor	5.65^{a}	5.17^{a}	5.49^{a}	5.67^{a}	5.27^{a}	5.57^{a}
Aftertaste	4.89^{ab}	4.44^{b}	4.87^{ab}	5.46^{a}	4.77^{ab}	5.33^{a}
Texture liking	6.45^{a}	6.01^{a}	6.60^{a}	6.31^{a}	6.24^{a}	6.00^{a}
Gumminess	3.78^{a}	3.49^{a}	3.53^{a}	4.08^{a}	3.60^{a}	3.89^{a}
Creamy texture	5.89^{a}	5.05^{b}	5.60^{ab}	5.94^{a}	5.63^{ab}	5.19^{b}
Smoothness	4.28^{a}	4.47^{a}	4.43^{a}	4.24^{a}	4.15^{a}	4.09^{a}
Mouth coating	5.21^{ab}	4.57^{b}	4.81^{ab}	5.43^{a}	4.75^{ab}	5.39^{a}
Overall liking	6.33^{a}	6.13^{a}	6.47^{a}	5.87^{a}	6.23^{a}	5.92^{a}

^{a,b} Values with the same letters in a row do not differ

4. Relation between ice cream mix rheology and ice recrystallization in okra polysaccharide-stabilized ice cream

4.1 Rheology and stability of ice cream mixes

Rheological properties of ice cream mix are important since they govern the quality development throughout the manufacturing process (Eisner, 2006). The previous studies showed that OKW did not much contribute the rheological properties as much as OKP so OKW was not determined in this part.

The structure deforming profiles of ice cream mix with different concentrations of OKP were measured with Physica MCR rheometer, amplitude sweep mode, strain sweeps from 0 to 100%, 1 s^{-1}, at -1°C. The results are shown in Figure 29. For all ice cream mixes there were a sigmoidal one-step transitions of G´ values as a function of shear stress, implying the deforming of the network structure in the samples. The G´ at critical transition point, was defined as the yield stress of ice cream mix. Yield stress, is the shear stress value at which the linear-elastic range is exceeded, describes the external forces acting on the material to start flowing. Sample begins to flow when the external forces acting on the material are larger than the internal structural forces (Mezger, 2002).

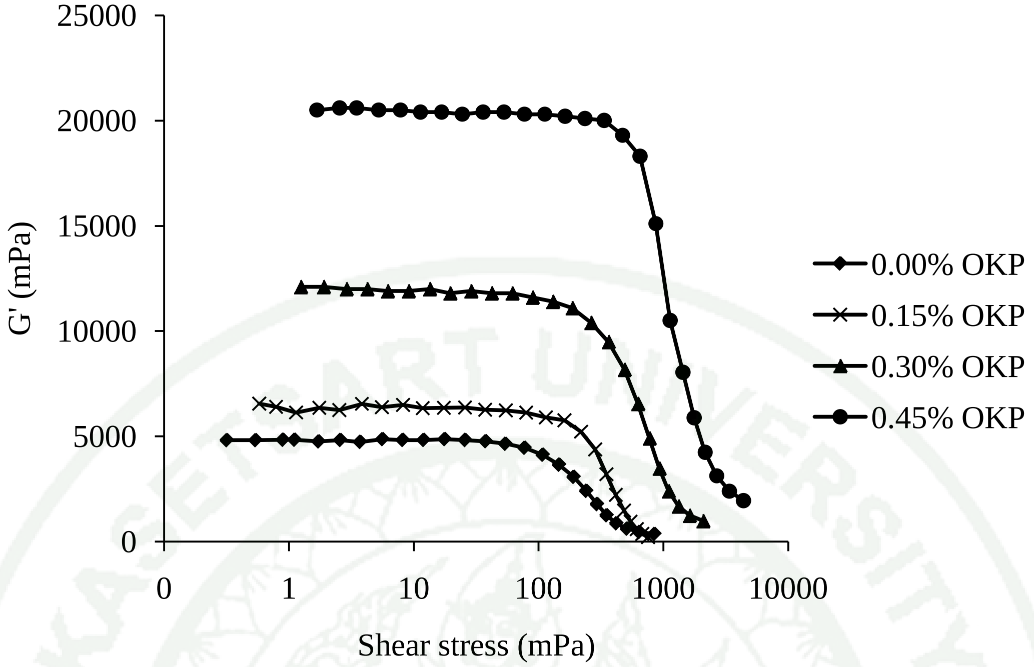


Figure 29 Deforming profiles of ice cream mixes determined at strain 0 to 100%, frequency 1 s^{-1}, temperature -1^{o}C

In order to study effect of temperature fluctuation on the stability of ice cream mix rheological properties, yield stress of ice cream mix were measured before and after temperature cycling. The yield stresses values of ice cream mix are shown in Table 13. The values ranged from 0.12 to 0.81 Pa. The yield stress increased with increasing OKP concentration. This implied that OKP provided stronger structure to the samples. The study about yield stress of ice cream mix was also done by Rosnani *et al*. (2007). They found that yield stress values of ice cream mixes that were made with 0.35% commercial blend of emulsifier/stabilizer and varying ratios of palm oil: milk fat blends (measured at 4^{o}C) were 0.04-0.08 Pa (Rosnani *et al*., 2007), which were slightly lower than yield stress of ice cream mix in this thesis. This is probably due to they were measured at higher temperature (4^{o}C).

After temperature cycling, the yield stress of ice cream mix with 0.00%, and 0.15% OKP increased significantly comparing to before temperature cycling. It was probably due to aggregation of casein after temperature cycling. During freezing process as ice crystals are formed, proteins are concentrated. The increase in concentration of the protein during freezing can result in protein aggregation (Wang, 2005).

Ice cream mix with 0.30% and 0.45% OKP were stable to temperature cycling, which their yield stress were not significantly changed by temperature cycling. It probably dues to the ice cream mix with high OKP concentrations had high viscosity (Figure 23) and rigidity (Table 13, 14) which cause the samples were stable to temperature cycling.

Table 13 Yield stresses of ice cream mix before and after temperature cycling (mean ± SD, *n* = 3)

OKP concentration (%)	Yield stress (Pa)		Change of yield stress (%)
	Before	After	
0.00	0.12 ± 0.02$^{c, A}$	0.23 ± 0.02$^{c, B}$	98.94 ± 9.90^{a}
0.15	0.16 ± 0.04$^{c, A}$	0.25 ± 0.04$^{c, B}$	65.02 ± 22.38^{b}
0.30	0.32 ± 0.01$^{b, A}$	0.42 ± 0.06$^{b, A}$	37.02 ± 18.16^{b}
0.45	0.81 ± 0.02$^{a, A}$	0.83 ± 0.04$^{a, A}$	1.94 ± 2.93^{c}

$^{A, B}$ Value with the same letter in a row do not differ ($P > 0.05$)

$^{a, b, c}$ Value with the same letter in a column in each group do not differ ($P > 0.05$)

The results of the dynamical analysis available through Physica MCR oscillation rheometer were correlated to microstructural information. Dynamic (oscillatory) rheological tests provide valuable information on the viscoelastic nature of ice cream mix. The dynamic rheological tests in the linear viscoelastic range, the storage modulus, G´, and the loss modulus, G´´, can be obtained (Rao, 1992). Figure 30 showed the storage and loss modulus (G´ and G´´) measured in an dynamic oscillation test with an increase of the angular frequency from 0.1 to 110 s^{-1}, conducting in the linear viscoelastic domain (0.1% strain), at -1°C. The graph was divided into three zones as follows:

Zone I: At low angular frequency (0.1 to 1 s^{-1}), the storage modulus G´, which describes the solid like behavior, and the loss modulus G´´, which describes the viscous or flow behavior, started to cross over (G´-G´´) which G´ started to dominate G´´. This indicated that material changed from viscous to viscoelastic in this frequency zone. It can be correlated to the rigidity of the ice cream mix structure. Interestingly, the sample with higher OKP concentration had G´-G´´ crossover at lower frequency. The ice cream mix with 0.45% OKP, G´ and G´ crossover occurred at a lowest frequency (at 0.09 s^{-1}). The dynamical rheological analysis of okra polysaccharide dispersion was also done by Sengkhamparn *et al*. (2010). They suggested that the crossover between viscous and elastic behavior of 0.2-0.5% of okra polysaccharide was at a frequency of about 0.1 s^{-1} (Sengkhamparn *et al*., 2010), which was similar to the study. The crossover of G´ and G´´ values provides a good indication of viscoelastic behavior of the material (Iagher *et al*., 2002) and defines the beginning of the elastic behavior or approaching gel state (Norziah *et al*., 2001).

All ice cream mixes could be characterized as concentrated dispersions, showing frequency dependence with G´´ predominating at low frequency, and G´-G´´ crossed over as frequency increases (Steffe, 1996). At the lower concentrations of OKP, the ice cream mix had a lower G´ and higher-frequency cross over compared to the ice cream mix with OKP at higher concentrations. These suggested that ice cream mix had less aggregation with the presence of the higher OKP.

Zone II: In the frequency range of 1 to 10 s^{-1}, the samples showed the viscoelastic properties, G´ > G´´ of whole range. In this case, the elastic behavior dominated over the viscous behavior. The ice cream mixes with OKP, the G´ and G´´ curve were almost parallel straight lines throughout the entire frequency range with a shallow slope.

Zone III: At angular frequency of 10 to110 s^{-1}, in this zone the G´ of ice cream mix with 0.00% to 0.30% OKP started to decline. The crossover of G´ and G´´ occurred at frequency 62.80, 92.10, and 92.20 s^{-1} for ice cream mix with 0.00%, 0.15%, and 0.30% OKP, respectively. This indicated that viscoelastic characteristic become viscous characteristic in this region. Whereas, the G´ and G´´ of ice cream mix with 0.45% OKP did not show the cross over point at the frequency study. This implied that 0.45% OKP sample had a highest frequency tolerance.

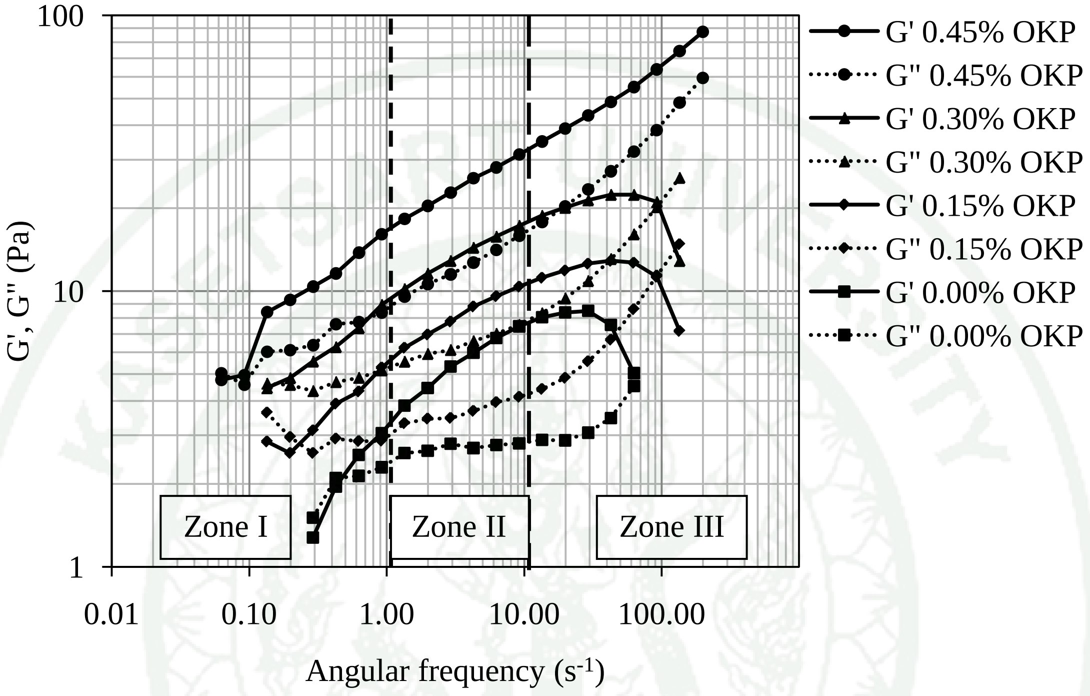


Figure 30 Mechanical spectra of ice cream mixes obtained at 0.1% strain, -1°C

The microstructure of ice cream mix may impact on the ice cream stability and the change of rheological properties of ice cream mix may significantly impact on the ice crystal growth of ice cream. The G´, G´´ and complex viscosity (ɳ*) (Pas) at fixed frequency are shown in Table 14. The G´, G´´, and ɳ* values of ice cream mix increased with increasing OKP concentrations.

The yield stress (from Table 13), G´, G´´, and ɳ* of ice cream mix before and after temperature cycling are shown in Table14. The high yield stress and G´ resulted from the increased OKP concentration in ice cream mix which 0.45% OKP provided the highest rigidity. This was attributed to a greater content of the okra water soluble polysaccharide. The okra large polysaccharide molecules effectively bind water in the system because of its negative charge (Sengkhamparn *et al.*, 2010) thus increased the flow resistant of the ice cream mix. At sufficient concentration, water soluble polysaccharide can interact with each other and become entangled which modifies the rheological characteristics of ice cream mix (Marshall *et al*., 2003; Hagiwara and Hartel, 1996). The minimum OKP concentration significantly affected the yield stress was 0.30% and when OKP was added at 0.45%, a dramatically stronger structure occurred.

The rheological characteristics of ice cream mix after temperature cycling were also measured to observe the heat shock stability of the mix (Table 14). G´, G´´ and η^* of ice cream mix with 0.00% and 0.15% OKP increased significantly ($P < 0.05$) after the temperature cycling whereas the rheological parameters of the mixes with 0.30 and 0.45% OKP did not change ($P > 0.05$). The results were similar to the yield stresses (Table 13). It seem like ice cream mix with 0.30% and 0.45% OKP were stable to temperature cycling. This was presumably due to low mobility of macromolecules in the high rigidity systems. Moreover, the rheological results were accordance to the study of Patmore *et al*. (2003). They found that yield stress of ice cream mix with 0.30% LBG (LBG/MSNF/sucrose/fat/emulsifier emulsions) did not significantly increase with temperature cycling (Appendix Table 5).

In this thesis, rheological properties of the ice cream mix were determined as well as ice crystal growth of the ice cream. Interestingly, it was found that rigidity (yield stress and G´) of ice cream mix increased with increasing OKP concentrations (Table 13 and 14), the ice crystal growth decrease with increasing OKP concentrations (Table 11). Moreover, the high stability (less change) of ice cream mix also observed with high OKP concentrations (Table 13 and 14). It will be an advantage for ice cream industry, if correlation between rheological properties and stability of ice cream mix, and ice crystal growth of ice cream be found, and the ice crystal growth of ice cream can be predicted. These were interested. The relationships were determined in this thesis, the details are shown in next subsection.

Table 14 Rheological properties of ice cream mixes measured before and after temperature cycling (mean ± SD, $n = 3$)

	OKP	Temperature cycling		Change of
	concentration (%)	Before	After	rheological (%)
G´ (Pa)	0.00	$2.50 \pm 0.26^{c, A}$	$5.95 \pm 0.51^{c, B}$	136.61 ± 14.99^{a}
	0.15	$4.73 \pm 1.18^{bc, A}$	$7.54 \pm 0.24^{c, B}$	64.74 ± 31.85^{b}
	0.30	$7.86 \pm 1.67^{b, A}$	$11.35 \pm 1.38^{b, A}$	47.11 ± 23.65^{bc}
	0.45	$20.3 \pm 4.23^{a, A}$	$22.37 \pm 4.20^{a, A}$	10.75 ± 9.26^{c}
G´´ (Pa)	0.00	$1.56 \pm 0.31^{c, A}$	$2.94 \pm 0.40^{c, B}$	90.39 ± 12.77^{a}
	0.15	$2.51 \pm 0.35^{c, A}$	$3.84 \pm 0.16^{c, B}$	54.88 ± 19.34^{a}
	0.30	$5.29 \pm 0.54^{b, A}$	$5.97 \pm 0.30^{b, A}$	13.78 ± 14.94^{b}
	0.45	$12.73 \pm 1.12^{a, A}$	$13.11 \pm 1.32^{a, A}$	2.93 ± 3.45^{b}
η * (mPa.s)	0.00	$2.13 \pm 0.61^{c, A}$	$3.62 \pm 0.88^{c, B}$	71.46 ± 7.94^{a}
	0.15	$3.28 \pm 0.52^{bc, A}$	$4.67 \pm 0.44^{c, B}$	43.30 ± 9.46^{b}
	0.30	$4.67 \pm 0.44^{b, A}$	$6.35 \pm 0.63^{b, B}$	36.21 ± 10.92^{bc}
	0.45	$12.68 \pm 1.84^{a, A}$	$13.93 \pm 1.31^{a, A}$	10.50 ± 8.31^{c}

$^{A, B}$ Value with the same letter in a row do not differ ($P > 0.05$)

$^{a, b, c}$ Value with the same letter in a column in each group do not differ ($P > 0.05$)

Values at 0.1% strain, 1 s^{-1} frequency

4.3 Correlation between rheological properties of ice cream mix and ice crystal growth of ice cream

The rheological properties of ice cream mix with different concentration of OKP were measured (Table 13 and 14) then were correlated with the ice crystal growth of ice cream (Table 11). The ice crystal growth of ice cream had negative correlation to rheological values (yield stress, G´, G´´, and η*) of ice cream mix before and after temperature cycling (Figure 31, and Appendix Table 6 and 7). The results suggested that the higher rheological values with addition of OKP related to the lower level of ice recrystallization.

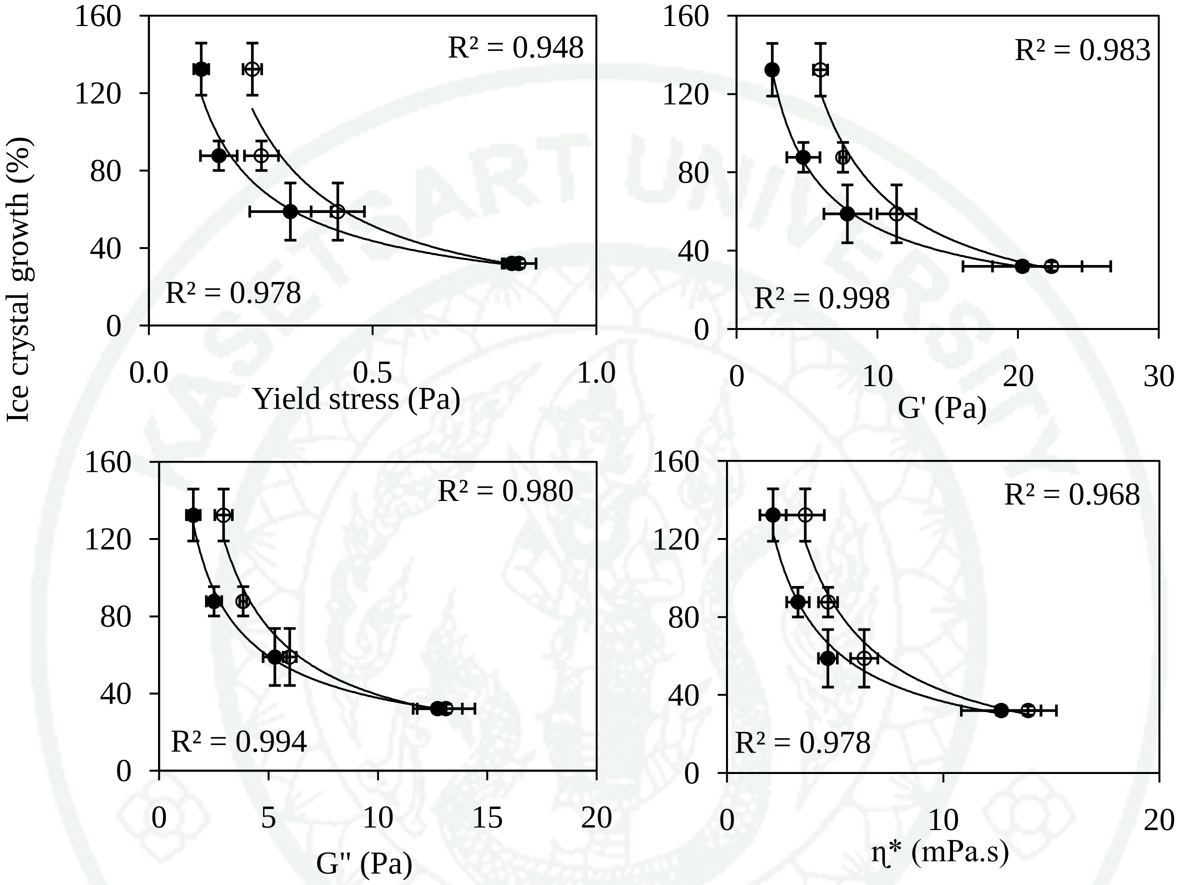


Figure 31 Relationship between ice crystal growth of ice cream and yield stress, G´, G´´ and ɳ* of ice cream mix; ● = before temperature cycling, and ○ = after temperature cycling. Error bars are standard deviations (n = 3)

The relationships between ice crystal growth of ice cream and rheological properties of ice cream mix before temperature cycling had the same pattern with the relationships between ice crystal growth of ice cream and rheological properties of ice cream mix after temperature cycling. However, the latter shifted to the right because of its higher rheological values.

In order to estimate the stability of ice cream mix in correlation to ice crystal growth of ice cream, the different between rheological values before and after temperature cycling was calculated as ice cream mix rheology change then correlated to the ice crystal growth of ice cream. Interestingly, a linear curve fit was found between change of rheological parameters of ice cream mix and the ice crystal growth of ice cream (Figure 32). The correlate was in the positive direction which the lower change of rheological parameters, the lower ice crystal growth.

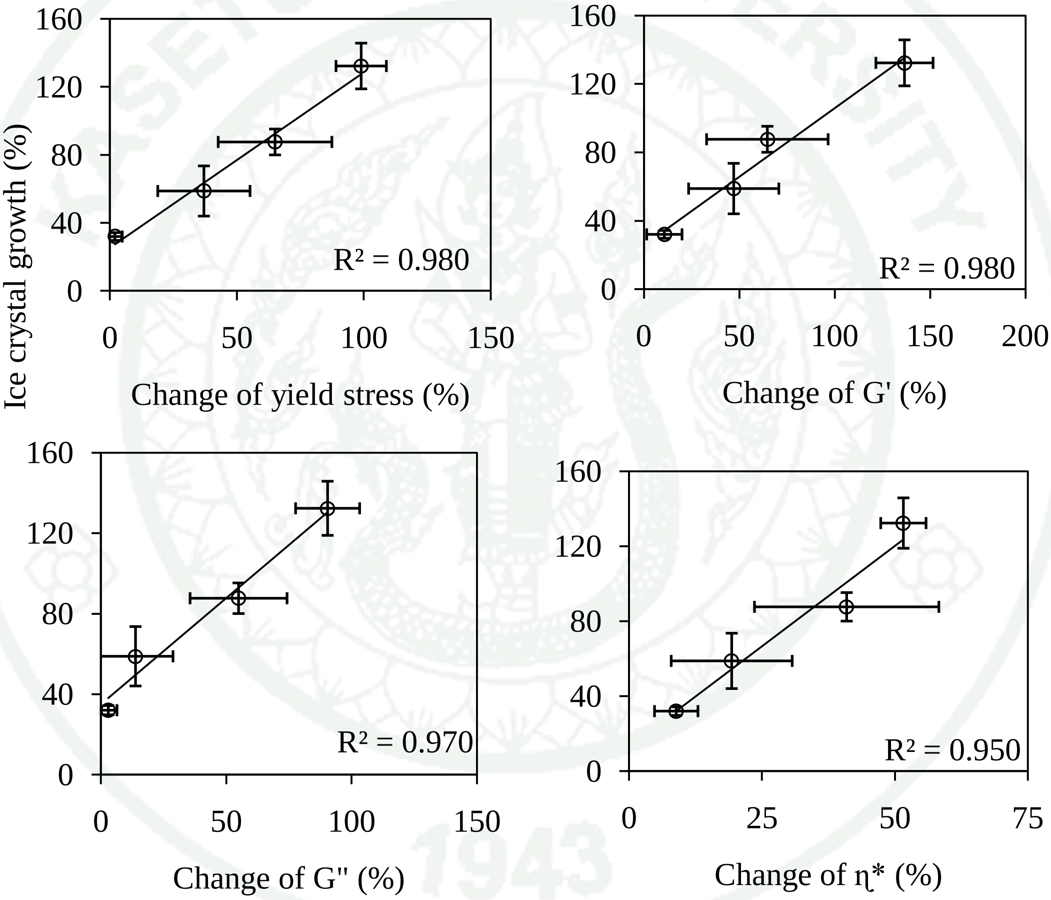


Figure 32 Relationship between ice crystal growth of ice cream and change of rheological properties (i.e., yield stress, G´, G´´, and ɳ*) of ice cream mixes. Error bars are standard deviations (n = 3)

The ice crystal growth could be predicted based on the change of rheological parameters of ice cream mix. The changes of all rheological parameters of ice cream mix were tested for the significant correlation with ice crystal growth of ice cream. Stepwise multiple linear regression was applied to establish the prediction equation. The analysis equipped with a variable selection scheme which four variables were inputted (x_1 = change of yield stress, x_2 = change of G´, x_3 = change of G´´, x_4 = change of η*). Conceptually, the procedure started by selecting the variable that had the highest correlation with y (y = ice crystal growth). Then a regression coefficient was obtained. If the coefficient was considered to be significant, the variable was retained, if not, they were eliminated from the equation (Spiegel and Stephens, 1999). In the study, two variables; change of yield stress and change of G´ had significant correlation with ice crystal growth so they were included to the regression equation (Appendix Table 9). Whereas, x_3 (change of G´´) and x_4 (change of η*) were excluded. This obviously revealed that the rigidity ice cream mix (yield stress and G´ values) significantly affected the ice recrystallization inhibition. The equation for ice crystal growth prediction that developed was given below (Eq. 11):

$$y = 25.438 + 0.667x_1 + 0.285x_2$$

Where y = ice crystal growth, x_1 = change of yield stress, x_2 = change of G´. The equation was then validated to determine whether the equation was usable. The detail of equation validation was shown in appendix.

The equation (Eq. 11) showed that ice crystal growth of ice cream was a dependent variable which was varied by change of yield stress and change of G´ of ice cream mix. The low change of yield stress and change of G´ of ice cream mix related to the low ice crystal growth of ice cream. Likely, the result of the experiment showed that ice cream mix with 0.45% OKP which had a low change of rheological parameters contributed the lowest ice crystal growth in the ice cream.

These could be concluded that the more stable ice cream mix (lower rheological values change) had the lower ice crystal growth. These suggested that the high rigidity ice cream mix caused less mobility of molecules in the system which contributed the stable system. Mechanisms of polysaccharide to retard ice recrystallization in ice cream have been reported 1) viscosity and molecular mobility (Hagiwara and Hartel, 1996; Miller-Livney and Hartel, 1997) 2) cryo-gel formation (Goff *et al*., 1999; Patmore *et al*., 2003) 3) hydrocolloid phase separation (Regand and Goff, 2002, 2003) 4) freeze concentration (Bolliger *et al*., 2000). The different polysaccharides may have different mechanism to slow down ice recrystallization. OKP may retard ice crystal growth by generating rigidity of ice cream unfrozen phase to retard molecular mobility. The generating the rheological properties around ice crystals possibly through entanglements of polysaccharide (Blond, 1994) may make ice crystal growth more difficult whereas LBG may slow down ice crystal growth by forming a cryo-gel (Patmore *et al*., 2003).

Each polysaccharide may have specific mechanism to retard ice recrystallization presumably because of differences in their structure, water binding capacity, viscosity, rigidity, and cryo-gel forming ability. Comparing ice cream mix model to ice cream mix may be important to provide the information about mechanism of ice recrystallization of the polysaccharide.

CONCLUSION AND RECOMMENDATION

Conclusion

The aim of this work was to determine the effect of OKW and OKP on the characteristics and stability of ice cream. The various concentrations of OKW and OKP were added into ice cream model and ice cream. The characteristics of ice cream including viscosity of ice cream mix, ice cream overrun, melting characteristics as well as ice crystal growth were determined. From the results and discussion, it could be concluded as below.

Part 1: Okra cell wall (OKW) and water soluble polysaccharide (OKP) contain different amount of chemical composition. OKW composted of higher amount of protein and clued fiber than OKP, whereas OKP had higher ash and total carbohydrate than OKW. OKW showed the better water holding capacity, foaming capacity than OKP. OKP had the high water solubility, foaming stability, apparent viscosity, pH tolerance, temperature tolerance, freeze-thaw stability, and synergism with guar gum. Yield of OKW and OKP were 4.11% and 1.06%, respectively.

Part 2: Ice cream model with OKW and OKP at concentration 0.10% - 0.60%, (w/w) showed the pseudoplastic or shear-thinning flow behavior. The apparent viscosity, consistency coefficient (*K*) values, and pseudoplasticity increased with increasing OKW and OKP concentrations.

Part 3: OKP and OKW could be successfully incorporated to control desirable ice cream quality and to retard ice crystal growth during storage. Based on ice cream mix viscosity, ice cream overrun, melting characteristics, ice crystal growth, and consumer sensory testing, 0.15% OKP or 0.15% OKW could be used to stabilize ice cream without affecting its sensory perception.

Part 4: The rheological properties of ice cream mix with OKP had a relationship with ice crystal growth in ice cream. The ice crystal growth of ice cream could be predicted by the change of yield stress and change of G´ of ice cream mix. The multiple regression equation was generated which explained 98% of the data variability so it provided a useful prediction of percentage of the ice crystal growth. The rheological properties of ice cream mix with OKP may be the factor affecting ice recrystallization retardation in ice cream.

Recommendation

For further investigation, the mixture of OKP-guar gum (GG) should be studied in ice cream. Since GG showed the synergistic effect with OKP in solution, it may be synergistic with OKP in ice cream system and affect on ice recrystallization retardation synergistically.

In this study, OKP mixed with nonionic polysaccharide; guar gum, and anionic polysaccharide; xanthan gum were already studied. However, the mixture of OKP with cationionic polysaccharide such as chitosan should be also determined. In those OKP and cationic polysaccharide mixture, a complex coaservation may occur which may has more synergistic effect.

Due to the good result obtained in the use of OKW and OKP in ice cream, further research, OKW and OKP should be tried in other ice cream formulation and other products such as acidified milk.

This study showed that OKP was more affective on ice recrystallization retardation and ice cream good characteristics than OKW. However, OKW may be good for use in reduced-fat ice cream or other food products, since OKW contain higher protein content and emulsifying capacity than OKP.

If the ice cream industry is actually going to use OKW or OKP as stabilizer, they would have to test it under commercial scale and test it with a variety of formulations.

Comparing ice cream mix model to ice cream mix may be important to provide the information about mechanism of ice recrystallization inhibition of the OKP. Ice crystal growth in ice cream mix model with OKP should be studied in the future.

The conjugations of protein and okra polysaccharide may improve physico-chemical properties of okra polysaccharide which may affect ice cream physical properties such as overrun and meltdown. Significance of protein in okra polysaccharide functional properties should be studied in future work.

LITERATURE CITED

Acquistucci, R. and R. Francisci. 2002. Effect of okra (*Hibiscus esculentus* L.) addition on the technological properties of a wheat flour. **Int J Food Sci Nutr.** 53: 375-379.

Adapa, S, H. Dingeldien, K.A. Schmidt and T.J. Herald. 2000a. Rheological properties of ice cream mixes and frozen ice creams containing fat and fat replacers. **J Dairy Sci.** 83: 2224-2229.

Adapa, S., K.A. Schmidt, I.J. Jeon, T.J. herald and R.A. Flores. 2000b. Mechanisms of ice crystallization and recrystallization in ice cream: a review. **Food Rev Int**. 16: 259-271.

Adebowale, K.O. and O.S. Lawal. 2003. Foaming, gelation and electrophoretic characteristics of macuna bean (*Macuna pruriens*) protein concentrate. **Food Chem.** 83: 237-246.

Aime, D.B., S.D. Arntfield, L.J. Malcolmson and D. Ryland. 2001. Textural analysis of fat reduced vanilla ice cream products. **Food Res Int.** 34: 237-246.

Akanbi, T.O., S. Nazamid and A.A. Adebowale. 2009. Functional and pasting properties of a tropical breadfruit (Artocarpus altilis) starch from Ile-Ife, Osun State, Nigeria. **Int Food Res J.** 16: 151-157.

Akerholm, M., B. Hinterstoisser and L. Salmen. 2004. Characterization of the crystalline structure of cellulose using static and dynamic FT – IR spectroscopy. **Carbohydr Res.** 339: 569-578.

Akhtar, M., B.S. Murray and E. Dickinson. 2006. Perception of creaminess of model oil-in-water dairy emulsions: influence of the shear-thinning nature of a viscosity-controlling hydrocolloid. **Food Hydrocolloid.** 20: 839-847.

Alamprese, C., R. Foschino, M. Rossi, C. Pompei and L. Savani. 2002. Survival of *Lactobacillus johnsoni* La1and influence of its addition in retail-manufactured ice cream produced with different sugar and fat concentrations**. Int Dairy J.** 12: 201-206.

Alba, K., C. Ritzoulis, N. Georgiadis and V. Kontogiorgos. 2014. Okra extracts as emulsifiers for acidic emulsion. **Food Res Int.** 54:1730-1737.

Aleong, J.M., S. Frochot and H.D. Goff. 2008. Ice recrystallization inhibition in ice cream by propylene glycol monostearate. **J Food Sci.** 73:463-468.

Aman, P. and H. Graham. 1990. Chemical evaluation of polysaccharides in animal feeds, pp. 161-195. *In* J. Wiseman and D.J. Cole, eds. **Feed stuff evaluation**. Butterworths, London.

Aman, P. and E.Westerlund. 1996. Cell wall polysaccharides: structural, chemical and analytical aspects, pp. 191-226. *In* A.C. Eliasson, eds. **Carbohydrate in Food**. Marcel Dekker, New York.

AOAC. 2006. Official methods of analysis of AOAC International, 17th ed. W, Horwitz ed. **Association of Official Analytical Chemists.** Gaithersburg, MD. USA.

Arapitsas, P. 2008. Identification and quantification of polyphenolic compounds from okra seeds and skins. **Food Chem.** 110: 1041-1045.

Arbuckle, W.S. 1986. **Ice cream**. Van Nostrand Reinhold, New York.

Aremu, M.O., O. Olaofe and E.T. Akintayo. 2007. Functional properties of some Nigerian varieties of legume seed flours and flour concentration effect on foaming and gelation properties. **J Food Technol.** 5: 109-115.

Aremu, M.O., O. Olaofe, E.T. Akintayo and E.I. Adeyeye. 2008. Foaming, water absorption, emulsification and gelation properties of kersting's groundnut (Kerstingiella geocarpa) amd bambara groundnut (Vigna subterranean) flours as influenced by neutral salts and their concentration. **Pak J Nutri.** 7: 194-201.

Arita, K., E. Babiker, E. Azakami and A. Kato. 2001. Effect of chemical and genetic attachment of polysaccharides to protein on the production of IgG and IgE. **J Agr Food Chem.** 49: 2030-2036.

BahramParvar, M., M.H. Haddad-Khodaparast and S.M.A. Razavi. 2009. The effect of *Lallemantia royleana* (Balangu) seed, palmate-tuber salep and carboxymethylcellulose gums on the physiochemical and sensory properties of typical soft ice cream. **Int J Dairy Technol**. 62: 571-576.

BahramParvar, M., S.M.A. Razaviai and M.H. Haddad-Khodaparast. 2010. Rheological characterization and sensory evaluation of typical soft ice cream made with selected food hydrocolloids. **Food Sci Technol Int.** 16: 79-88.

Baht, U.S. and R.N. Tharathan. 1987. Functional properties of okra (*Hibiscus esculentus*) mucilage. **Starch/starke**. 39: 165–167.

Barger, G.G. 1990. Ice cream, pp. 367-429. *In* K. Larsson and S.E. Friberg, 2nd eds. **Food emulsions.** Marcel Dekker, New York.

Batistuti, J.P. 1998. Functional properties of defatted chickpea (Cicer arietinum, L.) flour as influenced by thermoplastic extrusion. **Alim Nutr.** 9: 65-75.

BeMiller, J.N., R.L. Whistler and D.G. Barbalow. 1993. Aloe, China flexseed, okra, psyllium seed, quince seed, and tamarind gums, pp. 235-255. .*In* L.W. Roy and J.N. BeMiller, eds. **Industrial Gums Polysaccharide and their Derivatives.** Academic Press, Inc., San Diago.

Berger, K.G. 1990. Ice cream, pp. 367-444. *In* K. Larsson and S. Friberg, 2nd eds **Food Emulsions**. Marcel Dekker Inc., New York.

Blaine, R.L. and L.C. Thomas. 2013. **Optimization of the freeze drying process using modulated differential scanning colorimetry.** Available Source: http://www.tainstruments.co.jp/application/pdf/Thermal_Library/Applications_Briefs/TA354.PDF. May 12, 2013.

Bolliger, S, H. Wildmoser, H.D. Goff and B.W. Tharp. 2000. Relationships between ice cream mix viscoelasticity and ice crystal growth in ice cream. **Int Dairy J.** 10: 791-797.

Blond, G. 1994. Mechanical properties of frozen model solutions**. J Food Eng**. 22: 253-269.

Boone, J.A.L. 2006. **Around the world of food: Adventures in culinary history**. iUniverse., Nebraska.

Bourne, M.C. 2002. **Food texture and viscosity: concept and measurement**. 2nd ed. Academic Press, Inc., San Diago.

Bryant, L.A. , J.R. Montecalvo, K.S. Morey and B. Loy. 1988. Processing, functional and nutritional properties of okra seed products. **J Food Sci**. 53: 810-816.

Caldwell, K.B., H.D. Goff and D.W. Stanley. 1992. A low temperature scanning electron microscopy study of ice cream. I. Techniques and general microstructure. **Food Struct.** 11: 1-9.

Cardenas, A., F.M. Goycoolea and M. Rinaudo. 2008. On the gelling behavior of 'nopal' (*Opuntia ficus indica*) low methoxyl pectin. **Carbohyd Polym**. 73: 212-222.

Carp, D.J., G.B. Bartholomai and A.M.R. Pilasof. 2001. Effects of denaturation on soy protein-xanthan interaction: comparison of a whipping-rheological and bubbling method. **Colloid Surface B**. 21: 163-171.

Carp, D.J., R.I. baeza, G.B. Bartholomai and A.M.R. Pilosof. 2004. Impact of proteins- K-carrageenan interactions on foam properties. **Lebensm Wiss Technol**. 37: 573-580.

Carpita, N.C. and D.M. Gibeaut. 1993. Structural models of primary cell walls in flowering plants: Consistency of molecular structure with the physical properties of the walls during growth. **Plant J**. 3: 1-30.

Center for New Crops and Plant Products of Purdue University. 2007**. Okra plant**. Available source: http://www.hort.purdue.edu/newcrop/Crops/okra.html, July 26, 2011.

Canteri-Schemin, M.H., H.C.R. Fertonani, N. Waszczynskyj and G. Wosiacki. 2005. Extraction of pectin from apple pomace. **Braz Arch Biol Tchn**. 48: 259-266.

Chaisawang, M. and M. Suphantharika. 2005. Effect of guar gum and xanthan gum additions on physical and rheological properties of cationic tapioca starch. **Carbohyd Polym.** 61: 288-295.

Chakraborty, A. and S. Ray. 2011. Development of a process for the extraction of pectin fruit wastes viz. lime peel, sprent quava, apple pomace etc. **Int J Food Safety**. 13: 391-397.

Chang, Y. and R.W. Hartel. 2002(a). Measurement of air cell distributions in dairy foams. **Int Dairy J.** 12: 463-472.

Chang, Y. and R.W. Hartel. 2002(b). Development of air cells in a batch ice cream freezer**. J Food Eng.** 55: 71-78.

Chinnan, M.S., K.H. McWaters and V.N.M. Rao. 1985. Rheological characterization of grain legume pastes and effect of hydration time and water level on apparent viscosity. **J Food Sci**. 50: 1167-1171.

Clarke, C. 2004. **The Science of ice cream: The royal society of chemistry**. Cambridge, UK.

Constantino, A.J. and J.E. Romanchick-Cerpoviez. 2004. Physical and sensory measures indicate moderate fat replacement in frozen dairy dessert is feasible using okra gum as a milk-fat ingredient substitute. **J Am Diet Assoc.** 104: 44-50.

Cosgrove, D.J. 2001. Wall structure and wall loosening: A look backwards and forwards. **Plant Physio**. 125: 131-134.

Costa F.F., J.V. Resende, L.R. Abrue and H.D. Goff. 2008. Effect of calcium addition on ice cream structure and quality. **J Dairy Sci.** 91: 2165-2174.

Cottrell, J.I.L., P. Geoffrey and G.O. Philips. 1980. The effect of stabilizers on the viscosity of an ice cream mix. **J Sci Food and Agri**. 31: 1066-1070.

Cottrell, J.I.L., G. Pass, and G.O. Philips. 1979. Assessment of polysaccharides as ice cream stabilizers. **J Sci Food Agr**. 30: 1085-1088.

Davidson, M.W. 2013. **Cell biology and microscopy structure and function of cell and viruses.** Plant cell wall. Available Source: http://micro.magnet.fsu.edu/cells/plants/cellwall.html**,** June 6, 2013.

Delmer, D.P. 1987. Biochaemistry of cellulose biosynthesis. **Tappi J**. 70: 141-143.

Department of Agriculture Extension (Thailand). 2013. **Okra.** Available Source: http://www.doae.go.th/library/html/detail/okra/index.html. June 15, 2013.

Dickinson, E. 1993. Protein-polysaccharide interaction in food colloid. pp. 77-93. *In* E. Dickinson and P. Walstra eds, **Food colloids and polymers: Stability and mechanical properties**. Royal Society of Chemistry. Cambridge.

Dickinson, E. and B.B. Galazka. 1991. Emulsion stabilization by ionic and complexes of β-lactoglobulin with polysaccharides. **Food Hydrocolloid**. 5: 281-296.

Dickinson, E. and E. Izgi. 1996. Foam stabilization by protein-polysaccharide complexes. **Colloid Surface A**. 113: 191-201.

Dogan, M. and A.Kayacire. 2007. The effect of aging at low temperature on the rheological properties of kahramanmaras-type ice cream mix. **Int J Food Prop**. 10: 19-24.

Donhowe, D.P. and R.W. Hartel. 1996. Recrystallization of ice cream during controlled accelerated storage. **Int Dairy J.** 6: 1191-1208.

Eisner, M.D. 2006. **Fat structure development in low temperature extruded ice cream**. Ph.D. dissertation. University of Karlsruhe.

Eliasson, A. C. and H.R. Kim. 1992. Changes in rheological properties of hydroxyproply potato starch pastes during freeze-thaw treatments I. A rheological approach for evaluation of freeze-thaw stability. **J Texture Stud**. 23: 279-295.

FAO. 2012. **FAOSTAT statistics database: Okra**. Available Source: http://faostat.fao.org, December 15, 2012.

Farhoosh R. and A. Riazi. 2007. A compositional study on two types of salep in Iran and their rheological properties as a function of concentration and temperature, **Food Hydrocolloid.** 21: 660-666.

Fenema, O.R. 1973. Low-temperature preservation of foods and living matter, pp. 151-239. *In* O.R. Feneema, W.D. Powrie and E.H. Marth, eds. **Nature of the freezing process**. Marcel Dekker, New York.

Flores, A.A. and H.D. Goff. 1999. Recrystallization in ice cream after constant and cycling temperature storage conditions as affected by stabilizers. **J Dairy Sci.** 82: 1408-1415.

Flory, P.J. 1953. **Principles of polymer chemistry**. Cornell University Press. New York.

Fox, P.F. and P.L.H. McSweeney. 1998. **Dairy chemistry and biolochemistry**. Blackie academic and professional. New York.

Gilles, P. 2002. **Use of at least one protein fraction extracted from Hibiscus esculentus seeds and cosmetic composition containing such a fraction.** U.S. patent 6,379,719.

Glicksman, M. 1991. Hydrocolloids and the search for the oil grail. **Food Technol**. 45: 94-103.

Goff, H.D. 1997a. Colloid aspects of ice cream – a review. **Int Dairy J**. 7: 363-373.

Goff, H.D. and K.B. Caldwell. 1991. Stabilizers in ice cream: how do they work? **Mod Dairy**. 70: 14-15.

Goff, H.D. and R.W. Hartel. 2004. Ice cream and frozen dessertsj pp. 494-565. *In* Y.A., Hui, eds. **Handbook of Frozen Foods.** Marcel Dekker, New York.

Goff, H.D. and M.E. Sahagian. 1996. Glass transitions in aqueous carbohydrate solutions and their relevance to frozen food stability. [Special issue]. **Thermochemica Acta**. 280-281: 449-464.

Goff H.D. and P. Spagnuolo. 2001. Effect of stabilizers on fat destabilization measurements in ice cream. **Milchwissenschaft.** 8: 450-453.

Goff, H.D., B. Freslon, M.E. Sahagian, T.D. Hauber, A.P. Stone and D.W. Stanley. 1995. Structure development in ice cream-dynamic rheological measurements. **J Texture Stud.** 26: 517-536.

Goff, H.D. and V.J. Davidson. 1994. Controlling the viscosity of ice cream mixes at pasteurization temperatures. **Mod Dairy.** 73: 12-14.

Goff, H.D., D. Fernando and C. Schorsch. 1999. Fluorescence microscopy to study galactomannan structure in frozen sucrose and milk protein solutions. **Food hydrocolloid**. 13: 353-362.

Goff, H.D., K.B. Caldwell, D.W. Stanley and T.J. Maurice. 1993. The influence of polysaccharides on the glass transition in frozen sucrose solutions and ice cream. **J Dairy Sci.** 76: 1268-1275.

Goff, H.D. 1992. Low-temperature stability and the glassy state in frozen food. **Food Res Int.** 25: 317-325.

Hagiwara, T. and R.W. Hartel. 1996. Effect of sweetener, stabilizer, and storage temperature on ice recrystallization in ice cream. **J Dairy Sci.** 79: 735-744.

Haper, E.K. and C.F. Shoemaker. 1983. Effects of locust bean gum and selected sweetening agents on ice recrystallization rates. **J Food Sci.** 48: 1801-1809.

Hartel, R.W. 1996. Ice crystallization during the manufacture of ice cream. **Trends Food Sci Tech.** 7: 315-321.

Hartel, R.W. 1998. Mechanisms and kinetics of recrystallization in ice cream, pp. 287-319. *In* D.S. Reid, eds. **The properties of water in foods ISOPOW6**. Blackie Acad. Prof., New York.

Hartel, R.W. 2001. Recrystallization process. pp. 284-308. *In* G.V. Barbosa, eds **Crystallization in foods**. Aspen. New York.

Hermansson, A.M. 1997. Biopolymer microstructure and rheology, pp 18-26. *In* E.J. Windhab, eds. **Proceeding of the 1st International symposium on food rheology and structure.** Zurich, Vincentz, Hanover,

Herrera, M.L., J.I. M'Cann, C. Ferrero, T. Hagiwara, N.E. Zaritzky and R.W. Hartel. 2007. Thermal, mechanical, and molecular relaxation properties of frozen sucrose and fructose solutions containing hydrocolloids. **Food Biophys**. 2: 20-28.

Hong, J.H. and Y.H. Choi. 2007. Physico-chemical properties of protein-bound polysaccharide from *Agaricus blazei* Murill prepared by ultrafiltration and spray drying process. **Int J Food Sci Technol**. 42: 1-8.

HunterLab. 2008. CIE L*a*b* color scale. **Applications note**. 8: 1-4.

Iagher, F., F. Reicher and J.L.M.S. Ganter. 2002. Structural and rheological properties of polysaccharides from mango (*Mangifera indica* L.) pulp. **Int J Biol Macromol.** 31: 9-17.

Izydorczyk, M.S. and C.G. Biliaderis. 1992. Effect of molecular size on physicochemical properties of wheat arabinoxylans. **J Agri Food Chem.** 40: 561-568.

Jayani, R.S., S. Saxena and R. Gupta.2005. Microbial pectinolytic enzymes: A review. **Process Biochem**. 40: 2931-2944.

K-center. 2013. **Okra**. Available from: http://k-center.doae.go.th/index.jsp, January 15, 2013.

Kato, A. Y. Sasaki, R. Furuta and K. Kobayashi. 1990. Functional protein-polysaccharide conjugate prepared by controlled dry heating of ovalbumin-dextran mixtures. **Agr Biol Chem Tokyo**. 54: 107-112.

Kaya, S. and A.R. Tekin. 2001. The effect of salep content on the rheological characteristics of a typical ice cream mix. **J Food Eng**. 47: 59-62.

Khouryieh, H.A., T.J. Herald, F. Aramouni and S. Alavi. 2007. Intrinsic viscosity and viscoelastic properties of xanthan/guar mixtures in dilute solutions: Effect of salt concentration on the polymer interactions. **Food Res Int**. 40: 883-893.

Khule, N.R., N.B. Mahale, D.S. Shelar, M.M. Rokade and S.R. Chaudhari. 2012. Extraction of pectin from citrus peel and use as natural binder in paracetamol tablet. **Der Pharmacia Lettre**. 4: 558-564.

Kalara, A. and R.C. Chandan. 2008. Ice cream and frozen desserts, pp. 364-365.*In* R.C., Chandan, A. Kilara and N. Shah, eds. **Dairy processing & Quality assurance**. Wiley-Blackwell, New Deli.

Kim, S., C.H. Lim, C. Lee and G. An. 2009. Optimization of growth and storage conditions for lactic acid bacteria in yogurt and frozen yogurt. **J Korean Soc Appl Biol Chem**. 52: 76-79.

Kloek, W., T. van Vliet and M. Meinders. 2001. Effect of bulk and interfacial rheological properties on bubble dissolution. **J Colloid Interface Sci**. 237: 158-166.

Kontogiorgos V., I. Margelou, N. Georgiadis and C. Ritzoulis. 2012. Rheological characterization of okra pectins. **Food Hydrocolloid.** 29: 356-362.

Koxholt, N.M.R., B. Eisenmann and J. Hinrichs. 2001. Effect of the fat globule sizes on the meltdown of ice cream. **J Dairy Sci**. 84: 31-37.

Kumar, P.G.K., G. Battu and K.N.S.L. Raju, 2011. Isolation and evaluation of tamarind seed polysaccharide being used as a polymer in pharmaceutical dosage forms**. RJPBCS**. 2: 274-290.

Kus, S., A. Altan and A. Kaya. 2005. Rheological behavior and time-dependent characterization of ice cream mix with different salep content**. J Texture Stud**. 36: 273-288.

Lapasin, R. and S. Procl. 1995. **Rheology of industrial polysaccharides: Theory and applications.** Blackie Academic and Professional, Galsgow.

Launay, B., G., Cuvelier and S. Martinez-Reyes. 1997. Viscosity of locust bean, guar, and xanthan gum solutions in the Newtonian domain: a critical examination of the log($\square_{sp})_o$ - logC($\square)_o$ master curves. **Carbohyd Polym**. 34: 385–395.

Lawal, O.S. and K.O. Adebowale. 2004. Effect of acetylation and succinylation on solubility profile, water absorption capacity and emulsifying properties of muncuna bean (*Mucuna pruiens*) protein concentrate. **Nahrung/Food**. 48: 129-136.

Levine, H. and L. Slade. 1989. A food polymer of science approach to the practice of cryostabilization technology. **Comment Agric Food Chem.** 1: 315-396.

Lin, M.J., E.S. Humbert and F.W. Sosulski. 1974. Certain functional properties of sunflower meal products. **J Food Sci.** 39: 368-370.

Marcotte, M., A.R.T. Hoshahili and H.S. Ramaswamy. 2001. Evaluation of rheological properties of selected salt enriched food hydrocolloids. **J Food Eng**. 48: 157-167.

Marshall R.T., H.D. Goff and R.W. Hartel. 2003. **Ice cream**. 6th ed.: Kluwer Academic/Plenum Publishers, New York.

Marshall, R.T. and W.S. Arbuckle. 1996. **Ice cream**. 5th ed. Chapman & Hall, New York.

Martinez, K.D., R.I. Baeza, F. Millan and A.M.R. Pilosof. 2005. Effect of limited hydrolysis of sunflower protein on the interactions with polysaccharides in foams. **Food Hydrocolloid**. 19: 361-369.

Maskan, M. and F. Gogus. 2000. Effect of sugar on the rheological properties of sunflower oil – water emulsion. **J Food Eng**. 43: 173-177.

Mendez-Velasco C. and H.D. Goff. 2011. Enhancement of fat colloidal interaction for the preparation of ice cream high in unsaturated fat. **Int Dairy J.** 21: 540-547.

Mendez-Velasco C. and H.D. Goff. 2012. Fat structures as affected by unsaturated or saturated monoglyceride and their effect on ice cream structure, texture and stability. **Int dairy J.** 24: 33-39.

Mezger T.G. 2002. **The rheology handbook**. Vincentz Verlag, Hannover.

Miller-Livney, T. and R.W. Hartel. 1997. Ice recrystallization in ice cream: Interaction between sweeteners and stabilizers. **J Dairy Sci.** 80: 447-456.

Minhas, K.S., J.S. Sidhu, G.S. Mudahar and A.K. Singh. 2002. Flow behavior characteristics of ice cream mix made with buffalo milk and various stabilizers. **Plant Food Hum Nutr**. 57: 25-40.

Momen-Heravi, F., L. Balaj, S. Alian, A.J. Trachtenberg, F.H. Hochberg, J. Skog and W.P. Kuo. 2012. Impact of biofluid viscosity on size and sedimentation efficiency of the isolated microvesicles. **Frontiers in Physiology: Membrane Physiology and Biophysics**. 3: 1-6.

Monpien, W. 2005. **Modification of okra polysaccharides using ammonium hydroxide and methylamine**. M.S. Thesis, Kasetsart Univeristy.

Morris, E.R., A.N. Cutler, S.B. Ross-Murphy, D.A. Ress and J. Price. 1981. Concentration and shear rate dependence of viscosity in random coil polysaccharide solutions. **Carbohyd Polym.** 1: 5-21.

Morris, E.R. 1990. Mixed polymer gel, pp. 291-359. *In* P. Harris, eds. **Food gels**. Elsevier, London.

Morris V.J. 1991. Designing polysaccharides for synergistic interactions, pp. 161-171. *In* G.O. Philips, P.A Williams and D.J. Wedlok, eds. **Gums and stabilizers for the food industry** IRL Press, Oxford.

Morris, E.R. 1995. Polysaccharide rheology and in-mouth perception, pp.517-546. *In* A.M. Stephen, eds. **Food polysaccharides and their applications**. Marcel Dekker, New York.

Muller, G., M. Anhourrache, J. Lecourtier and G. Chauveteau. 1986. Salt dependence of the conformation of a single stranded xanthan. **Int J Biol Macromol.** 8: 167-172.

Muralikrishna, G., U.R. Bhat and R.N. Tharanathan. 1987. Functional characteristics of the mucilaginous polysaccharides derived from cow pea (Vigna sinensis), black gram (Phaseolus mungo) and linseed (Linum usitatissimum). **Starke.** 39: 107-109.

Muse M.R. and R.W. Hartel. 2004. Ice cream structural elements that affect melting rate and hardness. **J Dairy Sci.** 87: 1-10.

Ndjouenkeu, R., F.M. Goycoolea, E.R. Morris and J.O. Akingbala. 1996. Rheology of okra (*Hibiscus esculentus* L.) and dika nut (*Irvingia gabonensis*) polysaccharides. **Carbohyd Polym.** 29: 263-269.

Ndjouenkeu, R, J.O. Akingbala and G.B. Oguntimein. 1997. Emulsifying properties of three African food hydrocolloid: okra (*Hibiscus esculentus*), dika nut (*Irvingia gabonensis*), and khan (*Belschmiedia* sp.). **Plant Food Hum Nutr**. 51: 245-255.

Nelson, D.L and M.M. Cox. 2005. **Lehninger's principles of biochemistry**. 4th ed.: Freemam and company, New York.

Nor Hayati, I.,Y.B. Che Man, C.P. Tan and I. Nor Aini. 2007. Stability and rheology of concentrated O/W emulsions based soybean/palm kernel olein blends. **Food Res Int**. 40: 1041-1-51.

Norziah, M.H., S.S. Kong, A.A. karim and C.C. Seow. 2001. Pectin-cucrose-Ca^{2+} interaction: effects on rheological properties. **Food Hydrocolloid**. 15: 491-498.

Ogbonnaya, D., J. Calderon Vera and A. Heiwall. 2009. **Pectic polysaccharides isolated from fruit pods of okra**. EP 1854812 B1.

O'Neill, M.A. and W.S. York. 2003. The composition and structure of plant primary cell wall, pp. 1-44. *In* J.K.C. Rose, eds. **The plant cell wall**. Blackwell Publishing, Oxford.

Patmore, J.V., H.D. Goff and S. Fernandes. 2003. Cryo-gelation of galactomannans in ice cream model systems. **Food Hydrocolloid.** 17: 161-169.

Pearce, K.N. 2013. Milk powder. [Food Science Section, New Zealand Dairy Research Institute]. http://nzic.org.nz/ChemProcesses/dairy/3C.pdf. October, 11, 2013.

Prins, A. 1988. Principles of foam stability, pp. 91-122. *In* E. Dickinson and G. Stainsby, eds. **Advances in food emulsions and foams.** Elsevier, London.

Prins, A. 1999. Stagnant surface behavior and its effect on foam and film stability. **Colloid. Surface. A.** 149: 467-473.

Prommakool A., T. Sajjanantakul, T. Janjarasskul and J.M. Krochta. 2010. Whey protein-okra polysaccharide fraction blend edible file: tensile properties, water vapor permeability and oxygen permeability. **J Sci Food Agric.** 91: 362-369.

Rahimi, S., S. Abbasi, M.A. Sahari and M.H. Azizi. 2013. Characterization of an unknown exudates gum from Iran. **Persian gum**. 1: 1-5.

Ramsden, L. 2004. Plant and algal gums and mucilages, pp. 233-256. *In* P. Tomasik, eds. **Chemical and functional properties of food saccharides.** CRC press LLC, New York.

Rao, M.A. 1999. Introduction, pp. 1-24. *In* M.A. Rao, eds. **Rheology of fluid and semisolid foods**. Aspen Publishers, Gaithersburg.

Rao, M.A. 1992. Measurement of viscoelastic properties of fluid and semisolid foods, pp.*In* M.A. Rao and J.F. steffe, eds. **Viscoelastic properties of foods**. Elsevier Applied Science. Barking, England.

Rao, M.V.S.S.T.S., R.S. Manohar and G. Muralikrishna. 2004. Functional characteristics of non-starch polysaccharides (NSP) obtained from native (n) and malted (m) finger millet (ragi, *Eleusine coracana*, indaf-15). **Food Chem**. 88: 453-460.

Regand, A. and H.D. Goff. 2006. Ice recrystallization inhibition in ice cream as affected by ice structuring proteins from winter wheat grass. **J Dairy Sci.** 89: 49-57.

Regand, A. and H.D. Goff. 2002. Effect of biopolymers on structure and ice recrystallization in dynamically frozen ice cream model system. **J Dairy Sci.** 85: 2722-2732.

Regand, A. and H.D. Goff. 2003. Structure and ice recrystallization in frozen stabilized ice cream model systems. **Food Hydrocolloid.** 17: 95-102.

Rha, C. 1975. Theories and principles of viscosity, pp. 123-141. *In* C. Rha, eds. **Theory determination and contro; of physical properties of food materials.** Dordreretch, The reidel, Netherlands.

Rha, C. 1978. Rheology of foods. **Food Technol**. 32: 77-81.

Rochefort, W. E. and S. Middleman. 1987. Rheology of xanthan gum: sale, temperature, and strain effects in oscillatory and steady shear experiments. **J Rheol.** 31: 337-369.

Rodriguez-hernandez, A. I., S. Durand, C. Garnier, A. Tecante and J.L. Doublier. 2006. Rheology-structure properties of waxy maize starch-gellan mixtures. **Food Hydrocolloid**. 20: 1223-1230.

Romanchik-Cerpovicz J.E., R.W. Tilmon and K.A. Baldree. 2002. Moisture retention and consumer acceptability of chocolate bar cookies prepared with okra gum as a fat ingredient substitute. **J Am Diet Assoc.** 102: 1301-1303.

Rosnani, A.I.W., I.N. Aini. A.M.M. Yazid and M.H. Dzulkifly. 2007. Flow properties of ice cream mix prepared from palm oil: anhydrous milk fat blends. **Pakistan J Bio Sci.** 10: 1691-1696.

Rudrappa, U. 2013. **Okra.** Power your diet. Available Source: http://www.nutrition-and-you.com/okra.html, September 10, 2013.

Russel, A.B., P.E. Cheney and S.D. Wantling. 1999. Influence of freezing conditions on ice crystallization in ice cream. **J Food Eng**. 39: 179-191.

Sanderson, G.R. 1996. Gums and their use in food systems. **Food Technol**. 50: 81-84.

Sandford, P.A., J.E. Pittsley, P.R. Watson, K.A. Burton, M.C. Cadmus and A. Jeanes. 1978. Rheological and other physical characteristics of polysaccharides from the black yeast-like fungi. **J Appl Polym Sci**. 22: 701-710.

Sarker, D.K., P.J. Wiled and D.C. Clark. 1998. Enhancement of protein foam stability by formation of wheat arabinoxylan-protein cross-links. **Cereal Chem.** 75: 493-499.

Schols, H.A., C.J.M. Geraeds, M.F. Searle-Van Leeuwan, F.J.M. Kormelink and A.G.J. Voragen. 1990. Rhamnogalacturonase: a novel enzyme that degrades the hairy regions of pectins. **Carbohyd Res**. 206: 105-115.

Segall, K.I. and H.D. Goff. 2002. A modified ice cream processing routine that promotes fat destabilization in the absence of added emulsifier. **Int Dairy J**. 12: 1013-1018.

Sengkhamparn, N., E.J. Bakx, R. Verhoef, H.A. Schols, T. Sajjaanantakul and A.G.J. Voragen. 2009. Okra pectin contains an unusual substitution of its rhamnosyl residuals with acetyl and alpha-linked galactosyl groups. **Carbohyd Res.** 344: 1842-1851.

Sengkhamparn, N., L.M.C. Sagis, R.D. Vries, H.A. Schols, T. Sajjaanantakul and A.G.J. Voragen. 2010. Physicochemical properties of pectins from okra (*Abelmoschus esculentus* (L.) Moench). **Food Hydrocolloid.** 24: 35-41.

Shepherd, R. A. Robertson and D. Ofman. 2000. Dairy glyo-conjugate emulsifiers: casein-maltodextrins. **Food Hydrocolloid.** 14: 281-286.

Sofjan, R.P. and R.W. Hartel. 2004. Effect of overrun on structural and physical characteristics of ice cream. **Int Dairy J.** 14: 255-262.

Soukoulis, C., I. Chandrinos and C. Tzia. 2008. Study of the functionality of selected hydrocolloids and their blends with K-carrageenan on storagequality of vanilla ice cream, **Food Sci Technol.** 41: 1816-1827.

Soukoulis, C., D. lebesi and C. Txia. 2009. Enrichment of ice cream with dietary fiber: Effects on rheological properties, ice crystallization and glass transition phenomena. **Food Chem**. 115: 665-671.

Spiegel, M.R. and L.J. Stephens. 1999. **Theory and problem of statistics**, 3rd ed. McGRAW-HILL, New York.

Sravani, B., R. Deveswaran, S. Bharath, B.V. Basavaraj and V. Madhavan. 2011. Release characteristics of drugs from cross linked tamarind seed polysaccharide matrix tablets. **Der Pharnacia Sinica.** 2: 67-76.

Sriamornsak, P. and N. Wattanakorn. 2008. Rheological synergy in aqueous mixtures of pectin and mucin. **Carbohyd Polym**. 74: 474-481.

Stanley, N.I. and L.J. Taylor. 1993. Rheological basis of oral characteristics of fluid and semi-solid foods: a review. **Acta Psychol.** 84: 79-92.

Stanley, D.W., H.D. Goff and A.K. Smith. 1996. Texture-structure relationships in foam dairy emulsions. **Food. Res**. Int. 29: 1-13.

Steffe, J.F. 1996. **Rheological methods in food process engineering**, 2nd ed. Freeman Press, East Lansing.

Subba Rao, M.V.S.S.T., R. Sai manohar and G. Muralikrishna. 2004. Functional characteristics of non-starch polysaccharides (NSP) obtained from native (n) and malted (m) finger millet (ragi, Eleusine coracana, indaf-15). **Food Chem**. 88: 453-460.

Susheelamma, N.S., and M.V.L. Rao. 1979. Functional role of the arabinogalactan of black gram (Phaseolus mungo) in the texture of leavened foods (steamed puddings). **J Food Sci.** 44: 1309-1316.

Sutton, R.L. and J. Bracey. 1996. The blast factor. Dairy Industries International. 62(2):31-33.

Sutton, R.L.and J. Wilcox. 1998. Recrystallization in model ice cream solutions as affected by stabilizer concentration. **J Food Sci.** 63: 9-11.

Szczesniak, A.S. and E. Farkas. 1962. Objective characterization of the mouthfeel of gum solutions. **J Food Sci**. 27: 381-385.

Talmadage, K.W., K. Keegstra, W.D. Bauer and P. Albersheim. 1973. The structure of plant cell walls: I. The macromolecular compounents of the walls of suspension-cultured sycamore cells with a detailed analysis of the pectic polysaccharides. **Plant Physiol**. 51: 158-173.

Thakur, B.R., R.K. Sigh and A.K. Handa. 1997. Chemistry and uses of pectin: a review. **Crit Rev Food Sci Nutr**. 37: 47-73.

Togrul, H. and N. Arslan. 2003. Production of carboxymethyl cellulose from sugar beet pulp cellulose and rheological behavior of carboxymethyl cellulose. **Carbohyd Polym**. 54: 73-82.

Togrul, H. and N. Arslan. 2004. Mathematical model for prediction of apparent viscosity of molasses. **J Food Eng**. 62: 281-289.

Tomada, M., K. Shimada, Y. Saito and M. Sugi. 1980. Plant mucilages. XXVI Isolation and Structural Features of a mucilage, “Okra mucilage”, from the immature fruit of *Abelmoschus esculentus*. **Chem Pharm Bull**. 28: 2933-2940.

Tuinier, R., P. Zoon, M.A.C. Stuart, G.J. Fleer and C.G.D. Kruif. 1999. Concentration and shear rate dependence of the viscosity of an exocellular polysaccharide. **Neth Biopolym**. 50: 641-646.

Vardhanabhuti, B., S. Ikeda. 2006. Isolation and characterization of hydrocolloids from monoi (Cissampelos pareira) leaves. **Food Hydrocolloid**. 20: 885-891.

Vincken, J.P., A.G.J. Voragen and G. Beldman. 2003. Enzymes degrading rhamnogalacturonan and xylogalacturonan, pp. 930-941. *In* J.R. Whitaker, A.G.J. Voragen and D.W.S. Wong, eds. **Handbook of food enzymology**. Marcel Dekker, New York.

Wang, J.C. and J.E. Kinsella. 1976. Functional properties of novel proteins; alfalfa leaf protein**. J Food Sci**. 41: 286-289.

Wang, W. 2005. Protein aggregation and its inhibition in biopharmaceutics. Int J Pharm. 289: 1-30.

Whistler, R.L. and H.E. Conrad. 1954. **J Am Chem Soc.** 76: 1673-1974.

Wildmoser, H., S.A.K. Jeelani and E.J. Winghab. 2005. Serum separation in molten ice creams produced by low temperature extrusion process. **Int Dairy J.** 15: 1074-1085.

Willats, W.G.T., P. Knox and J.D. Mikkelsen. 2006. Pectin: new insights into an old polymer are starting to gel. **Trends Food Sci Technol**. 17: 97-104.

Woolfe, M.L., M.F. Chaplin and G. Otchere. 1977. Studies on the mucilages extracted from Okra fruis (*Hibiscus esculentus* L.) and Baobab Leaves (Adansonia digitata L.). **J Sci Food Agri.** 28: 519-529.

Yoo B, Rao MA. 1995. Yield stress and relative viscosity of tomato concentrates: effect of total solids and finisher screen size. **J Food Sci**. 60: 777-779.

Yoo, D. C. Kim and B. Yoo. 2005. Steady and dynamic shear rheology of rice starch-galactomannan mixtures. **Starch/Starke**. 57: 310-318.

Zhang, L.M. 1999. Synergistic blends from aqueous solutions of two celluloses derivatives. **Colloid Polym Sci.** 277: 886-890.

APPENDIX

Experiment 1: Preparation of okra cell wall (OKW) and okra polysaccharide (OKP), and investigation of their physico-chemical properties

1. Chemical composition analysis

1.1 AOAC official method 934.01: Moisture (loss on drying)

Samples around 2 g were dried at 95-100°C to constant weight. The moisture content was calculated with Eq.12.

$$\text{Moisture content (\%)} = \frac{\text{Weight loss on drying (g)}}{\text{Weight of test portion (g)}} \times 100$$

1.2 AOAC official method 942.05: Ash

Weighted 2 g test portion into crucible and placed in temperature controlled furnace preheated to 600°C. Then held at this temperature for 2 h, transferred crucible directly to desiccators, cooled, and weighted. The percent ash was calculated with Eq. 13.

$$\text{Ash (\%)} = \frac{\text{Weight of test portion (g)} - \text{weight loss on ashing (g)}}{\text{Weight of test portion (g)}} \times 100$$

1.3 AOAC official method 990.03: Crude protein (combustion method)

Nitrogen freed by combustion at high temperature in pure oxygen was measured by thermal conductivity and converted to equivalent protein by appropriate numerical factor. Protein content was calculated with Eq. 14.

$$\text{Crude protein (\%)} = \%\ \text{N} \times 6.25$$

1.4 AOAC official method 2003.06: Crude fat (Hexanes extraction-submersion method)

Weighted 1-5 g of test portions into cellulose thimbles, dried thimbles containing test portion at 102±2°C for 2 h. Placed defatted cotton plug on top of test portion to keep material immersed during the boiling step and prevented any loss of test portion from top of thimble. Placed five a 5 mm glass boiling beads into each cups, and dried cup for at least 30 min at 102±2°C. Transferred to desiccators and cooled to room temperature, weighted an extraction cup.

Preheated extractor and turned on condenser cooling water, then attached thimbles containing dried test portions to extraction column. Put sufficient amount of solvent into each extraction cup to cover test portion when thimbles were in boiling position. Placed cups under extraction column and secured in place. Lower thimbles into solvent and boil for 20 min, raised thimbles out of solvent and extracted in this position for 40 min. Then distilled as much as solvent as possible from cups to reclaim solvent and attained apparent dryness, removed extraction cups from extractor and placed in operating fume hood to finish evaporating solvent at low temperature. Dried extraction cups in 102±2°C oven for 30 min to remove moisture. Crude fat content was calculated with Eq. 15.

$$\text{Crude fat (\%)} = \frac{[\text{Weight of cup (g)} + \text{fat residue (g)}] - \text{Weight of empty cup (g)}}{\text{Weight of test portion (g)}} \times 100$$

1.5 AOAC official method 962.09: Crude fiber (Ceramic fiber filter method)

Crude fiber was loss on ignition of dried residue remaining after digestion of sample with 1.25% (w/v) sulfuric acid (H_2SO_4) solution and 1.25% (w/v) Sodium hydroxide (NaOH) solutions under specific conditions.

Around 1.5-2.0 g dry weight sample was transfer into 600 ml beaker, 200 ml boiling 1.25% H_2SO_4 and one drop diluted antifoam. Placed beaker on digestion apparatus with hot plate and boil exactly 30 min. Removed beaker, and filter in California Buchner, allowed to settle 5-10 s, turned on vacuum. Rinsed beaker with 50-75 ml boiling H_2O, poured into California Buchner and sucked dry. Removed residue and replaced in beaker. Added 200 ml boiling 1.25% NaOH, and boiled exactly 30 min, filter, washed with 25 ml of boiling 1.25% H_2SO_4, three 50 ml portion H_2O, and 25 ml alcohol, and removed residue to ashing dish. Residue was dried for 2 h at 130±2°C. Cooled in desiccators, weighted, and ignited 30 min at 600±15°C, re-weighted. Crude fiber was calculated with Eq. 16.

$$\text{Crude fiber (\%)} = \frac{\text{Loss in weight on ignition (g)} - \text{Loss in weight of ceramic fiber blank (g)}}{\text{Weight of test portion (g)}} \times 100$$

2. Preparation of buffer solutions

Buffer solutions for the measurement of effect of pH on apparent viscosity of OKW and OKP were prepared by mixing stick solution at different volumes as showed in Appendix Table 1 and 2.

Appendix Table 1 The volume of stock solutions of 0.2 M dibasic sodium phosphate and 0.1 M of citric acid for phosphate-citrate buffer preparation

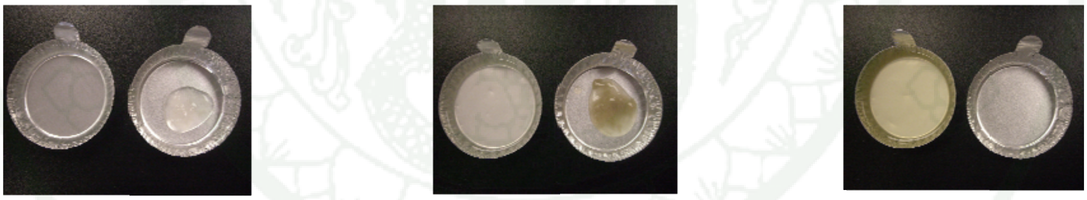
0.2 M dibasic sodium phosphate (ml)	0.1 M citric acid (ml)	pH
10.2	39.8	3.0
19.3	30.7	4.0
25.7	24.3	5.0
32.1	17.9	6.0
43.6	6.5	7.0

Appendix Table 2 The volume of stock solutions of 0.1 M sodium carbonate and 0.1 M of sodium bicarbonate for carbonate-bicarbonate buffer preparation

0.1 M sodium carbonate (ml)	0.1 M sodium bicarbonate (ml)	pH
4.0	46.0	9.0
27.5	22.5	10.0

3. Water solubility (WS) and water holding capacity (WHC) measurements of OKW and OKP

A detail of water solubility (WS) and water holding capacity (WHC) of OKW and OKP measurement was described in materials and methods section of the thesis that a centrifugal technique was used for this study. The visual appearance of supernatant and sediment of the sample are shown in Appendix Figure 1.



GG OKW OKP

Appendix Figure 1 Supernatant and sediment of 0.30% GG, OKW and OKP

4. Information for estimation of minerals in ice cream.

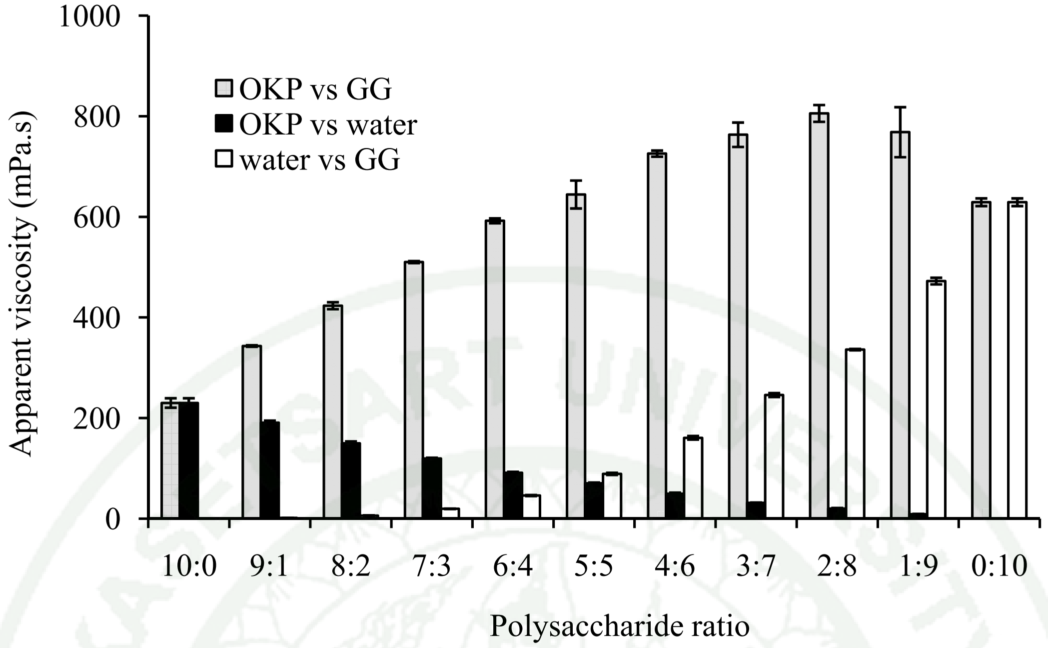
In results and discussion section of effect of salt ion on apparent viscosity, it was mentioned about the minerals in ice cream. This is information for estimation of minerals in ice cream.

The potassium sodium, magnesium, and calcium contain in typical ice cream mix was calculated from the fundamental data: approximately 13 kg of whole milk powder (WMP) or 9 kg of milk solid not fat (MSNF) can be made from 100 L of whole milk (Pearce, 2013).

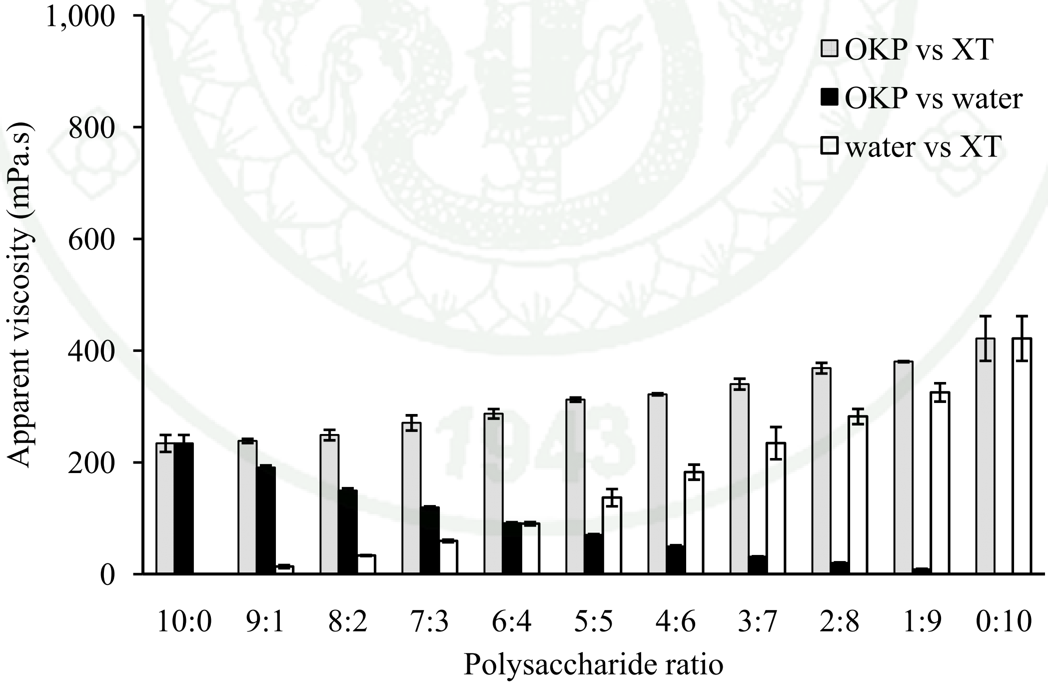
Appendix Table 3 Average amount of minerals in milk

Constituent	Amount (mg/100 ml)
Calcium	120
Magnesium	12
Phosphorus	95
Sodium	50
Potassium	150
Chloride	100
Sulphate	10
Citrate (as citric acid)	170

Source: Fox and McSweeney (1998)



Appendix Figure 2 Apparent viscosity of OKP and guar gum mixtures at different ratios, measured at 50 s^{-1}



Appendix Figure 3 Apparent viscosity of OKP and xanthan gum mixtures at different ratios

Experiment 3: Investigation the effect of OKW and OKP on physical properties and stability of ice cream

1. Fat particle size of ice cream mix

1.1 Determination of fat particle size

The fat particle size was determined by a method based on the procedure of Mendez-Velasco and Goff (2012). Ice cream was melted at 4°C for 2 h before analysis. The weighted average particle diameter ($d_{4,3}$) of ice cream mix and melted ice cream were estimated with Malvern Mastersizer 2000 (Malvern Instruments, Malvern, Worcestershire, UK), Measurements were made with refractive index of 1.46 and 1.33 for the fat and the dispersing medium, respectively, absorbance of 0.001 and obscuration value in the range of 12-18%.

1.2 Fat particle size of ice cream mix

The fat particle size of ice cream mix and melted ice cream are shown in Table 9. In the process of ice cream mix preparation, homogenization pressure is the dominating factor in emulsion formation, and there is sufficient surfactant present from either protein or emulsifier to produce satisfactory emulsion size distribution (Bolliger *et al*., 2000). The fat particle sizes of ice cream mix in this study ranged from 0.532-27.9 µm. The study of Goff *et al*. (1999) found that the fat particle size of ice cream mix varied from 1.80-8.67. Ice cream mix with OKW show the largest particle, the result may be interfered by the water-insoluble cell wall.

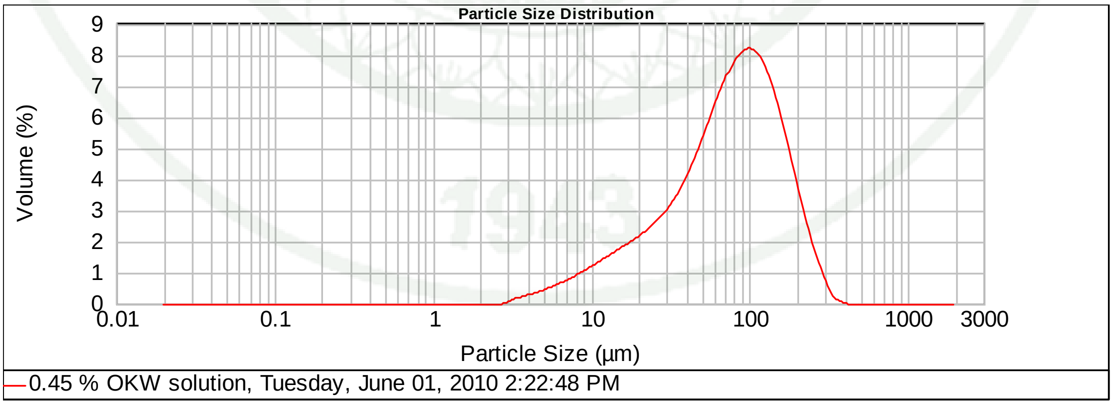
The results showed that the fat particle size of melted ice cream was bigger than of ice cream mix of all samples. Both OKW and OKP significantly increased the fat particle size of ice cream. It has been stated that fat destabilization ice cream is partial coalescence of fat globule due to the destabilizing effect during freezing conditions (Goff, 1997). Since the use of OKW and OKP increased the fat destabilization index, it was reasonable to consider that OKW and OKP could enhance the coalescence of fat globules. In general, ice cream products with high fat destabilization index and low particle size of fat globules were considered to be better quality (Goff, 1997).

Appendix Table 4 Fat particle size of ice cream mix and melted ice cream

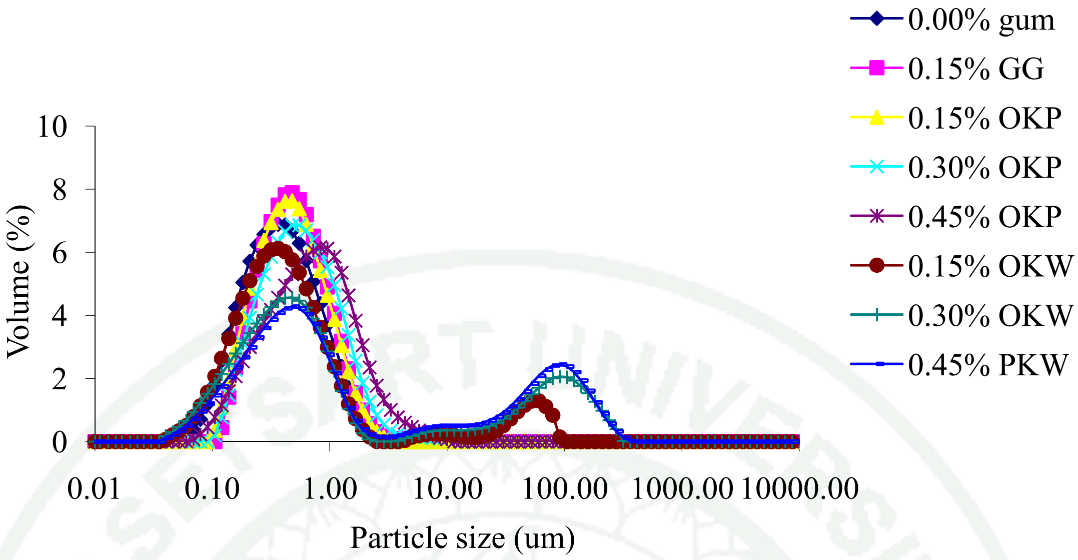
Ice cream sample	d[4,3]*	
	Ice cream mix	Melted ice cream
0.00% gum	0.532^{d} ± 0.011	1.347^{d} ±0.127
0.15% GG	0.648^{d} ± 0.034	1.444^{d} ± 0.181
0.15% OKP	0.604^{d} ± 0.011	1.395^{d} ± 0.183
0.30% OKP	0.858^{d} ± 0.071	3.579^{d} ± 0.777
0.45% OKP	1.454^{d} ± 0.755	7.461^{d} ± 1.184
0.15% OKW	6.192^{c} ± 0.636	11.504^{c} ± 0.446
0.30% OKW	19.425^{b} ± 0.653	23.120^{b} ± 0.653
0.45% OKW	27.979^{a} ± 0.677	31.942^{a} ± 0.781

*Values in the columns were mean ± SD

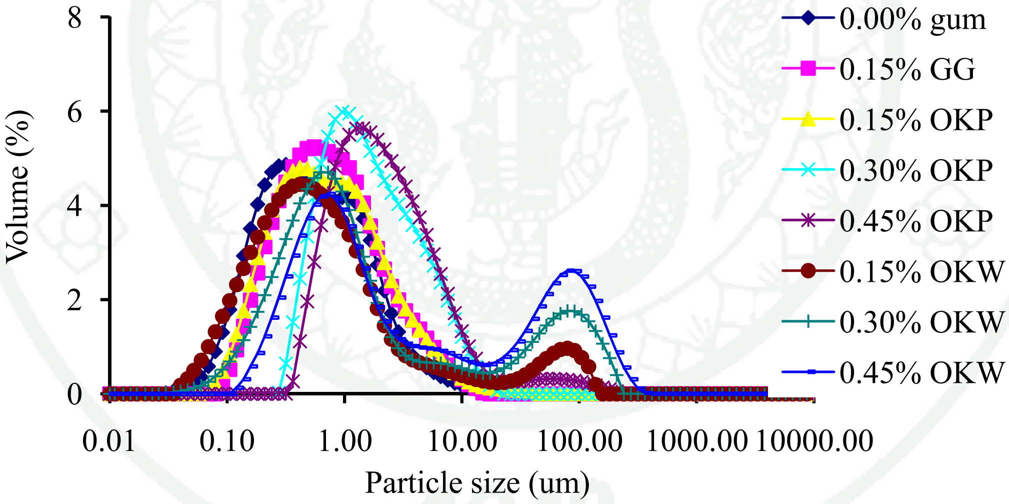
$^{a, b, c, d}$ Values with the same letter in a column do not differ



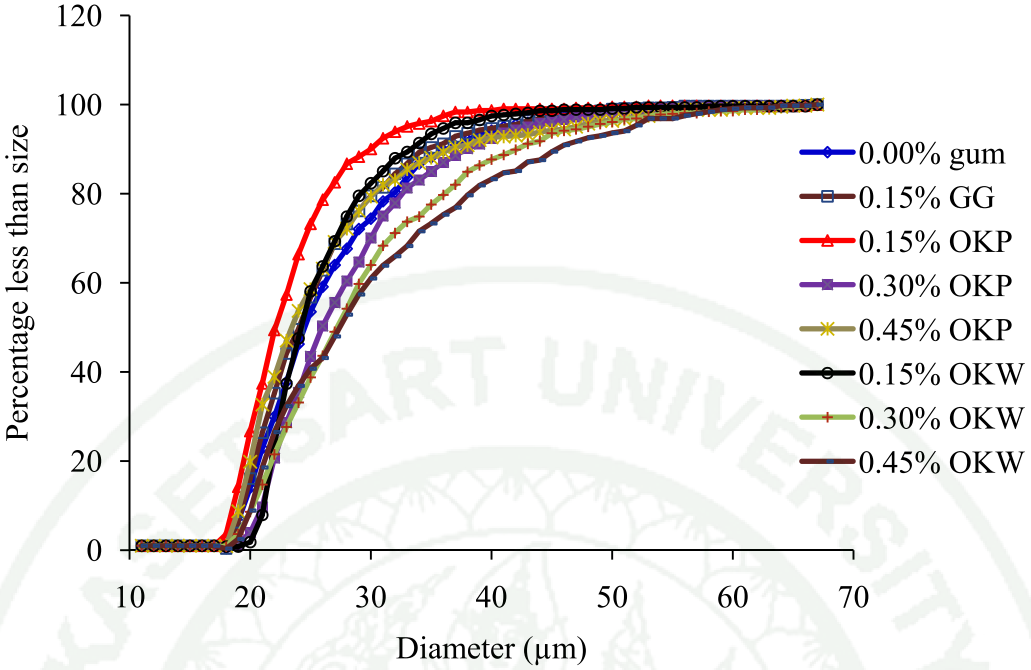
Appendix Figure 4 Particle size distribution of OKW solution



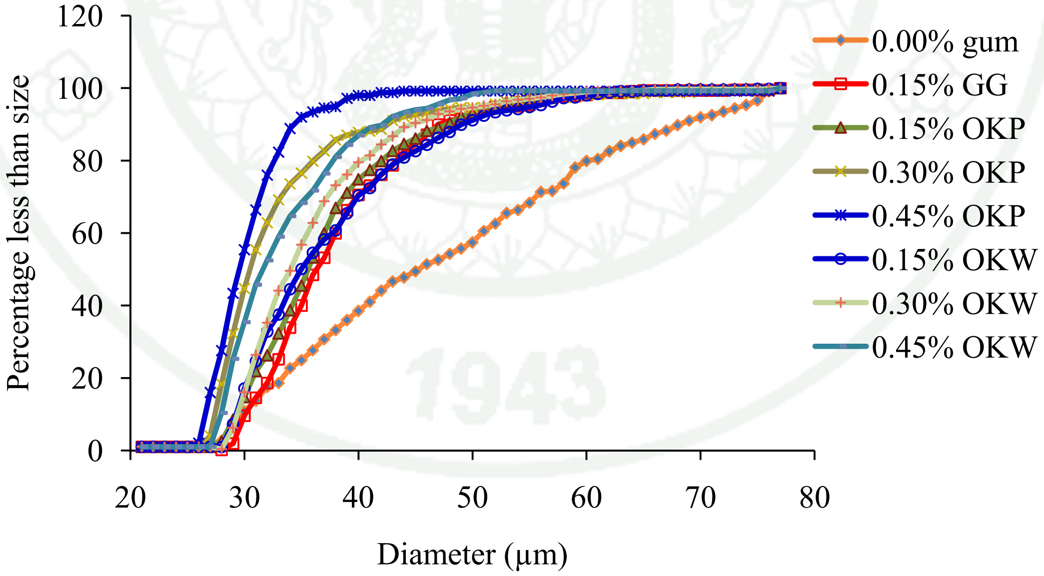
Appendix Figure 5 Fat particle size distribution of ice cream mix



Appendix Figure 6 Fat particle size distribution of melted ice cream



Appendix Figure 7 Logistic model distribution for ice cream at 0 cycles of temperature cycling



Appendix Figure 8 Logistic model distribution for ice cream at 10 cycles of temperature cycling

Ice cream taste panel

You will be trying six samples
Please taste them in the order indicated and answer the questions on the screen.
Between each sample you will have a 30 second break.
During that time, slide your sample back through the hatch and take a bite of cracker and a sip of water and wait for your next sample to be served to you.

To start the test, click on the continue button below:

Panelist Code: __________________
Panelist Name: ______________________________________
Question # 1 – Sample ____________
Please taste sample %01 and tell us how much you like or dislike the sample Overall
Overall Liking

dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	neither like nor dislike	like slightly	like moderately	Like very much	like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Question # 2 – Sample_____________
Taste sample %01. How much do you like or dislike the Flavour of this sample?
Flavour Liking

dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	neither like nor dislike	like slightly	like moderately	Like very much	like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Question # 3 – Sample_____________

Evaluate the strength of the following attributes for sample %01. Sweetness, Vanilla ice cream Flavour, Dairy Flavour and Aftertaste.

Sweetness

Weak Strong

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Vanilla ice cream flavor

Weak Strong

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dairy (cream) Flavour

No cream flavor Strong cream flavor

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Aftertaste

No aftertaste Strong aftertaste

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Question # 4 – Sample_____________

Taste sample %01. How much do you like or dislike the Texture of this sample?

Texture Liking

dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	neither like nor dislike	like slightly	like moderately	Like very much	like extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐

Question # 5 – Sample_____________

Evaluate Sample %01 for Guminess, Creamy Texture, Smootness and Mouthcoating.

Guminess

Not gummy Gummy

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Creamy texture

Not creamy Creamy

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Smoothness

Smooth Coarse

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Mouthcoating

None Extream

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

THANK YOU

Experiment 4: Investigation of relation between heat-shock ice cream mix rheology and ice crystal growth in OKP-stabilized ice cream

Appendix Table 5 Rheological parameters of ice cream mix with OKP, and ice cream mix model with LBG or GG

(Pa)	This study		The study of Patmore *et al*., (2003)					
	OKP/MSNF/fat emulsion		LBG/MSNF/sucrose solution		LBG/MSNF/fat/sucrose emulsion		GG/MSNF/sucrose solution	
	Be	Af	Be	Af	Be	Af	Be	Af
Yield stress	0.32^{A}	0.42^{A}	~0.1	~0.7	~0.1	~0.3	~0.1	~0.2
G′	7.86^{A}	11.35^{A}	4.80^{A}	3.31^{B}	3.78^{A}	1.32^{B}	3.95^{A}	4.50^{A}
G′′	5.29^{A}	5.97^{A}	6.42^{A}	2.84^{B}	4.51^{A}	1.47^{B}	3.91^{A}	4.19^{A}

*Emulsion with emulsifier, and stabilizer (OKP, LBG, and GG) at 0.30% concentration

$^{A, B}$ Letters indicated significant differences between before (Be) and after (Af) temperature cycling for 5 cycles within formulation groups ($P < 0.05$)

Source: adapted from Patmore *et al*., (2003)

Appendix Table 6 Pearson correlation coefficient of ice cream mixes rheological properties before temperature cycling and ice crystal growth of ice cream

	Crystal growth	OKP concentration	Yield point	G´	G´´
Concentration	-0.965**				
Yield point	-0.845**	0.895**			
G´	-0.823**	0.884**	0.951**		
G´´	-0.857**	0.919**	0.987**	0.954**	
Complex vis	-0.813**	0.875**	0.971**	0.962**	0.986**

** Correlation is significant at the 0.01 level (2-tailed).

Appendix Table 7 Pearson correlation coefficient of ice cream mixes rheological properties after temperature cycling and ice crystal growth of ice cream

	Crystal growth	OKP concentration	Yield point	G´	G´´
Concentration	-0.965**				
Yield point	-0.854**	0.906**			
G´	-0.820**	0.891**	0.939**		
G´´	-0.831**	0.905**	0.980**	0.973**	
Complex vis	-0.818**	0.889**	0.971**	0.978**	0.994**

** Correlation is significant at the 0.01 level (2-tailed).

Appendix Table 8 Pearson correlation coefficient of change of ice cream mixes rheological properties affected by temperature cycling, and ice crystal growth of ice cream

	Crystal growth	OKP concentration	Yield point	G´	G´´
Concentration	-0.965**				
Yield point	0.96988	-0.943**			
G´	0.922**	-0.900**	0.842**		
G´´	0.936**	-0.929**	0.928**	0.832**	
Complex vis	0.932**	-0.925**	0.913**	0.849**	0.854**

** Correlation is significant at the 0.01 level (2-tailed).

Appendix Table 9 Included parameters and coefficients estimated by multi-linear stepwise regression analysis of ice cream mix before temperature cycling

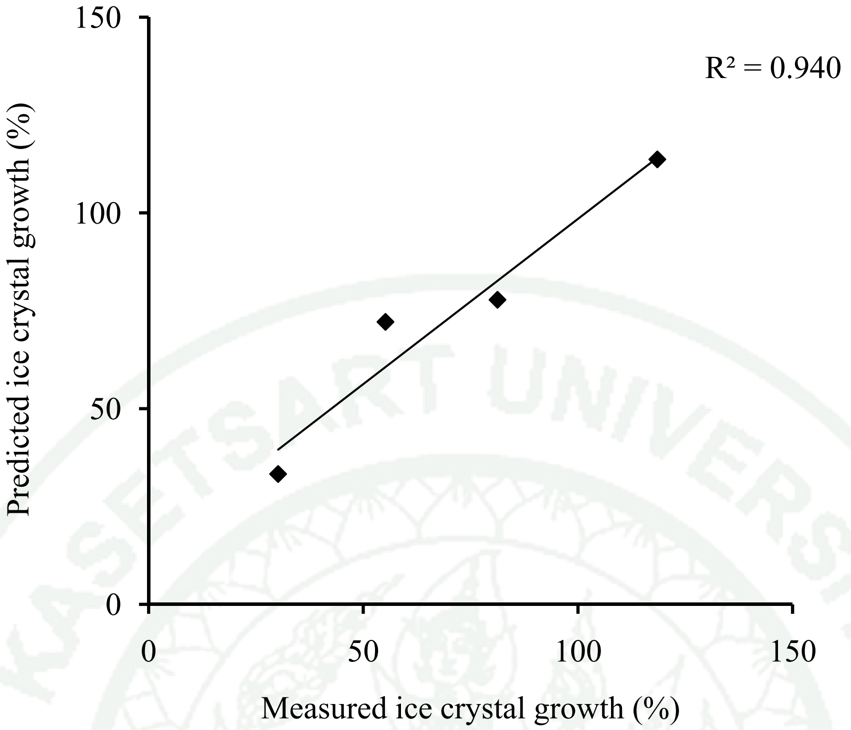
Independent variables	Unstandardized coefficient		*t*	Sig.	R^2
	B	Standard error			
Included variable					
Constant	25.438	3.292	7.727	0.000	0.98
x_1	0.667	0.094	7.061	0.000	
x_2	0.285	0.073	3.913	0.004	
Excluded variable					
x_3	0.149	-	1.081	0.311	
x_4	-0.044	-	-0.411	0.692	

x_1 = change of yield stress, x_2 = change of G´, x_3 = change of G´´, x_4 = change of □

1. Regression validation

The equation was validated to determine whether the equation was usable which the detail was shown in appendix. Another four ice cream mix and ice cream samples were prepared and tested for rheological parameters and measured for ice crystal growth. Their predicted ice crystal growths were calculated from their change of yield stress (x_1), and change of G′ (x_2).

The actual and predicted ice crystal growths were compared (Appendix Figure 9). The ice crystal growth predicted by the change of yield stress and change of G′ of ice cream mix showed a good correlation with the measured ice crystal growth ($R^2 = 0.94$). The results indicated that the regression equation provided a useful prediction of ice crystal growth. The equation explained 98% of the data variability (Appendix Table 9). The method had a potential to estimate ice crystal growth of ice cream from rheological parameter of ice cream mix. The concept of the studied can be elaborated for the widely use of ice cream industry. It will be useful if the stability of ice cream can be estimated from the ice cream mix, rather than the final product ice cream.



Appendix Figure 9 Relationship between predicted and measured ice crystal growths: the multiple regression equations of prediction were based on the change of yield stress and change of G′ of ice cream mix before temperature cycling

CURRICULUM VITAE

NAME : Miss Pilapa Yuennan

BIRTH DATE : October 02, 1982

BIRTH PLACE : Suratthani, Thailand

EDUCATION :

YEAR	INSTITUTE	DEGREE/DIPLOMA
2005	Walailak University	B.Sc. (Food Technology)
2007	Kasetsart University	M.S. (Food Science)

POSITION/TITLE : Research Assistant
WORK PLACE : Faculty of Agro-Industry, Kasetsart University
SCHOLARSHIP/AWARDS : The Royal Golden Jubilee Ph.D. scholarship (2007-2010)