PHYSICAL AND RHEOLOGICAL PROPERTIES OF STARCH-CHITOSAN MIXTURES DURING PASTING

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE
(BIOTECHNOLOGY)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2010

Thesis
entitled

PHYSICAL AND RHEOLOGICAL PROPERTIES OF STARCH-CHITOSAN MIXTURES DURING PASTING

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PHYSICAL AND RHEOLOGICAL PROPERTIES OF STARCH-CHITOSAN MIXTURES DURING PASTING

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ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and appreciation to my advisor, Assoc. Prof. Dr. Manop Suphantharika for his helpful guidance, continuous comments, discussion, encouragement and support through out this study. All his kindness and courtesy will be long remembered with respect.

I wish to thank to the members of advisory committee, Dr. Pairoj Luangpituksa, Asst.Prof. Dr. Atitaya Siripinyanond and Asst.Prof. Dr. Jirarat Tattiyakul for kindness in all guidance and valuable suggestions.

I am thankful to General Starch Co. Ltd. and National Starch Co. Ltd., Thailand for supporting of tapioca starch and cationic tapioca starch, respectively. I am grateful to Metrohm Siam Ltd. for kindly providing the rheometer used in these experiments.

Special thanks must be extended to all members of the room BT204 and, for friendship and their helps and providing facilities during my work. My thanks are also all of my friends for encouragement, enjoyment and cheerfulness.

This thesis is partially supported by the Center of Excellence for Agricultural Biotechnology, Postgraduate Education and Research Development Office, Commission on Higher Education, Ministry of Education.

Finally, I am grateful to my family who has grown me up with warmness, so much love, understanding, patience and encouragement through out my study.I would like to say thank you so much and love you so.

Supawadee Chawanthayatham

PHYSICAL AND RHEOLOGICAL PROPERTIES OF STARCH-CHITOSAN MIXTURES DURING PASTING

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ABSTRACT

The effects of molecular weights (M_w) of chitosan (CS) on the physical and rheological properties of native and modified tapioca starches were investigated. Rapid Visco Analysis (RVA) results showed that the addition of chitosan at various molecular weights increased the peak, breakdown, setback, and final viscosities, and pasting temperatures of native tapioca starch (NT), anionic tapioca starch (AT), and cationic tapioca starch (CT) to different degrees, except for the peak viscosities of CT/CS with medium and low M_w and the breakdown viscosities of all CT/CS which decreased as compared with those of the control CT alone. The presence of chitosan slightly increased swelling powers of NT and AT but greatly decreased those of CT due to electrostatic repulsion between the positively charged starch and gum molecules. CS formed a sheet structure in gel matrix and wrapped around NT and AT granules but did not wrap CT granules. These effects were more pronounced with increasing M_w of CS. Differential scanning calorimetry (DSC) data demonstrated that addition of CS regardless of their M_w resulted in a significant increase in the onset (T_o), peak (T_p) and conclusion (T_c) gelatinization temperatures of NT and AT but did not affect those of CT. The gelatinization enthalpies (ΔH_1) were affected by CS addition in different ways, i.e. decreased for NT, increased for AT, and no effect for CT. Dynamic viscoelasticity measurement indicated that the addition of any of these CS resulted in an increase in the storage modulus (G') and loss modulus (G'') of the fresh native and modified tapioca starch pastes. The tan δ values of NT and AT were increased by the addition of CS whereas those of CT tended to decrease. Steady flow tests illustrated the time-dependent shear-thinning (thixotropic) behaviour of all pastes. The hysteresis loop areas of NT and AT pastes were reduced by the addition of CS, indicating high shear resistant and structure recovery of starches in the presence of CS. In contrast, those of CT pastes increased, suggesting CS weakened the network structure of the CT pastes.

KEYWORDS: NATIVE TAPIOCA STARCH/ CATIONIC TAPIOCA STARCH/ ANIONIC TAPIOCA STARCH/ CHITOSAN/ STARCH-CHITOSAN MIXTURES/ SWELLING POWER/ GELATINIZATION/ VISCOELASTICITY

80 pages

การศึกษาคุณสมบัติทางกายภาพและรีโอโลจีของของผสมระหว่างแป้งและไคโตซานระหว่างให้ความร้อน

PHYSICAL AND RHEOLOGICAL PROPERTIES OF STACRH-CHITOSAN DURING PASTING

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บทคัดย่อ

การศึกษาผลกระทบของไคโตซานที่มีขนาดโมเลกุลต่างๆ ต่อคุณสมบัติทางกายภาพและรีโอโลจีของแป้งมันสำปะหลังและแป้งมันสำปะหลังดัดแปร โดยการศึกษาคุณสมบัติในระหว่างการต้มสุกของแป้งผลการทดลองโดยใช้เครื่องวัดความหนืดในขณะต้มสุก (RVA) แสดงให้เห็นว่า ไคโตซานแต่ละชนิดเพิ่มค่า ความหนืดสูงสุด ค่าความหนืดที่ลดลงในช่วงการให้ความร้อน ค่าความหนืดที่เพิ่มขึ้นในช่วงการลดอุณหภูมิ ค่าความหนืดสุดท้าย และอุณหภูมิที่เกิดการเจลาติไนเซชั่นของของผสมระหว่างแป้งมันสำปะหลังหรือแป้งมันสำปะหลังดัดแปรและไคโตซานในระดับที่แตกต่างกัน ยกเว้นค่าความหนืดสูงสุดของของผสมแป้งมันสำปะหลังชนิดประจุบวกและไคโตซานที่มีโมเลกุลขนาดปานกลางและเล็ก และค่าความหนืดที่ลดลงในช่วงการให้ความร้อนของแป้งมันสำปะหลังชนิดประจุบวกและไคโตซานทุกชนิดซึ่งมีค่าลดลง การเติมไคโตซานทำให้ค่าการพองตัวของแป้งมันสำปะหลังและแป้งมันสำปะหลังดัดแปรชนิดประจุลบเพิ่มขึ้นเล็กน้อย แต่ทำให้การพองตัวของแป้งมันสำปะหลังดัดแปรชนิดประจุบวกลดลงอย่างมากเนื่องจากแรงผลักของแป้งที่มีประจุบวกและไคโตซาน จากการศึกษาด้วยการใช้กล้องอิเล็กตรอนแบบส่องกราดพบว่า เม็ดแป้งมันสำปะหลังและแป้งมันสำปะหลังชนิดประจุลบแทรกอยู่ระหว่างแผ่นไคโตซานแต่แป้งมันสำปะหลังชนิดบวกไม่ถูกห่อหุ้มด้วยไคโตซาน โดยผลของการห่อหุ้มมากขึ้นเมื่อเพิ่มขนาดสายโมเลกุลของไคโตซานที่ใช้ ข้อมูลที่ได้จาก DSC แสดงให้เห็นว่า ไคโตซานมีผลเพิ่มอุณหภูมิที่เริ่มเกิดเจลาติไนเซชั่น (T_o) อุณหภูมิสูงสุด (T_p) และอุณหภูมิเมื่อสิ้นสุดการเกิดเจลาติไนเซชั่น (T_c) อย่างมีนัยสำคัญต่อแป้งมันสำหลังและแป้งมันสำปะหลังดัดแปรชนิดมีประจุลบ แต่ไม่มีผลต่อแป้งมันสำปะหลังชนิดมีประจุบวกไคโตซานมีผลลดค่าพลังงานความร้อน (enthalpy) ที่ใช้ในการเจลาติไนเซชั่น (ΔH_1) ของแป้งมันสำปะหลัง แต่ในเพิ่มแป้งมันสำปะหลังชนิดประจุลบและไม่มีผลกระทบต่อแป้งมันสำปะหลังชนิดประจุบวก การวัดรีโอโลยีแบบไดนามิคแสดงให้เห็นว่าไคโตซานทุกชนิดมีผลเพิ่มค่า G' และ G'' ของเจลแป้งมันสำปะหลังและแป้งมันสำปะหลังดัดแปร ไคโตซานมีผลลดค่า tan δ ของแป้งมันสำหลังและแป้งมันสำปะหลังดัดแปรชนิดมีประจุลบ แต่มีแนวโน้มเพิ่มค่า tan δ ในแป้งมันสำปะหลังชนิดประจุบวกลดลง การศึกษาคุณสมบัติการไหลแสดงให้เห็นว่าของผสมทุกชนิดมีพฤติกรรมการไหลแบบ Time-dependent shear-thinning (thixotropic) ค่า hysteresis loop area ของเจลแป้งมันสำปะหลังและแป้งมันสำปะหลังดัดแปรชนิดประจุลบจะลดลงโดยการเติมไคโตซาน ในทางตรงกันข้าม ไคโตซานเพิ่มค่า hysteresis loop area ของเจลแป้งมันสำปะหลังชนิดประจุบวกซึ่งทำให้โครงสร้างของเจลอ่อนแอลง จากการศึกษาสรุปได้ว่า การกระทำระหว่างแรงไอออนิกของกัมกับแป้ง มีบทบาทสำคัญในการเกิดเจลาติไนเซชัน และสมบัติทางรีโอโลยีของแป้งสุก

80 หน้า

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LIST OF ABBREVIATIONS

α	Alpha
&	And
AACC	American Association of Cereal Chemists
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of variance
AT	Anionic tapioca strach
β	Beta
CS	Chitosan
CSH	High molecular weight of chitosan
CSL	Low molecular weight of chitosan
CSM	Medium molecular weight of chitosan
CT	Cationic tapioca starch
DP	Degree of polymerization
DP3	Cellotriosyl residue
DP4	Cellotetraosyl residue
DS	Degree of substitution
DSC	Differential scanning calorimeter
Ed. (Eds.)	Editor, editors, edited by
et al.	Et alii (Latin), and others
g	Gram
G'	Storage modulus, Pa
G''	Loss modulus, Pa
GT	Gelatinization temperature
h	Hour
Hz	Hertz
ΔH	Enthalpy, J/g
i.e.	Id est (Latin), that is

LIST OF ABBREVATIONS (cont.)

J	Joule
K	Consistency coefficient, Pa s^n
mg	Milligram
ml	Millimeter
min	Minute
M_W	Molecular weight
N	Normality
N	Newton
nm	Nanometer
n	Flow behavior index, dimensionless
NMR	Nuclear Magnetic Resonance
p.(pp.)	Page (pages)
Pa	Pascal
rad	Radian
RVA	Rapid Visco Analyzer
RVU	Rapid Viscosity Units
R^2	Coefficient of determination
s	Second
SD	Standard deviation
SEM	Scaning electron microscope
SPSS	Statistical Package for the Social Science
STP	Sodium tripolyphosphate
$\tan\delta$	Loss tangent, dimensionless
$T_c - T_o$	Phase transition temperature range, °C
T_o, T_p, T_c	Onset, peak, conclusion temperature, °C
UV	Ultraviolet

LIST OF ABBREVATIONS (cont.)

w/v	Weight by volume
w/w	Weight by weight
/	Per
%	Percent
°C	Degree Celsius
$\dot{\gamma}$	Shear rate, 1/s
σ	Shear stress, Pa
σ_0	Yield stress, Pa
ω	Angular frequency, rad
θ	Zeta

CHAPTER I
INTRODUCTION

Tapioca starch is the major energy reserve in plants and serves as the most important carbohydrate source. It is used innumerous industrial and food application, including as a gelling and thickening agent and paper making. However, its price in the world market is low when compared to other starch. Native starch component in a product may cause several problems. This is usually desirable and can be control by chemical modifications to obtain the better properties demanded. However, the expensive and chemical reagents will be disadvantages. An alternative to blending of native starch with polysaccharide hydrocolloids (gums) can alter the gelatinization and rheological characteristics of starches. It is well known that addition of hydrocolloids to starch suspension causes a synergistic increase in viscosity (Alloncle et.al., 1989; Lui et.al., 2003). These mixtures are increasingly important ingredients in the modern health-promote food industry.

Chitosan is a linear copolymer polysaccharide consisting of D-glucosamine and *N*-acetyl-D-glucosamine units, obtained by deacetylation of chitin. Chitin is usually obtained from crustaceans (crab, shrimp and crayfish), especially because a large amount of their exoskeleton is available as a by-product of seafood processing. Chitosan is biocompatible, nonantigenic, bioactive, nontoxic, biodegradable, and biofunctional compound (Hirano et.al., 1990; Li et.al., 1992). Thus, chitosan has been approved as a food and non-food additive. Chitosan having amino groups performs as a cationic polymer. Moreover, it is generally accepted that each hydrocolloid affects in a different way in the pasting properties of starch (Bahnassey & Breene, 1994; Christianson et. al., 1981; Rojas, Rosell, & Barber, 1999). This can be ascribed to many factors mainly the molecular structure of hydrocolloids (Abdulmola et.al., 1996; Murphy, 1995; Sudhakar et.al., 1996; Viturawong et.al., 2008) and/or ionic charges of both starches and hydrocolloids. The information of the effect of ionic charges of starches and hydrocolloids on of pasting and rheological characteristics of the starch- hydrocolloid mixtures is rare in literature.

Wei et.al., (2001); Lee et.al., (2002) reported that negatively charge xanthan gum reduced the paste viscosity of sweet potato starch (negatively charge) significantly, possibly through strong network formation with starch, whereas guar gum and alginate (nonionic charge) increased the viscosity. This result is consistent with Shi & BeMiller (2002). They suggested that the greatly decrease peak viscosity when negative charge gums were added to potato starch was due to the repulsion between phosphate groups on potato starch and negative charge on the gum molecules. Chaisawang & Suphantharika (2006) reported that the ionic interaction between xanthan gum and cationic starch played an important role in the gelatinization characteristics of mixtures and also rheological properties of the paste. However, there are a few reports on the interaction between gums and modified starch (Abdulmola et.al., 1996; Shi & BeMiller, 2002; Tecante & Doublier, 1999) and morphology of gelatinization of starch gum mixtures by using scanning electron microscope (SEM) (Jing-ming & Sen-lin, 1990; Mandala et.al., 2002; Chaisawang & Suphantharika, 2005). The aim of this study was to investigate the effects of chitosan on the physical and rheological properties of native tapioca starch (non-ionic starch), anionic tapioca starch (negatively charge), and cationic tapioca starch (positively charge) by using a Rapid Visco-Analyzer (RVA), Scanning Electron Microscope (SEM), Rheometer and Differential Scanning Calorimetry.

CHAPTER II
LITERATURE REVIEW

2.1 Starch

Starch is one of the naturally occurring biomaterials that are abundant and is a major food reserve providing a bulk nutrient and often at a low cost for commercial application. Commercial starches are obtained from seeds (corn, wheat, sorghum, rice), the tubes or root (cassava, potato, arrowroot, yam), and the pith of the sago palm. The character of the starch varied with plant source from which is derived. In addition, starches and modified starches can be used to affect the physical properties of many foods such as a gelling agent, thickener, adhesion agent, moisture-retention agent, stabilizer, and anti-staling agent.

2.1.1 Molecular structure and properties of starch

Starch molecules are radically in the granules which are arranged in semi-crystallize characteristic. Starch granules consist of amorphous and crystalline regions (Figure 2.1). The starches are composed primarily of D-glucopyranose polymers linked together by α-1, 4 and α-1, 6 glycosidic bond. Two structurally distinct molecules in starch are amylose and amylopectin (Figure 2.2).The ratio of these two polymers within a given type of starch is very important point to consider with respect to application.

Amylose is a linear polymer molecule composed almost entirely of α-1, 4 linked D-glucopyranose; many amylose molecules have a few α-1, 6 linked D-glucopyranose branches, perhaps 0.3-0.5% (Whistler & BeMeiller, 1997). Amylose chain gives the molecules a right-handed spiral or helical shape and molecular weight of amylose is between 10^5 and 10^6 Da (Mua & Jackson, 1997). The interior of the helix contains hydrogen atoms and is there form hydrophobic which can form complex with free fatty acid, fatty acid components, some alcohol and iodine (Fennema, 1985)

Another well-known attribute of amylose is its ability to form a gel after the starch granule has been cooked, i.e. gelatinized and pasted.

Amylopectin is a highly branched glucopyranose units containing both α-1, 4 linear and α-1, 6 branched linkages. Average molecular weights and molecular weight ranges of amylopectins vary with the botanical source (Whistler & BeMiller, 1999). The amylopectin chains can be classified into three different types of sub-chains termed A, B and C, according to their length and branching points.

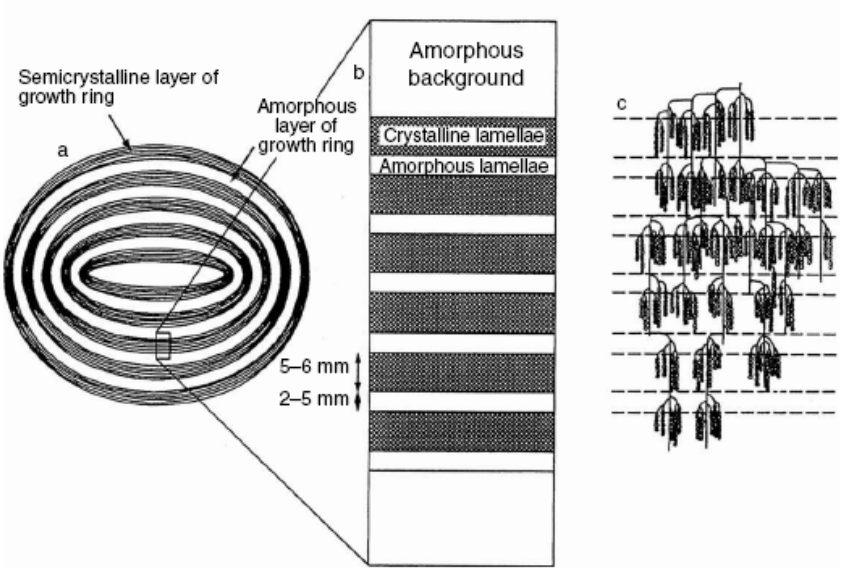

Figure 2.1 Schematic diagram of starch granule structure. (a) A single granule, comprising concentric rings of alternating amorphous and semi-crystalline composition. (b) Expanded view of the internal structure. The semi-crystalline growth ring contains stacks of amorphous and crystalline lamellae. (c) The currently accepted cluster structure for amylopectin within the semi-crystalline growth ring. A-chain sections of amylopectin form double helices, which are regularly packed into crystalline lamellae. B-chains of amylopectin provide intercluster connections. Branching points for both A and B chains are predominantly located within the amorphous lameilae (Jenkins & Donald, 1995).

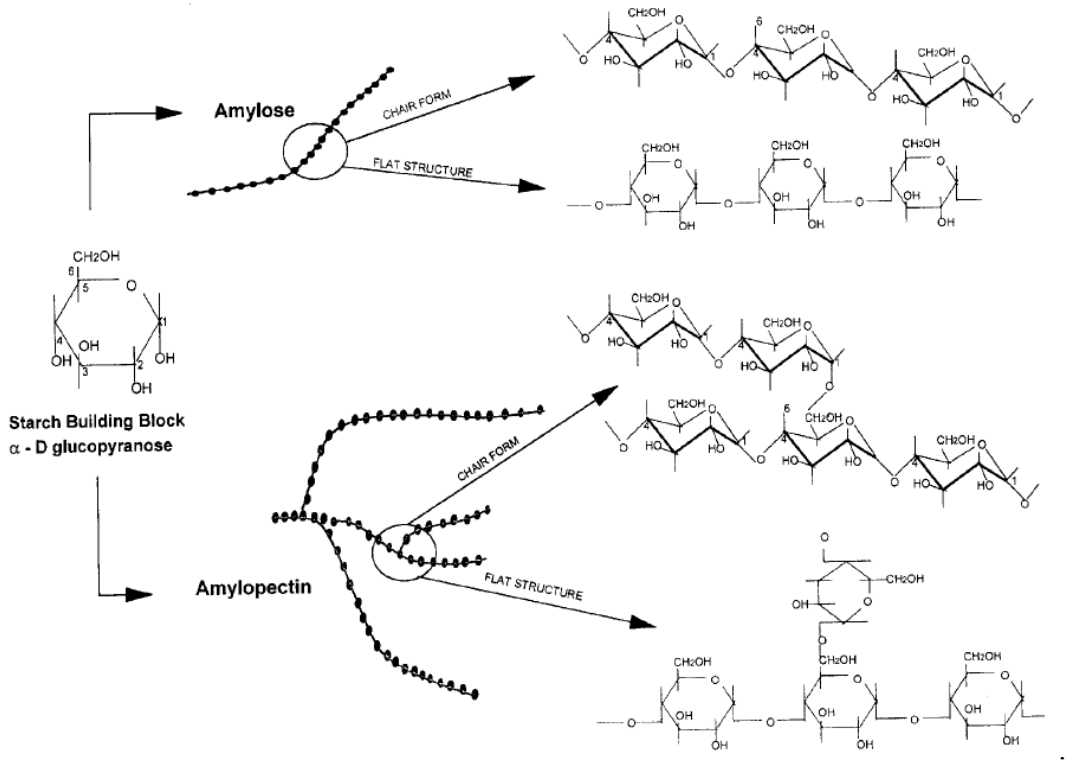

Figure 2.2 Linear and branched starch polymers (Taggart, 2004).

2.1.2 Tapioca starch

Tapioca (*Manihot esculent Crantz*) is a perennial plant widely grown in many tropical countries, including Thailand as one of the most important commercial crops. This starch is used for the production of foods, drugs, adhesives, alcohol and animal feed. The most important characteristics of tapioca starch are odorless, paste clarity, and stickiness. As a food ingredient, tapioca starch has long been used to thicken soups and to make other in Thai dishes. In addition to its low cost and plentiful supply, the properties of tapioca starch make it a choice of ingradient in many food industries.

2.1.2.1. Morphology

Tapioca starch granules are mostly round with a flat surface on one side containing a conical pit, which extends to a well-defined eccentric hilum (Moorthy, 2002). The native surface of most granules appears smooth without observable pores as shown in Figure 2.3 (Whistler, Bemiller & Paschall, 1984). Granular sizes of tapioca starch are middle with diameter ranges of 5-40 μm.

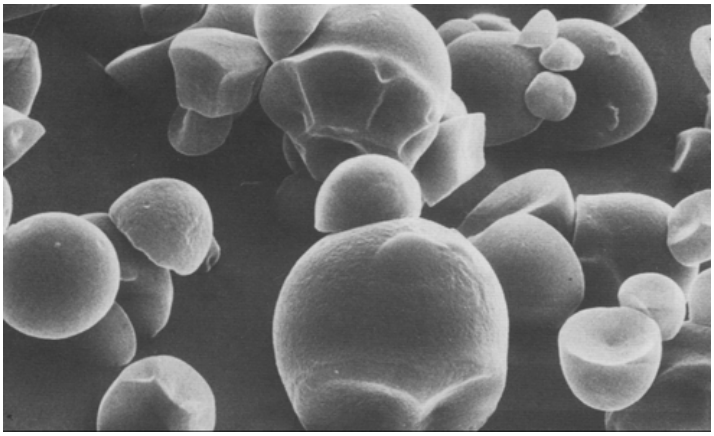

Figure 2.3. Tapioca starch granules with magnification 1500x (Whistler, Bemiller & Paschall, 1984).

2.1.2.2. Amylose and amylopectin

The amylose and amylopectin content and structure affect the architecture of the starch granules, gelatinization and pasting profiles, and textural attributes. The approximate amylose and amylopectin content of several starches are shown in Table 2.1. Tapioca starch has very tiny lipid and phosphorus content. The amylose content in the starch is in the range of 13.6-23.8% similar to most other starches. The amylose content shows variation among varieties, but the age of the crop and environmental factors do not affect the amylose content to any large extent (Moorthy, 2002). The soluble amylose content forms approximately 40% of the total amylose. Gel formation is primarily the results of the re-association of solubilized starch polymers after cooking and can occur quite rapidly with the linear polymer amylose. Tapioca amylopectin is a highly branched molecule. Because of the highly branched nature of amylopectin, its properties differ from those of amylose. For example, given the size of the molecule and its “tumbleweed-like” structure, retrogradation is slowed and gel formation can either be delayed or prevented.

Table 2.1 Approximate amylose and amylopectin content of common food starches.

Starch source	Amylose content (%)	Amylopectin content (%)
Waxy rice	0	>99
High amylose corn	70	30
Corn	28	72
Tapioca	17	83
Waxy sorghum	0	>99
Wheat	25	75
Sweet potato	18	82
Arrowroot	21	79
Sago	26	74
Potato	20	80
Rice	19	81

2.1.2.3. Swelling power and solubility

When starch is heated in excess water, the crystalline structure is disrupted due to the breakage of hydrogen bonds, and water molecules become linked by hydrogen bonding to the exposed hydroxyl group of amylose and amylopectin. This causes an increase in granule swelling and solubility. Swelling power and solubility provide evidence of the magnitude of interaction between starch chains within the amorphous and crystalline domains. The extent of this interaction is thought to be influenced by a sample's amylose content, amylose and amylopectin structure, degree of granulation and other factors. For example, amylose-lipid complexes have been shown to restrict swelling and solubilization. The large degree of polymerization (DP) molecules might have a lower tendency to leach out of the granule during heating or may trap other molecules. The swelling power and solubility of starch provide evidence of non-covalent bonding between starch molecules. Factors like amylose-amylopectin ratio, chain length and molecular weight distribution, degree/length of branching and conformation decide the swelling and solubility. Tapioca starch has medium swelling power compared to potato and cereal

starches - a property in conformity with itsobserved viscosity. The reported values for the swelling power of cassava starch vary considerably from 42-71 (Rickard, Asaoka & Blanshard, 1991). Soni et al. (1985) have a reported a two stage swelling for cassava starch and attributed it to the two types of forces that require different energy input to cause relaxation of the starch molecules.

2.1.2.4. Gelatinization

Starch granules are insoluble in cold water, but can imbibe water reversibly; that is, they can swell slightly, and then return to their original size on drying. Starch gelatinization is the collapse (disruption) of molecular orders within the starch granule, when starch is heated in the presence of water, manifested by irreversible changes in properties such as granular swelling, native crystalline melting, loss of birefringence, and starch solubilization. Leaching of amylose occurs during gelatinization, but some leaching of amylose can also occur prior to gelatinization (Shi & BeMiller, 2002) (Figure 2.4). The point of initial gelatinization and the range over which it occurs is governed by starch concentration, method of observation, granular type, and heterogeneities within the granule population under observation (Atwell, Hood, Lineback, Varriano-Marston, & Zobel, 1988). For gelatinization to occur the regions of amorphous starch must first melt or undergo glass transition (Slade & Levine, 1988). The temperature at which starch begins to undergo these changes is referred to as the gelatinization temperature. In general, the gelatinization temperature of tuber and root starches such as potato and tapioca is slightly lower than that of cereal starches such as corn and wheat. One of the most common techniques used in studying starch gelatinization event is differential scanning calorimetry (DSC) which reveals gelatinization temperature (GT). DSC measures the range in transition temperature required for gelatinization to occur. Thermal properties typically reported using DSC include gelatinization onset (T_o), peak (T_p), conclusion (T_c) and enthalpy (ΔH). DSC is particularly well suited to investigate the phase transition of starch-water systems because it allows: (1) study of starch gelatinization over a wide range of starch-water ratio; (2) determination of gelatinization temperature above 100 °C; and (3) estimation of gelatinization enthalpies (Biliaderis, Maurice, & Vose, 1997). In this method the sample is submitted to linear heating and the rate of heat flow in sample, proportional to the instantaneous specific heat, is measured continuously.

In other words the temperature of sample is kept at the same value with reference sample and the electrical input necessary for the maintenance of isothermal conditions is measured. The gelatinization enthalpy of tapioca starch was found in many literature ranged from 6.8 to 16.6 J/g (Asaoka, Blanshard & Rickard, 1992; Moorthy, 2002; Atichokudomchai, Varavinit & Chinachoti, 2002).

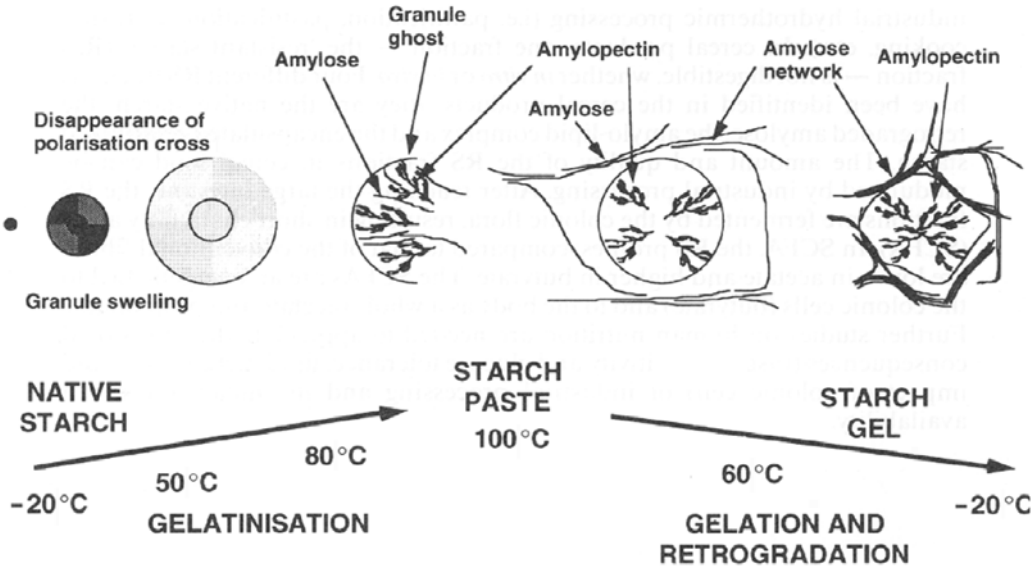

Figure 2.4 Gelatinization and retrogradation on physical starch characteristics. (Bornet, 1993)

2.1.2.5. Pasting

Pasting is the phenomenon following gelatinization in the dissolution of starch. It involves granular swelling, exudation of molecular components from the granule, and eventually, total disruption of the granules (Atwell et al, 1988). When the starch granule is heated up to the GT in excess water, heat transfer and moisture transfer phenomena occur. The granule swells to several times its initial size as a result of the loss of the crystalline order and the absorption of water inside the granular structure. The pasting viscosity during swelling and gelatinization can be recorded using a Brabender Visco Amylograph, Rapid Visco Analyzer (RVA), or other viscometers, which record the viscosity continuously as the temperature is increased, held constant for a time, and then decreased (Figure 2.5). At the initial step, the viscosity increases rapidly with the increase of temperature as the granule swells.

The peak viscosity is reached when granules swelling have been balanced with the granules broken by stirring. With continued stirring, more granules rupture and fragment, causing a further decrease in viscosity. On cooling, some starch molecules partially reassociate to form a precipitate or gel (retrogradation), in which amylose molecules aggregate into a network, embedding remnants of starch granules.

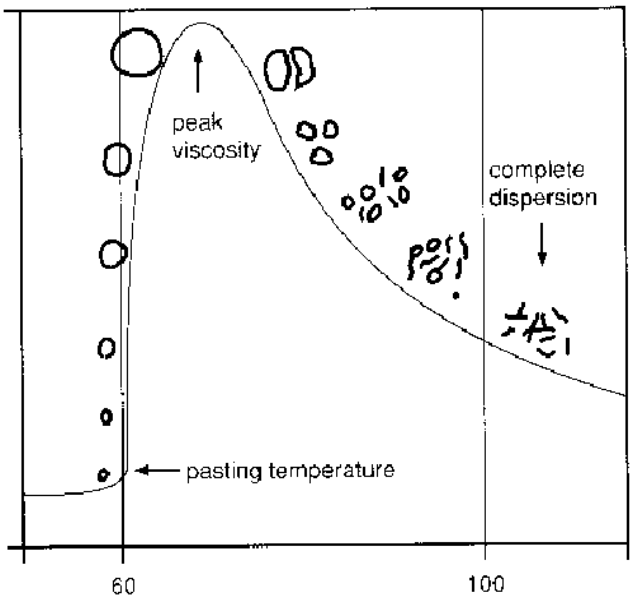

Figure 2.5 Swelling, disruption and dispersion of a starch granule during gelatinization (Sanders, 1996)

2.1.2.6. Retrogradation

Starch after heating starch paste produces a firm viscoelastic gel, which was the consequent of the molecular interaction (mainly hydrogen bonding between starch chain). Atwell, et al. (1988) defined starch retrogradation as a process which the starch chains begin to reassociate in an ordered structure. Two or more starch chains initially form a simple junction point, which then may develop into more extensively ordered regions and ultimately, under favorable conditions, to a crystalline order. This process results in viscosity increase, gel firming, syneresis (starch gel loss its ability to remain hydrated, thus releasing of water) and textural staling of predominantly starch-containing systems. Retrogradation of cooked starch involved in crystallization or recrystallization process both of two constituent polymers, amylose and amylopectin. Amylose retrogadration is more rapid due to structurally linear molecule allowing polymers to orient in parallel fashion that permit hydrogen bonding

between polymers and lowering the affinity for water. Thus, amylose is responsible for short-term (less than one day) changes during retrogradation (Zhou, Robards, Helliwell, & Blanchard, 2002). Whereas, amylopectin crystallization results from association of the outermost short branches (Ring, Colonna, Anson, Kalicheversky, Miles, Morris & Oxfird, 1987) and tends to occur more slowly due to size and branched nature of amylopectin, which interfere with the mobility of the molecules (Wurzberge, 1986). The extent of retrogradation may be affected by starch source (Orford, Ring, Carroll, Miles & Morris, 1987), concentration (Zeleznak & Hoseney 1987, Orford, Ring, Carroll, Miles & Morris, 1987), storage temperature (Slade & Levine, 1986), time and other intergradient.

Starch gels are metastable and non equilibrium systems and therefore undergo structural changes during storage (Ferrero, Martino & Zaritzky, 1994). Though intermolecular association, the hydroxyl groups of starch chains become bound and starch to losing their ability to remain hydrated and thus releasing water. Hence, the amount of retrogradation of gelatinized starch is of great importance to the food industry because it can influence the texture and acceptability of that food (Miles, Morris, Orford & Ring, 1985).

The retrogradation properties can be measured by DSC, rheological properties, starch gel hardness, and NMR. Methods to study retrogradation of starch have been reviewed by Karim, Norziah, & Seow (2000). However, the retrogradation kinetics of starch has received wide attention though the underlying mechanism of retrogradation has not been concluded. Generally, the amylopectin systems showed two stages of retrogradation behavior during early ($\leq$ 7 days) and late ($\geq$ 7days) storage. Correlation analysis suggested that the kinetics of early stage retrogradation were more correlated than the late stage retrogradation with the number-average molecular weight and chain lengths of the amylopectin molecules.

2.1.3 Starch modifications

However native starches lack the versatility to function adequately in the entire range of products currently available in the marketplace. The diversity of the modern industry and the enormous variety of products require that

starch be able to tolerate a wide range of processing techniques as well as various distribution, storage, and final preparation condition. These demands are met by modifying native starches by chemical, physical and biotechnological modification. The main types of modified starch are shown in Table 2.2.

Table 2.2 Modification of starch (Wurzburg, 1986)

Type of modification	Treatment	Main objectives
Chemical modification		
(a) Starch derivatives		
- Substitution starch	Esterification, Etherification	Improve viscosity, stability
- Cross-linked starch	Crosslink in suspension	Modification of cooking characteristic
(b) Acid-modified starch	Acid hydrolysis (suspension)	Lower viscosity, high gel tendency
(c) Dextrin	Dry heat treatment with acid	Lower viscosity, stability
(d) Oxidized starch	Oxidation (suspension/paste)	Lower viscosity, stability
(e) Starch sugars	Acid and/or enzymes	Sweet saccharide
Physical modification		
(a) Pre-galatinized starch	Drum-drying Extrusion	Cold water dispersibility
(b) Heat-treated starch	Heat-moisture treatment	Viscosity stability
Biotechnology modification		
(a) Waxy starch	Genetic engineering	Lower/no amylose
(b) High amylose starch	Genetic engineering	higher amylose

2.1.3.1 Cationic starch

Cationic starches are starch ester derivative of nitrogen such as amino, imino, ammonium, sulfonium, or phosphonium groups, all which can carry apositive charge. Cationic starches are prepared by reacting hydroxyl groups of starch under highly alkaline conditions. Specific reagents have been

approved by the Food and Drug Administration for making cationic starches used in paper or paperboard coming in contact with foods. There are (4-chlorobutene-2)-trimethylammonium chloride, 2-diethylaminoethyl chloride, and 2, 3-(epoxylpropyl)-trimethylammonium chloride (Solarek, 1986).

1) Preparation of cationic starch

Cationic starches can be prepared by the reaction of native starches with various reagents possessing positively charged groups. Cationic starches containing tertiary amino or quaternary ammonium groups are the most important commercial derivative. 2, 3-epoxypropyl-trimethylammonium chloride is normally used for adding quaternary ammonium groups to the starch molecule. The reagent stored in water in the chlorohydrin (3-chloro-2-hydroxy propyl-trimethyl-ammonium chloride) and rapidly converted to exoxide form by adding sodium hydroxide at pH 11.0 to 12.0 as shown in Figure 2.6. (Auzely-Velty & Rinaudo, 2003)

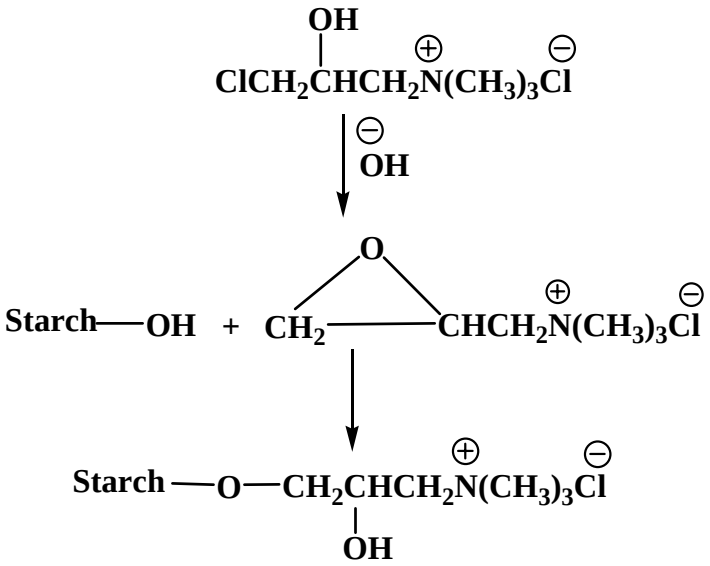

Figure 2.6 Reaction of 3-chloro-2-hydroxy propyltrimethylammonium chloride in alkaline condition (Solarek, 1986).

2) Functional properties

Cationic starches show a decrease in peak intensity in X-ray diffraction pattern with increase in DS. Gelatinization temperature decrease with increase DS and starches become cold water swelling at DS

0.07. The effect of cationization on the pasting properties of potato and tapioca starches is markedly reduced the pasting temperature. Cationic starches exhibit higher solubility and dispersibility with improved clarity and stability (Solarek, 1986).

3) Utilization of cationic starch

Dispersions of cationic starches display improved clarity and stability (Paschall, 1967). Due to their ionic attraction to anionic, cellulosic fibers, cationic starches have become important wet-end additives in paper making to improve retention and drainage rate of pulp and strength of the finished sheets. They are also used as coating binders in paper making, wrap sizing agents in textile manufacture, binders in laundry detergents. Moreover, cationic starches are excellent flocculants for suspensions of inorganic matter having a negative charge. The cationic starches also used for pharmaceutical compositions (Solarek, 1986).

2.1.3.2 Starch phosphates

Starch phosphates are starch ester derivatives of phosphoric acid such as mono-, di-, and tri-starch phosphate ester. Native starches contain small amounts of phosphorus whereas potato starch contains 0.07 to 0.09 % phosphorus covalently bound to the amylopectin fraction.

1) Preparation of starch phosphate monoester

Two steps are involved in the preparation of starch phosphate. Starch is first spray or soaked with an aqueous solution of a mixture of sodium tripolyphosphate (STP) and sodium sulphate together. In the second step, the impregnated starch is roasted at 130°C, washed with water and dried. The pH of the STP-starch mixture drops about less than 7.0 to 9.0 during reaction. However, the pyrophosphate can be present from hydrolysis of the STP and then can also react with starch (Solarek, 1986) which is shown in Figure 2.7. Depending on the phosphate concentration, varying amount of phosphate salt are retained in the starch filter cake (Table 2.3).

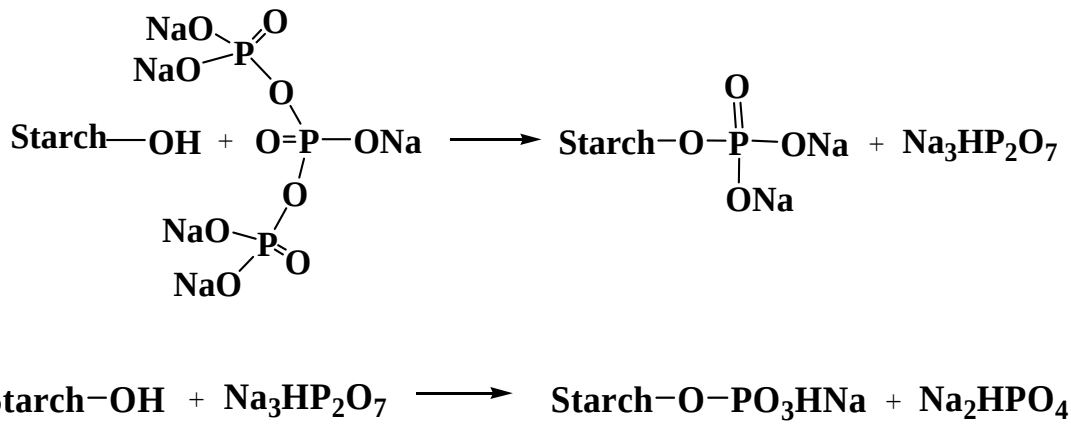

Figure 2.7 Reaction of sodium trimetaphosphate in alkaline condition (Solarek, 1986).

Table 2.3 Slurry impregnation[a] of starch with phosphate salts (Solarek, 1986).

Starch (g)	Salt (type)	Salt in slurry (g)	Slurry water (ml)	Salt retained (g (%))
180	STP[b]	15.5	215	9 (58.1)
180	STP	30.0	400	6 (20.0)
180	STP	15.0	400	3 (20.0)
180	STP	7.5	400	1.5 (20.0)
180	SHMP[c]	12.8	215	7 (57.4)
162	NaH_2PO_4	27.6	240	12.1 (43.8)

[a] The phosphate salt was dissolved in the water followed by suspension of the starch, mixing, and filtration.

[b] Sodium tripolyphosphate

[c] Sodium hexametaphosphate

2) Functional properties

Starch phosphates are strongly anionic polymers, which yield higher viscosity, more clear and stable dispersions with long cohesive texture and resistance to retrogradation. The viscosities are reduced by the presence of salts. The gelatinization temperature decreases with the increasing degree of substitution, and the monoester becomes cold water swelling when DS increased to 0.07. Dispersion of starch phosphate has superior freeze-thaw stability than other modified starch (Kerr & Cleveland, 1962; Albrecht, Nelson & Steinberg, 1960). Starch phosphates are good emulsification agents because of the ionic properties (Rutenberg & Solarek, 1984)

3) Utilization of phosphorylated starch

Starch phosphates are used in food as emulsion stabilizers for vegetable oil in water and thickening agents with good freeze-thaw stability. A combination of starch phosphate, guar gum, and propylene glycol has been used as an emulsion stabilizer for vinegar and vegetable oil in water (Solarek, 1986). Cold water swelling starch is dry mixed with sugar and flavoring agent, which is added to cold milk to form pudding with smooth, soft, even texture, and superior eating quality (Neukom, 1958). Starch phosphate are useful wet-end additive which can be found in various kinds of applications such as in a paper making industry, the initial adhesive strength for weed pieces, in personal care and pharmaceutical products, a hydrophobic powder (filler). It also used to substitute with potato starch noodle (Muhammad, Kusnandar, Hashim & Rahman, 1999).

2.2. Hydrocolloids (gums)

Hydrocolloids, or gums, are substances consisting of hydrophilic long-chain, high molecular weight molecules which have linear or branched molecules, as biopolymers. This term also refers to a range of polysaccharides and proteins (gelatin) because its functionality is very similar to that of the polysaccharide-based gums.

Moreover, gums are used in low concentration which can improve achieve cost reductions (Ward & Andon, 2002). The diversity of structural features of polysaccharides, which originates from differences in the monosaccharide composition, linkage types and patterns, chain shapes, and the degree of polymerization, dictates their physical properties including solubility, flow behavior, gelling potential, and/or surface and interfacial properties. Hydrocolloids, which are commercially available for use in food and non-food industries as stabilizers, thickening and gelling agents, crystallization inhibitors, and encapsulating agents, etc. Most hydrocolloids occur naturally, but there are also several natural hydrocolloids that have been chemically modified. The commercially important hydrocolloids and their origin are given in Table 2.4.

When hydrocolloids are in solution, one can visualize a cylinder of organized water surrounding the molecule. The water molecules are oriented with respect to the hydroxyl groups found on the individual sugar units of the hydrocolloid molecule as shown in Figure 2.5. The main effects of hydrocolloids result from their ability to organize water and/or form networks. Visualize a hydrocolloid molecule as looking like a long, flexible piece of yarn. Now visualize a cylinder of water surrounding yarn, to some arbitrary distance, such that this layer of organized water of hydration actually moves around with the gum molecule. This water is organized in the sense of being associated with the long, thin gum molecule, particularly at hydroxyl group along the polysaccharide chain and at any of the anionic groups that present on some gums, and moves around with gum molecule to some extent. Increased associations generally lead to increases in volume and swelling. The chain length, or degree of polymerization (DP), influences a gum's viscosity and hydration rate. Long molecules tend to produce higher viscosities and take longer to hydrate than short ones. A highly branched molecule takes up less space than straight one with the same molecular weight and therefore provides less viscosity. Longer hydrocolloids sweep out a much greater volume as they randomly tumble in solution, leading to more collisions with neighbors and resulting in an increase in viscosity.

Hydrocolloid can be roughly divided into categories on the basis of their functionality in food systems: thickening agents and gelling agents (Figure 2.9) (Hoefler, 2004). The thickening agents provide viscosity in food system, but they are

not capable of suspending particulates or the rising of oil droplets, but they cannot stop the separation from occurring. Gelling agents form links between their molecules, building a three dimensional lattice in a food system. The result is that particulates or oil droplets become permanently trapped in the lattice and do not separate out. Most gelling agent can be used at low concentrations so that the food system pours or flow without separation of oil droplets or ingredients such as strawberries or spices.

Table 2.4 Source of commercially important hydrocolloids (Williams & Phillip, 2000)

Botanical	
	trees
	cellulose
	tree gum exudates
	gum arabic, gum karaya, gum ghatti, gum tragacanth
	plants
	starch, pectin, cellulose
	seeds
	guar gum, locust bean gum, tara gum, tamarind gum
	tubers
	konjac mannan
Algal	
	red seaweeds
	agar, carrageenan
	brown seaweeds
	alginate
Microbial	
	xanthan gum, curdlan, dextran, gellan gum, cellulose
Animal	
	Gelatin, caseinate, whey protein, chitosan

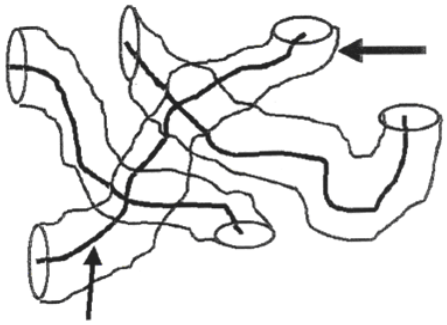

Figure 2.8 Hydrocolloid molecules surrounded by organized water (Hoefler, 2004).

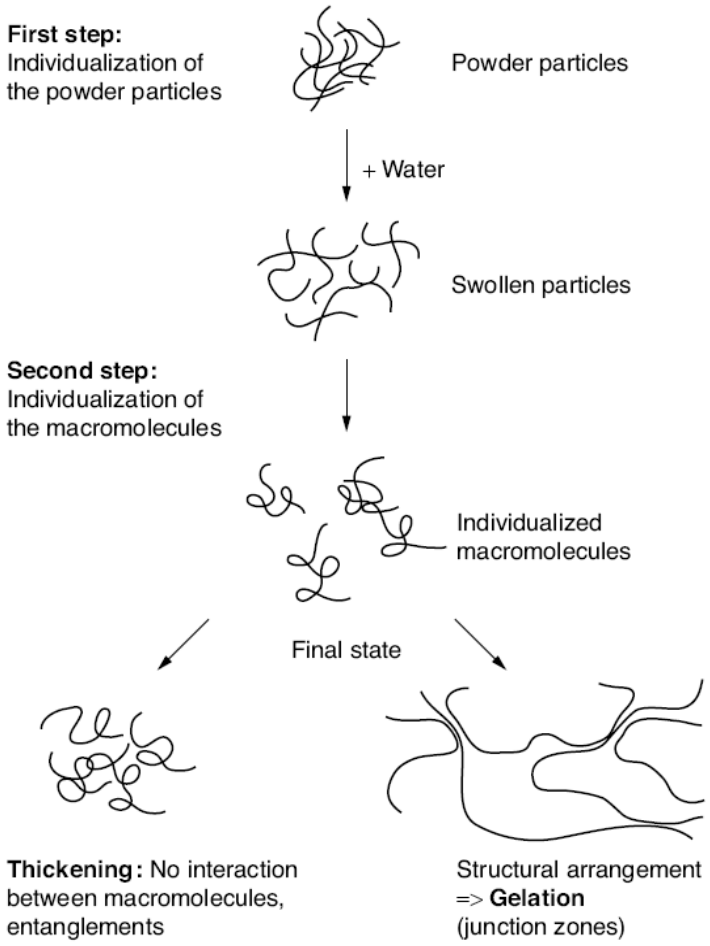

Figure 2.9 Schematic description of the different steps of the solubilization process of polysaccharides (Doublier & Cuvelier, 2006)

2.3. Chitosan

Chitosan is a non-branched linear polymer, obtained as a result of partial deacetylation of chitin. After cellulose, chitin is the second most abundant natural biopolymer (No & Meyers, 1989). Chitin is found in a wide range of natural sources such as crustaceans, fungi, insects and some algae. Chitin is usually obtained from crustaceans (crab, shrimp and crayfish), especially because a large amount of their exoskeleton is available as a by-product of seafood processing (Methacanon, Prasitsilp, Pothsree & Pattaraarchachai, 2003). Different source of chitin are shown in Table 2.5.

Table 2.5 Different sources of chitin

Arthropods	Insect	Microrganisms
Annelida	Scorpion	Green algae
Molluska	Spider	Yeast (β-type)
Coelantera	Brachipods	Fungi (cell wall)[a]
Lobster[a]	Cockroach	Penicillium (spore)
Shrimp[a]	Beetle	Cyano-bacteria
Crustecean	Ant	Chytridiacee
Prawn[a]		Blastocladiaceae
Krill		Ascomycota
Crab[a]		

[a] Main raw material

2.3.1. Structure and General Application

Chitosan is a linear copolymer polysaccharide consisting of β (1→4)-linked 2-amino-2-deoxy-D-glucose (D-glucosamine) and β (1→4)-linked 2-acetamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine) units, obtained as a result of deacetylation of chitin by treatment with hot strong alkali which contain unique characteristics including biodegradability, biocompatibility, and bioactivity, and it is therefore interesting not only as abundant resource but also as a novel type of functional material (Muzzarelli, 1977). For the preparation of chitosan, chitin is deacetylated under basic conditions to yield a co-polymeric chain with variable glucosamine to *N*–acetylglucosamine ratios. Most commonly used chitosans have 70 to 95% of their repeat units deacetylated. Chitosan has reactive hydroxyl and amino groups which are responsible for chemical modifications, achieved to obtaining suitable materials for different applications (Fig. 2.10). Amino groups give the chitosan cationic characteristic. At low pH, amino groups are protonated and positively charged.

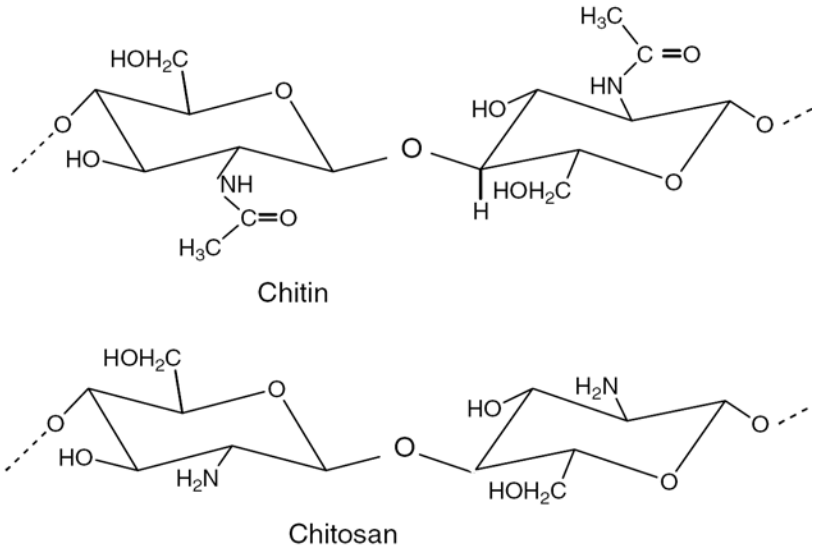

Figure 2.10 Chemical structures of chitin and chitosan

Over the last several years, chitinous polymers, especially chitosan, have received increased attention as one of the promising renewable polymeric materials for their extensive applications in the pharmaceutical and biomedical industries as bandage, in controlled release preparation, for enzyme immobilization, in chemical plants for wastewater treatment, and in food industries in food formulations as binding, gelling, thickening and stabilizing agent (Knorr, 1984). In general, three types of chitin can be observed by difference molecular conformation: α-, β-, and γ-chitins. The most abundant and easily α-chitin, where the molecules are aligned in an anti-parallel fashion as disclosed by X-ray diffraction studies (Fig. 2.11) (Blackwell, Gardner, Kolpak, Minke, & Claffey, 1980). This molecules arrangement is favorable for the formation of strong intermolecular hydrogen bonding, and α-chitin is the most stable form of the three crystalline variations which is found in mollusk. In β-chitin, the molecules are packed in a pararelle arrangement, leading to weaker intermolecular force which is found mostly in the crab and shrimp shell (Mazeau, Winter & Chanzy, 1994). β-chitin is thus assumed to be less stable than α-chitin while γ-chitin has the disorder pattern and hardly to find in the nature. It is considered to be a mixture (or intermediate form) of the α- and β-forms and has both pararelle and anti-pararelle arrangements. Chitosan is

also crystalline and shows polymorphism depending on its physical state. The structures for various forms including an anhydrous form, a hydrated form, and various salts were recently refined by X-ray diffraction analyses (Yui, Imada, Okuyama, Obata, Suzuki & Ogawa, 1994; Okuyama, Noguchi Miyazawa, Yui & Ogawa, 1997).

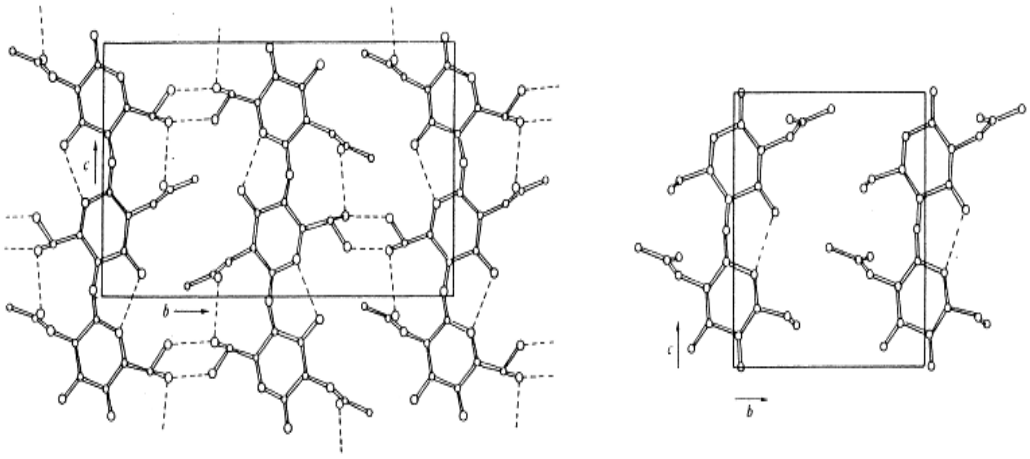

Figure 2.11 Arrangements of chitin molecules in α-chitin (left) and β-chitin (right) (Blackwell & Kolpak, 1980)

2.3.2 Production of Chitin/Chitosan

Both chitin and chitosan are processed industrially in large scale from crustacean outer shell waste especially crab, pen squid and shrimp shells. Isolation of chitin from crustacean shell waste consists of three basic steps (1) protein removal-deproteinization, (2) calcium carbonate (and calcium phosphate) removal-demineralisation, and (3) removal of pigments - decoloration. The former two steps also can be conducted in the reverse order, i.e., demineralization, followed by deproteinization. However, if protein recovery is an objective, its extraction before demineralization is preferred so as to maximize protein yield and its quality and the recovered protein can be used as a high-grade additive to livestock starter feeds, thus contributing to decrease the overall manufacturing cost of chitin (Johnson & Peniston, 1982). Deacetylation of chitin to chitosan is performed by treatment with hot concentrated NaOH solution.

2.3.3 Physical and Chemical Properties of Chitosan

For the preparation of chitosan, chitin is deacetylated under basic conditions to yield a copolymeric chain with variable glucosamine to *N*-acetylglucosamine ratios.

2.3.3.1 Degree of deacetylation

Degree of deacetylation is the factor which is used to identify the difference between chitin and chitosan. Chitin and chitosan is less easily defined in term of its exactly chemical composition. Since chitin and chitosan are copolymer of N-acetyl-D-glucosamine and D-glucosamine, degree of deacetylation is the ratio between these two monomers. The more D-glucosamine groups, the higher of degree of deacetylation can be observed in which representing more chitosan properties. In general, the degree of deacetylation is more than 70% for chitosan. Chitosan is the term used for the considerably deacetylated chitin that is soluble in dilute acid such as formic, acetic, oxalic, lactic, phosphoric, sulfuric, and citric acid but insoluble in either water or organic solvents. Therefore, chitin samples have different degrees of deacetylation, 5-15%, depending on their origin and mode of isolation. Many methods have been reported for assessing the degree of deacetylation including elemental/CHN analysis, hydrolysis of acetamide groups, and titration of free amino groups, dye adsorption, UV, IR, CD-spectroscopy, NMR, enzymatic degradation, and pyrolysis (Kurita, 2001).

2.3.3.2 Molecular weight

The molecular weights of chitin and chitosan are also important factors for characterization, but poor solubility and structural ambiguities in connection with the content and distribution of acetyl groups are major obstacles to quantitatively determining molecular weight values. The molecular weight of native chitin is usually larger than $1x10^6$ while that of the commercial chitosan product is 2×10^5 to 1.2×10^6. Several methods are used to estimate the molecular weight of chitin and chitosan. It is difficult to determine the molecular weight of native chitin, since chitin is usually present in closely associated from with proteins and other substances. The molecular weight of isolated chitin can be estimated by light scattering, gel-permeation chromatography (GPC), high performance liquid chromatography

(HPLC), and viscosity measurement. Chitosan is soluble in aqueous acid solutions, and the molecular weight is estimated by GPC or viscometry. Several Mark-Houwink equations have been proposed for aqueous solutions of acetic acid, acetic acid/sodium acetate, and lactic acid (Roberts, & Domszy, 1982; Shimojoh, Fukushima & Kurita 1998).

2.3.3.3 Solubility

Chitosan is not cationic or water-soluble unless the pH is below 1.0. It is polyamine and soluble in aqueous dilute acids. It dissolves in hydrochloric acid and aqueous organic acids such as formic, acetic, oxalic, and lactic acids. The extent of solubility depends on the concentration and on the type of acid. The solubility decreases with increasing concentration of acids, and aqueous solutions of some acids such as phosphoric acid, sulfuric, citric, and sebacic are not good solvents (Gross, Konrad & Mager, 1983).

2.3.3.4 Enzymatic degradation

Both chitin and chitosan are biodegraded in nature by many varieties of microorganisms. Most of the chitinases in microorganism hydrolyze *N*-acetyl-β-(1→4)-glucosaminide linkages randomly. Chitinases hydrolyze chitosan in an endo-splitting fashion. Compared to the chitinases, chitosanese have been studies less extensively. It should be noted that chitosan is susceptible to be broad range of enzyme preparations such as glycanases, lipase, and proteases from various sources (Pantaleone, Yalpani, & Scollar, 1992).

2. 3.4 Potential applications of chitosan in foods

To date chitosan has attracted notable interest due to its biological activities such as antimicrobial, biocompatible, biodegradable, and health-promoting that makes it a very good candidate to improve the quality and safety of perishable food. Chitosan has already been approved as a food in some countries, for instance in Japan and Korea.

2.3.4.1 Modifications of physical properties

Chitosan has been proposed as a texturing, emulsifying, foaming, gelling, and coating agent in a number of recent food related patent

and publications. Chitosan has been employed as an emulsifying in the following cases: amino acids and reducing sugars for microwave-browning food products (Haynes, Levien, Otterburn, & Mathewson, 1990), soybean oil and monoglyceryl fatty acid esters with aqueous solution of defatted milk in margarine (Endo, Suzaki, & Marui, 1990), and a mixture of sugar esters, monoglyceride, whey protein, lactic acid, and water in marbled beef (Hanawa, Nishtani, Tamaoki, Tatsumi, & Imon, 1990). A hamburger, which did not drip with oil, has been made from chitosan, tallow, bread crumbs, minced meat, onion, and, egg (Oonishi, Nishtani, & Hanawa, 1991). Presumably, the chitosan makes the emulsion more stable in these applications by increasing the viscosity of the aqueous phase (the continuous phase of colloid) or imparting a positive charge, which prevents coalescence, to the surface of droplets. Lee, et al. (1996) reported that addition of chitosan increased emulsifying capacity of egg yolk by about 10% and enhanced emulsion stability of mayonnaise by 9.4% compared with control. Kim & Hur (2002) also suggested the use of chitosan as an emulsion stabilizer in commercial mayonnaise preparation. Hart, (1989) and Poole, (1989) described the use of chitosan of low molecular weight to stabilize foams based on solution of acidic proteins. Fats and oil diminish protein foam stability, but the addition of chitosan to acidic protein solutions permits the development of stable foams even when lipids are present to the extent of 30% by weight. This technology could allow for the development of chocolate-flavored meringues and marsh-mallows and foam containing fruit-flavored oils. There are many examples in which chitosan have functioned as a gelling or shape-retaining agent. An ice cream made with chitosan present at level of 0.01-0.5% by weight exhibited little melting when stored at 20 °C for 60 min (Hanawa, Ro, Takeuchi, Nishitani & Kanazawa, 1991). It has been suggested that chitosan may be used in the coating of fruits such as apples, and tomatoes (Taguchi & Sato, 1989). Moon et al., (1997) suggested that the acorn starch jelly formulation increase its hardness by added 0.5% of chitosan. The observation through scanning electron microscope (SEM) revealed that acorn starch jelly containing chitosan showed fiber and more fibrous structure than that of the control without chitosan.

2.3.4.2 Antispoilage agents

Chitosan has attracted attention as a potential food preservative of natural origin due to its antimicrobial activity against a wide range of food born filamentous fungi, yeast, and bacteria (Sagoo, Board & Roller, 2002). Lee and No (2002) observed that the wet noodle containing chitosan could be stored longer than control. These studies clearly demonstrated that chitosan can be used as an effective preservative in wet noodle due to its antimicrobials activity. The use of chitosan as an antimicrobial agent to extend the shelf life of starch has been demonstrated. Moon et al., (1997) studied the preservative effect of chitosan on acorn starch jelly. The shelf life of acorn starch jelly containing 0.5% of chitosan dissolved in 1.0% acetic acid was extended twice longer than the control.

2.3.4.3 Health-promoting agents

The cholesterol-lowering action of oral chitosan has been reported by many (Sugano, Watanabe, Kishi, Izume, & Ohtakara, 1988). Information regarding digestion and absorption of chitin and chitosan in the gastrointestinal tract (GI) is limited. In an in *vivo* study in canine GI tract, it was shown that chitosan 10% decrease in weight and formed a film.

2.4. Interaction between starches and hydrocolloids

The physical properties of native starches and their colloidal limit their usefulness in many commercial applications (Liu, Eskin, & Cui, 2003). Chemical modification of starch can improve cooking characteristics, decrease retrogradation as well as increase freeze-thaw stability of starch pastes. However, obtained with consumer a concern regarding the chemical treatment of foods has led to alternative ways to modify starch such as blending with other hydrocolloids. The function of hydrocolloid to starch including inhibition of retrogradation or the improvement of water-holding capacity for the starch system, depending on the macromolecule characteristics of hydrocolloid. In addition, presence of hydrocolloids usually results in a certainly increase in starch viscosity (Alloncle, Lefevre, Llamas & Doublier, 1989; Tecante & Doublier, 1999; Sasaki, Yasui & Matsuki, 2000; Chaisawang & Suphantharika, 2005; Satrapai & Suphantharika, 2007). The viscocity of starch

increases due to synergistic interaction of starch and gum (Alloncle et al., 1989; Christianson, Hodge, Osborne & Detroy, 1981; Christianson, 1982; Bahnessey & Breene, 1994; Lui & Eskin, 1998; Shi & BeMiller, 2002). Christianson et al., (1981) and Christianson (1982) believe that hydrocolloid form stable associations with starch soluble, which increase the viscosity of starch-gum mixtures.

Khanna & Tester (2006) reported that the gelatinization temperature of starch-konjac glucomannan tended to be shifted to higher temperatures but decrease in enthalpy when compared with starch alone. Water is reduced in the mixed system because the non-starch polysaccharide readily hydrates and consequently reduces the amount of water available for gelatinization (Ferrero, Martino & Zaritzky, 1996; Lelievre, 1976). Karim et al., (2000) suggested that the retrogradation results in reassociation of gelatinized starch molecules, but in less ordered and hence less perfect or stable forms than those present in the native starch granules. Chaisawang and Suphantharika (2005) reported that gelatinization enthalpy of starch decreased with gum addition.

A small change in molecular conformation and structure of starch can bring about dramatic change in function and rheological properties of starch (Djakovic & Dokic, 1972). The rheology of starch-hydrocolloid combinations is particular to each mixture and depends on experimental conditions, namely, those involved in paste preparation. Therefore, it is necessary to determine it for particular case (Tecante & Doublier, 1999). Maximum viscosity is attained in systems where the granules are not completely broken and still keep their identity. Therefore, granule size influences the rheological behavior of gelatinized starch paste (Evans & Lips, 1992; Okechukwu & Rao, 1995). Alloncle and Doublier (1991) described such starch-gum dispersions as composites whose viscoelastic properties in the pasted and gelled states are governed primarily by the volume occupied by swollen particles. Thus increasing concentrations of hydrocolloids within the continuous phase will increase the viscoelastic behavior of the phase in to the starch granule increase the gum concentration surrounding them. Hence, viscosity increases because of competition for water rather than to any physical interaction between starch and gum. It is possible that both of these mechanisms are involved.

2.5. Rapid Visco Analyzer (RVA)

Pasting properties of the starch are commonly measured with instrument called Rapid Visco Analyzer (RVA). It is used to produce viscogram profiles which controlling mixing and heating, holding and cooling process to starch dispersion. The RVA has the advantage of using a small size, short testing time, and ability to modify testing conditions. As starch slurry is heated in viscoamylograph, typically under a constant rate of shear, the increase in viscosity is measured as a curve is traced. The curve shows in changes in the viscosity, breakdown, holding strength, final viscosity, and set back as shown in Figure 2.12 The key point to remember is that different starches generate different viscosity profiles.

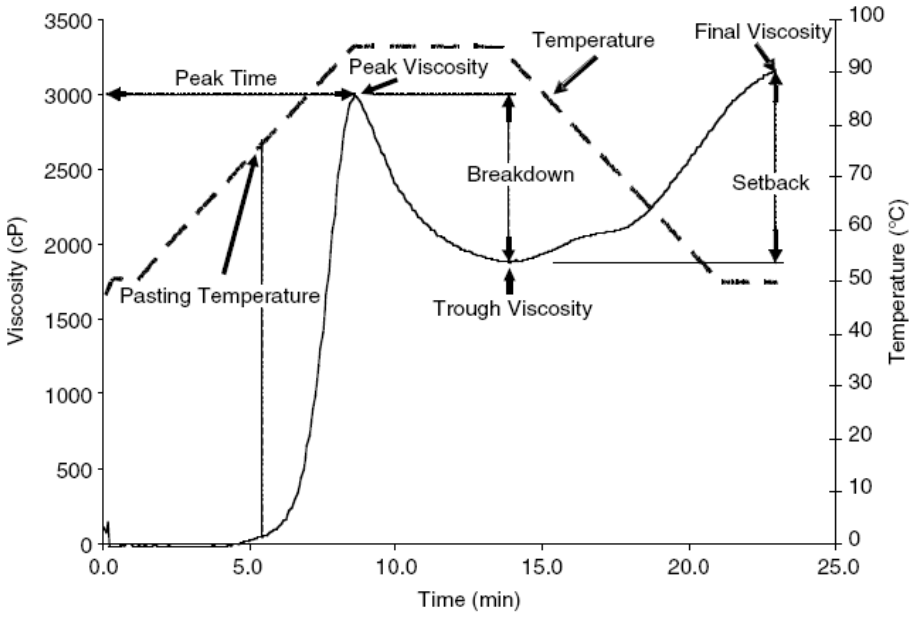

Figure 2.12 Typical RVA pasting profile of starch for viscosity (—) and temperature (---) as a function of time (Qiang, 2005).

- Peak viscosity = maximum viscosity developed during or soon after the heating portion of the test, in cP.
- Peak time = time at which the peak viscosity occurred, in minutes.
- Pasting temperature = temperature where viscosity first increases by at least 2 RVU over a 20 s period, in °C.

- Peak temperature = temperature at which the peak, normally occurring around the commencement of sample cooling, in cP.
- Breakdown = peak viscosity minus trough viscosity, in cP.
- Final viscosity = viscosity at the end of the test, in cP.
- Setback from peak = final viscosity minus peak viscosity, in cP.
- Setback from trough = final viscosity minus trough viscosity, in cP.

Granular starches are insoluble in water at room temperature, or little happens and the viscosity remains low. Until heat is applied above the gelatinization temperature, the granules absorb large amount of water and the viscosity of medium increase. At this point, polymers with lower molecular weights, particularly amylose molecules, being to leach from the granules. Peak viscosity is obtained during pasting when there is a majority of fully swollen. The peak viscosity is considered to represent the equilibrium point between swelling and rupture of starch granules (Newport Scientific, 1995).

During the high temperature holding phase (95°C) the granules being to break down, polymer solubilization continues, and molecular alignment occurs within the shear filed. Therefore, the reduction in the viscosity after appearance of peak was likely to be caused by mechanical rupture of starch granules. In the cooling phase at 50°C solubilized amylose and amylopectin polymer begin to reassociate, viscosity increased to a final viscosity at the end of RVA experiment, which was attributed to reassociation of amylose molecules or short-term retrogradation. The extent of short – term retrogradation evaluated from the RVA pasting curves is called setback.

Viscosity profiling is extremely helpful in determining starch behavior under various conditions and for comparing relative differences between starches. One of the most important aspects of viscosity profiling is measurement of the effect of starch-modifying reagents or processes on gelatinization and pasting.

2.6. Thermal analysis by differential scanning calorimetry (DSC)

DSC is a techniques used for characterization of polysaccharide gels. Thermo-reversible gels will melt if the gels are heated to exceed a certain temperature (melting temperature). This technique monitoring change in physical and chemical properties of materials as a function of temperature by detecting the heat changes

associated with such processes. In DSC, when a thermal transition occurs, the energy absorbed by the sample is replenished by increased energy input to the sample to maintain the temperature balance. Because the energy input is precisely equivalent in magnitude to the energy absorbed in the transition, a recording of this balancing energy yields a direct calorimetric measurement of energy transition which is then recorded as a peak. The area under the peak is directly proportion to the enthalpic change (ΔH) and its direction indicates whether the thermal event is endothermic or exothermic. DSC is commonly used for both measuring gelatinization and retrogradation of starch. In the case of retrograded starch, value of ΔH provides a quantitative measure of the energy transformation that occur during the melting of recrystallized amylopectin as well as precise measurement of the transition temperatures (i.e. onset, T_o; peak, T_p; and conclusion, T_c) of this endothermic event.

The retrogradation temperature range (T_c - T_o) is usually broader than the gelatinization temperature range for a given sample. Furthermore, the endothermic transition temperatures (T_o, T_p, and T_c) associated with melting of retrograded starch occur at temperature 10-26°C lower than those for gelatinization of starch granules (Baker & Rayas-Duarte, 1998; White, Abbas, & Johnson, 1989; Yuan et al., 1993), suggesting that retrogradation results in crystalline forms that are different in nature from those present in the native starch granules.

The gelatinzation enthaphy of tapioca starch was found in many literature ranged from 6.8 to 16.6 J/g (Asaoka, Blandshard & Rickard, 1992; Moorthy, 2002; Atichokudomchai & Varavinit & Chainachoti, 2002, Sae-kang & Suphantharika, 2006; Chaisawang & Suphantharika, 2006).

2.7. Rheology

By definition, rheology is the study of deformation and flow of materials. The amount of deformation will clearly depend on the area over which the factors are acts. The rheology is concerned with two physical quantities: stress and strain (solid) or shear rate (fluid)

$$\text{Shear stress } (\sigma) = \frac{\text{shear (force)}}{\text{area}} \qquad (\text{Pa or N/m}^2) \qquad (\text{shear})$$

$$\text{Shear rate} = \frac{\text{velocity}}{\text{distance}} \quad (1/s)$$

$$\text{Strain} = \frac{\text{defection}}{\text{distance}} \quad (-)$$

The general instrument commonly used for measuring viscosity and the rheological properties is a rotational viscometer. The rotational viscometer operates by rotating a spindle of define geometry within the fluid. Therefore, cone and plate rheometer was used in this study. It is a rotational viscometer that composes of a rotational cone and a fixed flat plate. The types of cone are determined by using the cone radius (R) and the cone angle (α). The ISO standard recommends using $\alpha = 1^o$ and excludes $\alpha > 4^o$ (Mezger, 2002). Moreover, temperature is environmental parameter, which influences viscosity or any rheological properties.

Various terms are used to describe the rheological properties of materials depending on the mathematical form of the relationship between stress and strain. There are many reasons that the rheology is important: 1. allowed insight into structure, 2. used for quality control during processing, 3. designed processing equipment, and 4. revealed food properties.

Rheological properties of starch are widely dependent upon the nature and the type of molecular arrangement of starch and its chemical structure, conformation and the forces acting between them (Bhandari et al., 2002). The rheology of starch modified by gum additions is particular to each mixture and depends on experiment conditions, namely, those involved in paste preparation. The most important aspects examined are their steady flow and dynamic viscoelastic behaviors.

2.7.1 Steady flow measurement

A fluid sample is subjected to different values of shear rate, γ, and the corresponding values of shear stress, σ are measured in a well-defined flow geometry. Fluid may be described as Newtonian or non-Newtonian depending on their rheology (flow) characteristics.

Newtonian fluid obeys Newton's law of viscous flow, the viscosity does not depend on the shear rate. Newtonian fluid has a constant viscosity regardless

of shear, therefore the viscosity of a Newtonian fluid will not vary with agitation rate (Figure 2.13).

Non-Newtonian fluid dose not have a constant viscosity. It is composed of dilatant, pseudoplastic, Bingham-plastic and Herschel-Bulkley plastic fluids. This measured viscosity is called apparent viscosity. The apparent viscosity varies with the shear rate, it can be distinguished into 2 types of behavior, shear thinning (pseudoplasticity) behavior, where the viscosity decreases with increasing shear rate such as starch paste; and shear thickening (dilatancy) behavior, where the viscosity increases with increasing shear rate (Chen & Ramaswamy, 1999).

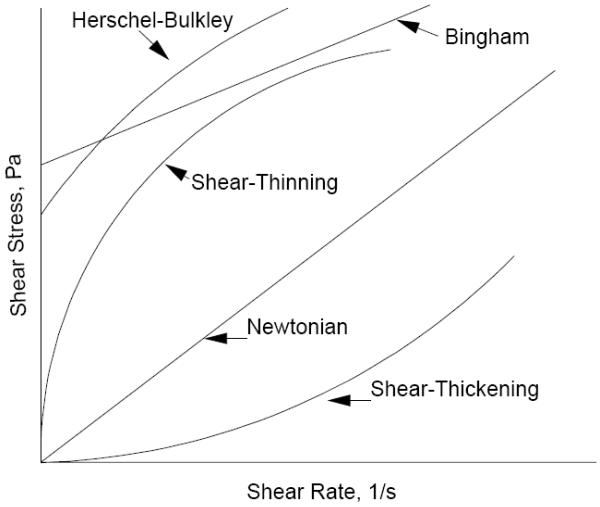

Figure 2.13. Comparison of the shear stress-rate of shear relationships for non-Newtonian fluids and Newtonian fluids (Steffe, 1996).

Non-Newtonian fluids are usually considered to be those for which the relation connecting shear stress and shear rate is not linear, that is, the “viscosity” of a non-Newtonian fluid is not constant at a given temperature and pressure but depends on the rate of shear or, more generally, on the previous kinematic history of the fluid. Non-linear fluids in shear flow may be classified into three broad types (a) fluids for which the rate of shear at any point is some function of the shear stress at that point and depends on nothing else; (time-independent fluids); (b) more complex systems for which the relation between shear stress and shear rate depends on the time the fluid has been sheared; (time-dependent fluids) and (c) systems which have characteristics

of both solids and fluids and exhibit partial elastic recovery after deformation; these are called viscoelastic fluids. Time-dependent non-Newtonian fluids show viscosity of more complex fluids depends not only on the rate of shear but also on the time the (constant) shearing has been applied. These fluids may be subdivided into two classes (Figure 2.14):

(a) thixotropic fluids – breakdown of structure by shear. If a thixotropic material is sheared at a constant rate after a period of rest, the structure will be progressively broken down and the viscosity will decrease with time.

(b) rheopectic fluids – formation of structure by shear. This is a case of gradual formation of structure by shear.

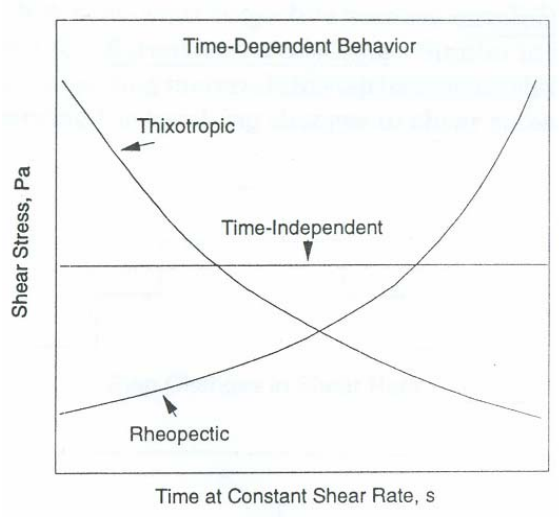

Figure 2.14 Time-dependent behavior of fluids (Steffe, 1996).

2.7.2 Dynamic oscillatory measurement

Dynamic tests were used to characterize property of viscoelastic behavior; starch pastes or gels can have both viscous (liquid-like) and elastic (solid-like) properties. These small-amplitude oscillatory tests are commonly performed in order of 1 to 3 or 5%. Basically, the gel specimen is subjected to a periodic, small amplitude sinusoidal torque (stress), the applied stress being altered at a given frequency (cycles s^{-1} or ω, radians s^{-1}). The frequency dependent functions $G'(\omega)$ and $G''(\omega)$ are shear elastic (storage) modulus and shear elastic (loss) modulus,

respectively. G' is a measure of the energy stored and subsequently release per cycle of deformation per unit volume. G″ is the property that relates to the molecule events of elastic nature (Rao, 1999). G'' is a measure of the energy dissipated as heat per cycle deformation per unit volume. This loss modulus is a property related to the molecular events of viscous nature (Rao, 1999). Another commonly used dynamic viscoelastic property is the loss tangent [tan δ (ω) = G''/G'] denoted relative effects of viscous and elastic components in a viscoelastic behavior. It is the ratio of the energy lost to the energy stored for each cycle of the deformation. It is a useful indicator of the relative contributions of the viscous (G'') and elastic (G') components to the viscoelastic properties of a material. The logarithmic plot of the loss tangent gives rise to several characteristics, those two moduli can be defined: (a) stress in-phase/strain or storage modulus (G') and (b) stress out-of-phase/strain or loss modulus (G'').

$$G' = \frac{\sigma_0 \cos\delta}{\gamma_0}$$

$$G'' = \frac{\sigma_0 \cos\delta}{\gamma_0}$$

where σ_0 is the yield stress (Pa), and γ_0 is the shear rate (s^{-1})

Storage modulus (G') is a measure of the energy stored in the material and recovered from it per cycle. On a molecular basis, the magnitude of G' is dependent upon what rearrangements can take place within the period of oscillation (Ferry, 1980), and is taken as an indication of the solid or elastic character of the material. Loss modulus (G'') is defined as the stress 90° out-of-phase with the strain divided by the strain and is a measure of the energy dissipated or lost (as heat) per cycle of sinusoidal deformation. It is, therefore, taken as an indication of liquid or viscous behavior. Another parameter which is often useful in indicating the physical behavior of a system is the loss tangent ($\tan\delta = G''/G'$). It is the ratio of the energy lost to the energy stored for each cycle of the deformation. It is a useful

indicator of the relative contributions of the viscous (G'') and elastic (G') components to the viscoelastic properties of a material. The logarithmic plot of the loss tangent gives rise to several characteristics. Gels formed at fast heating rate had higher stress and strain at fracture due to network structure, while those formed at a slow heating rate had a higher storage modulus, G'. If G' is much greater than G'', the material will behave more like a solid, i.e., the deformations will be essentially elastic or recoverable. When G'' is much higher than G' the energy used to deform the material is dissipated viscosity and the materials behavior is liquid like. For strain values within the linear range of deformation, G' and G'' are independent of the magnitude of the applied strain. The dynamic shear rheological test for deformation, in general, have been used to obtain valuable information on the viscoelastics properties of starch-gum mixtures to explain the sample's molecular structure (Gunasekaran & Ak, 2000). There are often three selected applications such as:

(a) deformation sweep at constant frequency (G' and G'' vs. strain) to determine the maximum deformation attainable by a sample in the linear viscoelastic region.

(b) frequency sweep (G' and G'' vs. ω) at constant deformation within the linear viscoelastic range to determine the viscoelastic character of the gel and

(c) temperature sweep at constant frequency and deformation within the linear viscoelastic range (G' and G'' vs. Temperature) to evaluate thermal characteristics.

In starch-gum mixed pastes, the increase of dynamic moduli can be attributed to the increase in viscoelastic properties of added gum, which is concentrated within the continuous phase in the starch-gum mixed systems, due to its thickening properties, as indicated by Alloncle and Doublier (1991).

CHAPTER III
MATERIALS AND METHODS

3.1 Materials

Native tapioca starch (NT) was kindly supplied by General Starch Co. Ltd., (Bangkok, Thailand) and had moisture (AOAC, 2000) contents of 11.23%. Commercial cationic tapioca starch used (Batch No. FHB8735, National Starch and Chemical Co., Ltd., Bangkok, Thailand) had 11.45% moisture content. The cationic tapioca starch was quaternary ammonium starch ether. Commercial grade-chitosan flake with high (viscosity 800 cP), medium (viscosity 200 cP) and low (viscosity 20 cP) molecular weight (approximately 85%, 75% and 75% degree of deacetylation, respectively) obtained from Sigma-Aldrich (MO, USA) of all had 7.2% moisture content.

3.2 Methods

3.2.1 Preparation of anionic tapioca starch

Native tapioca starch was phosphorylated by the procedure of Lim and Seib (1993). Accurately weighted amounts of sodium tripolyphosphate (4.5g) were dissolved in 241 ml of distilled water. The starch (301.5g, dry basis) was mixed into the solution by stirring continuously. The slurry was stirred for 1 h at room temperature and dried in an oven at 40 °C to retain 10-15% moisture. To effect phosphorylation, the dried starch cake was heated for 2 h at 130 °C in a hot air oven. After cooling to room temperature, the starch cake was washed many times by suspending the starch in distilled water and recovering the starch by centrifugation at 2300 rpm for 4 min. Finally, the starch was dried at 40 °C in a hot air oven. The phosphate content was determined by using the modified method of AOAC, 991.25 (2000). Starch was burned in furnace overnight (16h) at 525 °C, and cooled down in a desiccator. Ash was dissolved in 1 ml of 0.1 M HNO_3. After cooling, ash was

transferred to 250 ml volumetric flask and dilute to given volume. Determine phosphorus by using the UV-visible detector at 400 nm.

3.2.2 Determination of pasting properties

Pasting properties of starches or starch/gum mixtures suspended in 0.5% acetic acid were determined by a Rapid Visco-Analyzer (Model RVA-4C, Newport Scientific Pty. Ltd., Warriewood, Australia). The slurry concentration of 5.5% w/v (dry basis) starches (native, anionic, or cationic tapioca starches) was prepared by dispersing 1.5 g (dry basis) of starch in 25 ml of 0.5% acetic acid or 0.5% w/v (dry basis) chitosan solutions. In the case of starch/chitosan mixtures, chitosans were first dispersed in 0.5% acetic acid under continuous stirrings to dissolve chitosan completely. The starch was then slurried in the gum solutions. The dispersions were stirred with a spatula for sufficient duration to avoid formation of lumps. The starch-gum suspensions were poured into aluminum containers and stirred manually using plastic paddle for 20-30 s before insertion into the RVA machine. The heating and cooling cycles were programmed in the following manner; the slurry was held at 50 °C for 1 min, heated to 95 °C within 3 min and then held at 95 °C for 2 min. It was subsequently cooled to 50 °C within 3 min and then held at 50 °C for 2 min, while maintaining a rotation speed of 160 rpm.

3.2.3 Determination of swelling power

The swelling power (SP) was determined by modifying the method of Mandala and Bayas (2004). The concentration of starch used was less than the close packing concentration (~3.0%) of starch granules (Vandeputte, Derycke, Geeroms & Delcour, 2003). Only starch (1.25% w/w) or starch/chitosan (1.125% w/w starch and 0.125% w/w chitosan) suspensions were put into 50-ml centrifuge tube and heated in water bath at 60-90°C for 10 min with minimum shear condition. After heating, the centrifugal tubes were immediately immersed in an ice bath for 5 min to quickly cool the dispersion to room temperature and then centrifuged at 7,000 g at 5 °C for 15 min. The supernatant was removed for the measurement of solubilized starch by drying to constant weight in a hot air oven at 105°C. Precipitated paste and dried supernatant were weighed. The swelling power (SP) was calculated based on the

assumption that the total amount of gum remained in the supernatant. The SP is the ratio of the wet weight of precipitated starch gel to its dry weight.

$$SP = \frac{Wp}{Wps}$$

Where; SP = swelling power

Wp = hydrated precipitated paste (g)

Wps = dry precipitated paste (g)

3.2.4 Scanning electron microscopy (SEM)

In this assay, 0.6 g (dry basis) of starch was dispersed in 10 ml of 0.5% acetic acid or 0.5% (w/v) chitosan solutions. The samples were put in centrifuge tubes and heated in a water bath at 60 °C for 5 min for cationic starch samples and 68 °C for 5 min for native and anionic starch with and without chitosan under minimum shear condition. After heating, the samples were mixed with an equal volume (10 ml) of 2 % agarose solution of the same temperature as fast as possible and were immediately immersed in an ice bath. For the SEM observation, the samples were prepared by the technique of the critical point drying modified from the method of Egelandsdal et al. (2001). The samples were chemically fixed in 6% glutaraldehyde in 0.1 M phosphate buffer (pH 7) and were post fixed overnight in 2% osmium tetraoxide with 0.1 M imidazole and then dehydrated through a graded series of ethanol before being critical point drided through CO_2 in a critical point dryer (Hitachi HCP-2, Ibaraki, Japan). The agars were cut with a razor blade and torn before observation by SEM. Finally, the samples were mounted on aluminum nails and coated with copper. For the observation of the sample the SEM (JSM−5400, Hitachi Science Systems, Ibaraki, Japan) was used. The images were captured at magnification 1000× and at an accelerating voltage of 15 keV.

3.2.5 Differential scanning calorimetry (DSC) measurement

Gelatinization temperatures and enthalpy of the starches with and without added various molecular weights ($M_{w)}$ of chitosan was measured by a differential scanning calorimeter (DSC 822e, Mettler Toledo, Schwerzenbach, Switzerland).

The total solids content of samples was selected to be 12%, w/w (dry basis), due to the sensitivity of the instrument. The starch/chitosan mixtures with 5.5/0.5 mixing ratio was prepared following the method described above. After hydration for 1 h at room temperature, 10–15 mg of the well stirred starch/chitosan dispersions was exactly weighed into 40 µl aluminum crucibles and hermetically sealed immediately to prevent moisture loss. Scans were performed from 25 to 100°C at a controlled constant rate of 10°C/min. A sealed empty pan was used as a reference and the DSC was calibrated using indium. The enthalpy and transition temperatures; the onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c) were determined based on the first-run heating DSC curves. The gelatinization enthalpy was evaluated based on the area of the main endothermic peak and expressed in terms of J/g of dry starch using the equipment software.

3.2.6 Rheological properties

3.2.6.1 Dynamic viscoelastic measurement

The fresh starch/chitosan mixed pastes (3.5%, w/w) obtained from the RVA were cooled to room temperature (25°C). The samples were determined for viscoelastic properties by using a rheometer (Physica MCR 301, Anton Paar GmbH, Stuttgart, Germany). The samples were placed into the rheometer measuring system (cone and plate geometry, 50 mm diameter, 1° cone angle, and 0.05 mm gap) which was equilibrated to 25°C. Two steps of rheological measurements were performed: (1) deformation sweeps at a constant angular frequency (10 rad/s) to determine the maximum deformation attainable by a sample in the linear viscoelastic range and (2) frequency sweeps at a constant deformation (0.5% strain) within the linear viscoelastic range. The mechanical spectra were obtained recording the storage modulus (G'), loss modulus (G''), and loss tangent (tan $\delta = G''/G'$) as a function of frequency (ω).

2.6.2 Steady flow test

The steady flow tests were performed on the same sample after the frequency sweep tests to obtain shear rate versus shear stress (flow curves) data. The

cone was programmed to ramp the shear rate from 0 to 300 s^{-1} (up curve) in 3 min followed immediately by the down curve from 300 to 0 s^{-1} in 3 min. Data from the ascending and descending segments of the shear cycle were used to characterize the flow of the paste samples and fitted to the Power law model using the following equation:

$$\sigma = \sigma_0 + K\dot{\gamma}^n$$

where σ is the shear stress (Pa), σ_0 is the yield stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency coefficient ($Pa.s^n$), and η is the flow behavior index (dimensionless). In addition, hysteresis loop area of all starches and starch/chitosan pastes were calculated by the equipment software, indicating their structural changes.

3.2.7 Statistical analysis

For three replicates, the data were subjected to statistical analysis using SPSS 17.0 for Windows Evaluation Version (SPSS Inc., Chicago, IL, USA). Mean and standard deviations for each treatment were calculated. Tukey's test was used to compare differences among the mean values at 0.05 level of confidence.

CHAPTER IV
RESULTS

4.1 RVA pasting properties of starch and starch/chitosan mixtures

The typical pasting profiles of native (NT), anionic (AT), and cationic (CT) tapioca starches in the presence or absence of chitosan (CS) with various molecular weight (M_w), i.e. high (CSH), medium (CSM), and low (CSL), suspended in 0.5% of acetic acid as determined by RVA analysis are shown in Figure 4.1. Statistical analyses of all pasting parameters were also performed and are summarized in Table 4.1. CSH, CSM, and CSL dispersions in 0.5% of acetic acid without starch were also run under the same RVA conditions to establish a control viscosity curve. The viscosities of CSH, CSM, and CSL dispersions remained almost constant, at 2, 5, and 9 RVU(s), respectively. The phosphorus contents of native and anionic tapioca starches were 2 and 20 mg/100g starch (dry basis), respectively.

Pasting properties of NT, AT, and CT starches were greatly affected by the addition of CS. Addition of various CS to the NT starch resulted in significant ($p<0.05$) increase in peak, breakdown, final, and setback viscosities, pasting temperature, and peak times as compared with the NT alone. In general, this effect was more pronounced with increasing M_w of CS. A similar result was also found for the AT/CS mixtures.

In the case of CT starch, addition of CS with high M_w resulted in a significant ($p<0.05$) increase in peak viscosity, whereas addition of medium and low M_w chitosan resulted in a significant decrease in the peak viscosities as compared with the control CT alone. The breakdown viscosity of CT was significantly decreased by the addition of various CS. All other pasting properties of CT were significantly increased by the addition of these CS.

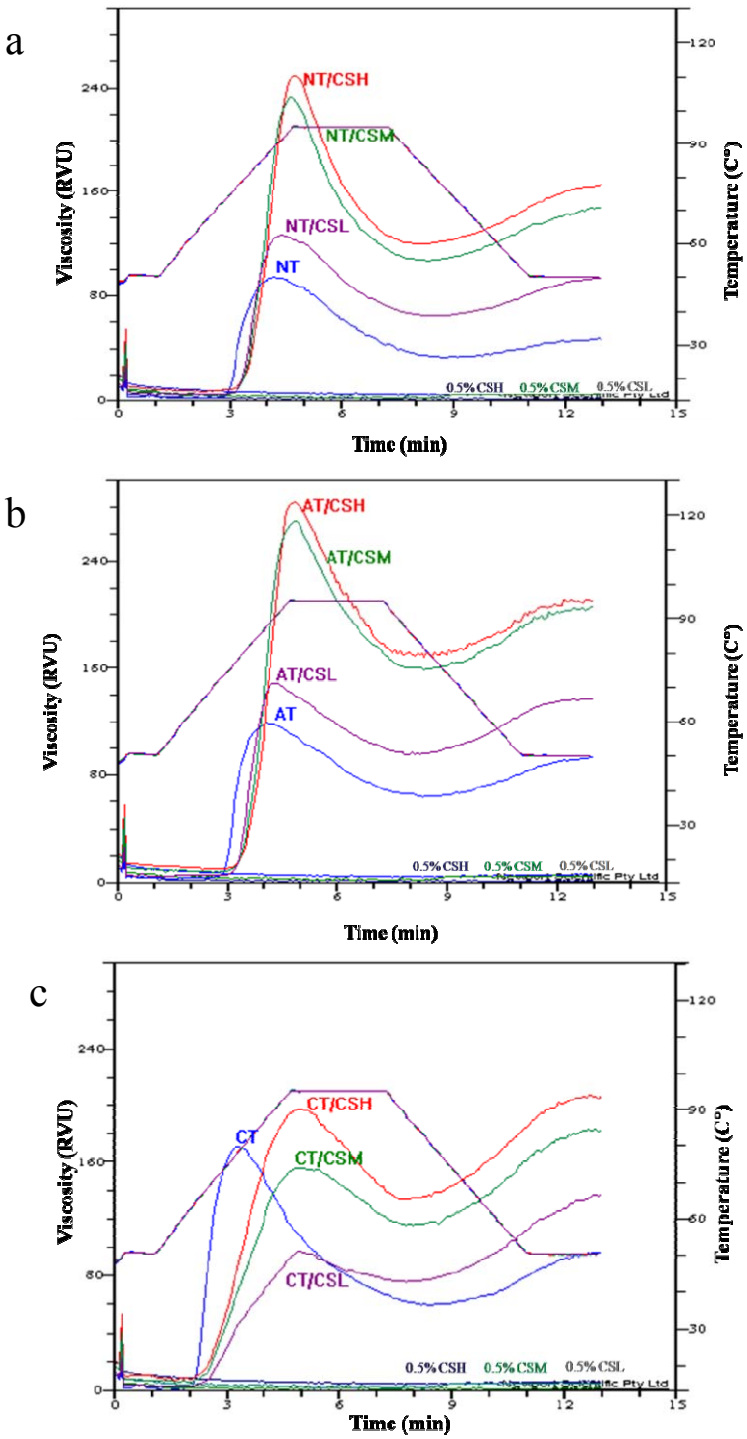

Figure 4.1. Typical pasting properties of 6% (w/w) NT, AT, CT alone, 5.5% NT, AT, CT in the presence of 0.5% chitosan with various M_w, and 0.5% chitosan in 0.5% acetic acid aqueous solution. Refer to Table 4.1 for the sample codes of various sample preparations.

Table 4.1 Pasting properties of 6% (w/w) native, anionic, and cationic tapioca starch alone and starch (5.5%) in the presence of 0.5% chitosan in 0.5% acetic acid solution measured by the Rapid Visco-Analyzer (RVA)[1]

Sample	Peak viscosity (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Setback (RVU)	Pasting temperature (°C)	Peak time (min)
Native tapioca starch alone (NT)	92.4 ± 2.3d	60.0 ± 1.7^{c}	45.4 ± 1.8^{d}	13.6 ± 0.7^{d}	72.7 ± 0.0^{b}	4.2 ± 0.0^{b}
Native tapioca starch/ Low M_w chitosan mixture (NT/CSL)	126.3 ± 1.6^{c}	62.5 ± 2.2^{c}	92.1 ± 1.3^{c}	28.4 ± 0.5^{c}	76.4 ± 0.5^{a}	4.4 ± 0.0^{b}
Native tapioca starch/ Medium M_w chitosan mixture (NT/CSM)	242.9 ± 1.6^{b}	133.3 ± 1.4^{a}	149.0 ± 2.2^{b}	40.4 ± 0.5^{ab}	76.2 ± 1.0^{a}	4.7 ± 0.0^{a}
Native tapioca starch/ High M_w chitosan mixture (NT/CSH)	249.3 ± 1.2^{a}	129.9 ± 0.3^{b}	162.5 ± 1.1^{a}	42.1 ± 1.0^{a}	76.3 ± 0.4^{a}	4.7 ± 0.1^{a}
Anionic tapioca starch alone (AT)	109.9 ± 1.2^{d}	44.7 ± 0.5^{c}	86.7 ± 0.3^{d}	25.7 ± 0.8^{c}	71.6 ± 1.2^{b}	4.0 ± 0.0^{c}
Anionic tapioca starch/ Low M_w chitosan mixture (AT/CSL)	146.9 ± 2.8^{c}	53.8 ± 0.7^{b}	135.8 ± 1.4^{c}	41.2 ± 0.5^{ab}	76.6 ± 0.2^{a}	4.3 ± 0.1^{c}
Anionic tapioca starch/ Medium M_w chitosan mixture (AT/CSM)	256.6 ± 2.6^{b}	108.8 ± 2.9^{a}	190.9 ± 0.9^{b}	43.7 ± 1.3^{a}	75.9 ± 0.7^{a}	4.8 ± 0.1^{b}
Anionic tapioca starch/ High M_w chitosan mixture (AT/CSH)	262.3 ± 0.4^{a}	105.5 ± 0.3^{a}	199.5 ± 0.4^{a}	42.5 ± 0.5^{ab}	75.6 ± 0.1^{a}	5.0 ± 0.0^{a}
Cationic tapioca starch alone (CT)	171.2 ± 0.5^{b}	113.3 ± 2.6^{a}	96.2 ± 2.8^{d}	37.0 ± 2.5^{c}	63.2 ± 0.5^{c}	3.3 ± 0.1^{b}
Cationic tapioca starch/ Low M_w chitosan mixture (CT/CSL)	95.7 ± 3.0^{d}	19.7 ± 3.2^{d}	134.6 ± 0.8^{c}	60.0 ± 2.0^{a}	68.0 ± 0.4^{a}	5.0 ± 0.1^{a}
Cationic tapioca starch/ Medium M_w chitosan mixture (CT/CSM)	154.0 ± 1.2^{c}	40.3 ± 0.8^{c}	167.2 ± 1.5^{b}	56.6 ± 2.2^{b}	65.5 ± 0.3^{b}	5.0 ± 0.1^{a}
Cationic tapioca starch/ High M_w chitosan mixture (CT/CSH)	193.0 ± 2.8^{a}	61.1 ± 1.8^{b}	194.5 ± 2.1^{a}	57.9 ± 2.2^{b}	65.3 ± 0.0^{b}	5.0 ± 0.1^{a}

[1] Assays were performed in triplicate. Mean ± standard deviation values in the same column followed by the same superscripts are not significantly different ($p > 0.05$).

4.2 Swelling power

The effect of chitosan addition on swelling power (SP) of native, anionic, and cationic starches as a function of heating temperatures ranged from 60 to 90°C is shown in Figure 4.2. All samples exhibited a sharp increase in SP with increasing heating temperatures from 60 to 90°C except for the CT alone which exhibited a significant reduction in SP from 80 to 90°C. The CT starch exhibited the highest SP values as compared with the NT and AT starches in the range of temperatures tested. Statistical analysis was performed comparing SP values of starch alone and starch-chitosan mixtures at each temperature from 60 to 90°C (Table 4.2). According to these results the SP of NT/CS mixtures was slightly higher than that of NT alone at temperatures 70 to 90°C and the effect was more pronounced with increasing M_w of CS as shown in Figure 4.2a. For the AT starch with CS addition during 60 to 70°C the SP profiles were nearly the same as those of the AT alone, but after a certain temperature (>80°C), addition of CS resulted in a slightly higher SP values in the order of CSH > CSM > CSL as compared with the control (Figure 4.2b). All samples of CT starch with CSH, CSM, and CSL additions at 60°C showed significantly higher SP values, whereas at higher temperatures (70-90°C) these samples exhibited significantly lower SP values than the control CT alone (Figure 4.2c). However, this effect seemed to be unaffected by the molecular weight of chitosan.

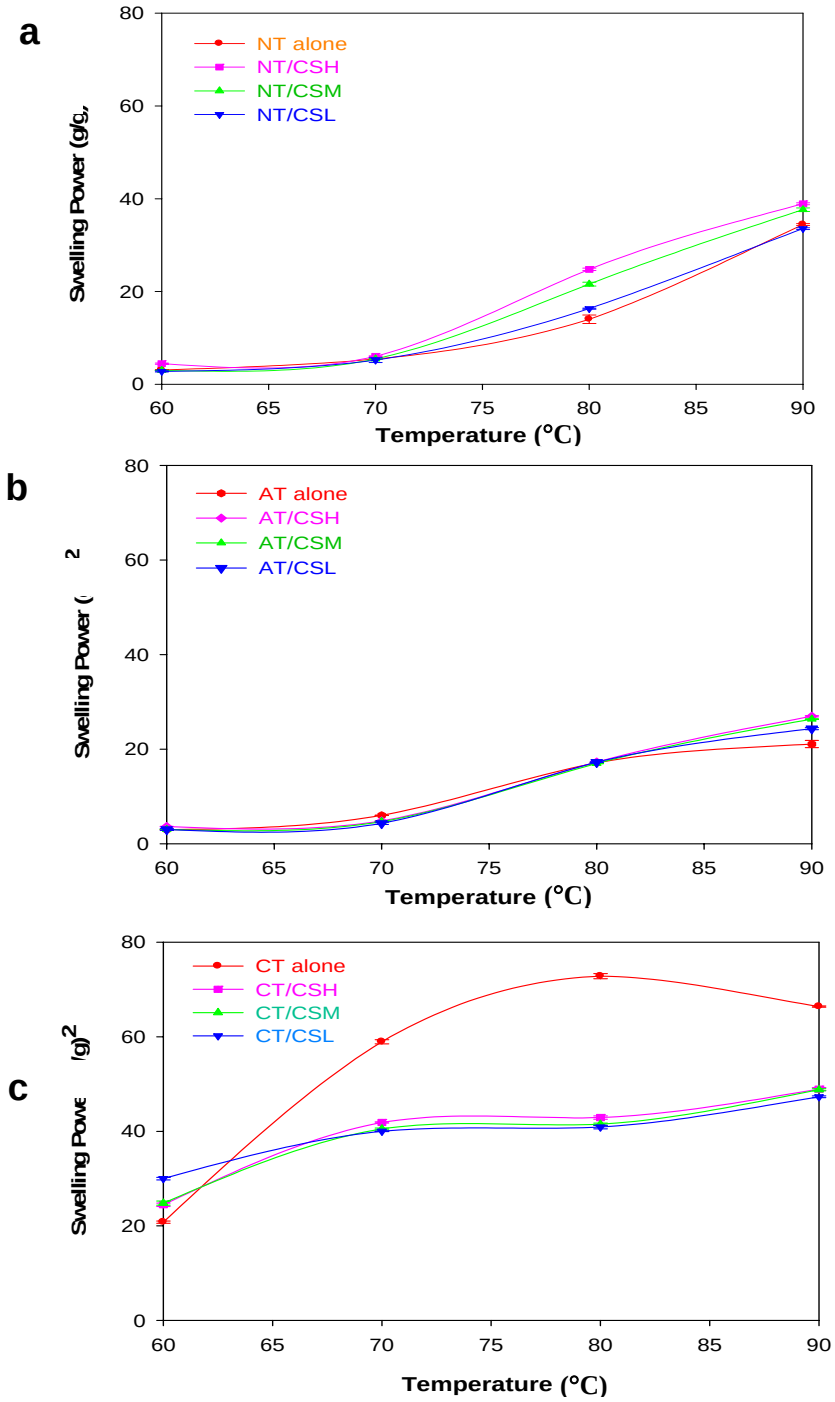

Figure 4.2 Swelling power (g/g) at different heating temperature of 1.25% tapioca starch alone (●) and starch (1.125%) in the presence of 0.125% high M_w (■), medium M_w (▲), and low M_w chitosans (▼). (a) native starch, (b) anionic starch, and (c) cationic starch. Error bars represent standard deviations.

Table 4.2 Swelling power (g/g) of NT, AT, and CT with and without additions of chitosan in 0.5% of acetic acid solution[1].

Samples[2]	Temperature (°C)			
	60	**70**	**80**	**90**
NT	3.08 ± 0.06^{b}	5.42 ± 0.12^{b}	14.00 ± 0.09^{c}	34.41 ± 0.22^{c}
NT/CSL	2.77 ± 0.11^{b}	5.19 ± 0.05^{b}	16.31 ± 0.13^{c}	33.54 ± 0.19^{c}
NT/CSM	3.01 ± 0.02^{b}	5.43 ± 0.10^{a}	21.57 ± 0.41^{b}	37.64 ± 0.38^{b}
NT/CSH	4.45 ± 0.14^{a}	6.00 ± 0.10^{a}	24.77 ± 0.26^{a}	38.91 ± 0.22^{a}
AT	2.98 ± 0.03^{b}	6.02 ± 0.04^{a}	17.13 ± 0.06^{a}	21.11 ± 0.77^{c}
AT/CSL	3.03 ± 0.18^{b}	4.33 ± 0.25^{b}	17.26 ± 0.03^{a}	24.34 ± 0.23^{b}
AT/CSM	3.04 ± 0.04^{b}	4.69 ± 0.05^{b}	16.99 ± 0.07^{a}	26.36 ± 0.04^{a}
AT/CSH	3.65 ± 0.24^{a}	4.84 ± 0.06^{b}	17.29 ± 0.14^{a}	26.95 ± 0.13^{a}
CT	20.75 ± 0.23^{c}	58.94 ± 0.44^{a}	72.83 ± 0.53^{a}	66.38 ± 0.14^{a}
CT/CSL	29.99 ± 0.27^{a}	40.01 ± 0.06^{c}	40.91 ± 0.37^{c}	47.30 ± 0.19^{c}
CT/CSM	24.80 ± 0.43^{b}	40.51 ± 0.18^{c}	41.51 ± 0.25^{c}	48.79 ± 0.22^{b}
CT/CSH	24.45 ± 0.36^{b}	41.87 ± 0.13^{b}	42.90 ± 0.35^{b}	48.92 ± 0.32^{b}

[1] Assays were performed in triplicate. Mean ± standard deviation values in the same column followed by the same superscripts are not significantly different ($p > 0.05$).

[2] Refer of Table 4.1 for the sample codes.

4.3 Morphological structures of starch pastes in the presence and absence of chitosan

The purpose of these experiments was to examine the microstructures of starch granules and starch granules in gum matrix by using the scanning electron microscope. The temperature of 68 °C was chosen which is lower than the pasting temperatures of most samples, except for cationic starch which is lower (see Table 4.1) in which a temperature of 60 °C was used for these samples. Scanning electron micrographs of starch samples in the presence and absence of chitosan are shown in Figures 4.3 to 4.5. The starch granules gelatinized in 0.5% acetic acid solution exhibited only tiny coverage of gum layer due to the presence of agarose which was used for fixation of the pastes.

In the case of NT and AT starches in the presence of chitosan, the results show that chitosan formed a sheet structure in gel matrix and packed around starch granules. The effect was more pronounced with increasing the M_w of chitosan used. In the case of CT starch, on the contrary, chitosan did not wrap the starch granules but formed a sheet structure in the continuous phase.

The granular size of starches within gel matrix of chitosan was smaller than those within the continuous phase.

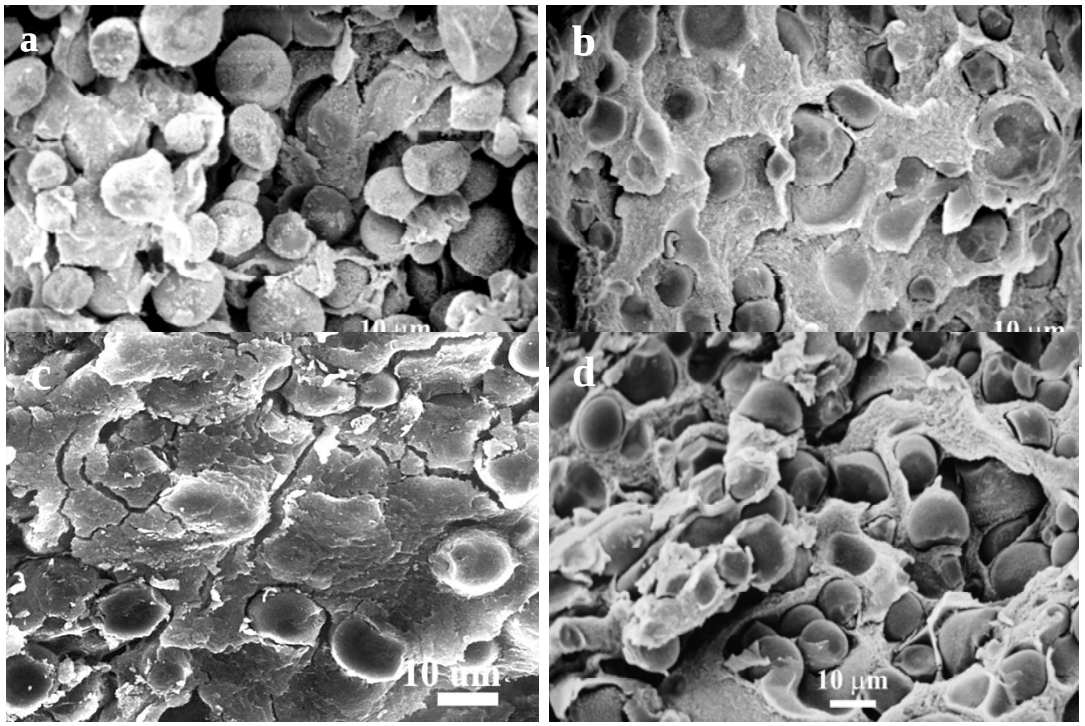

Figure 4.3 Scanning electron micrographs (SEM) of native tapioca starch in presence and absence of various M_w of chitosan in 0.5% acetic acid solution: (a) native tapioca starch alone, (b) native starch/ high M_w of chitosan mixture, (c) native starch/ medium M_w of chitosan mixture, and (d) native starch/low M_w of chitosan mixture (1000x, Bar = 10 μm).

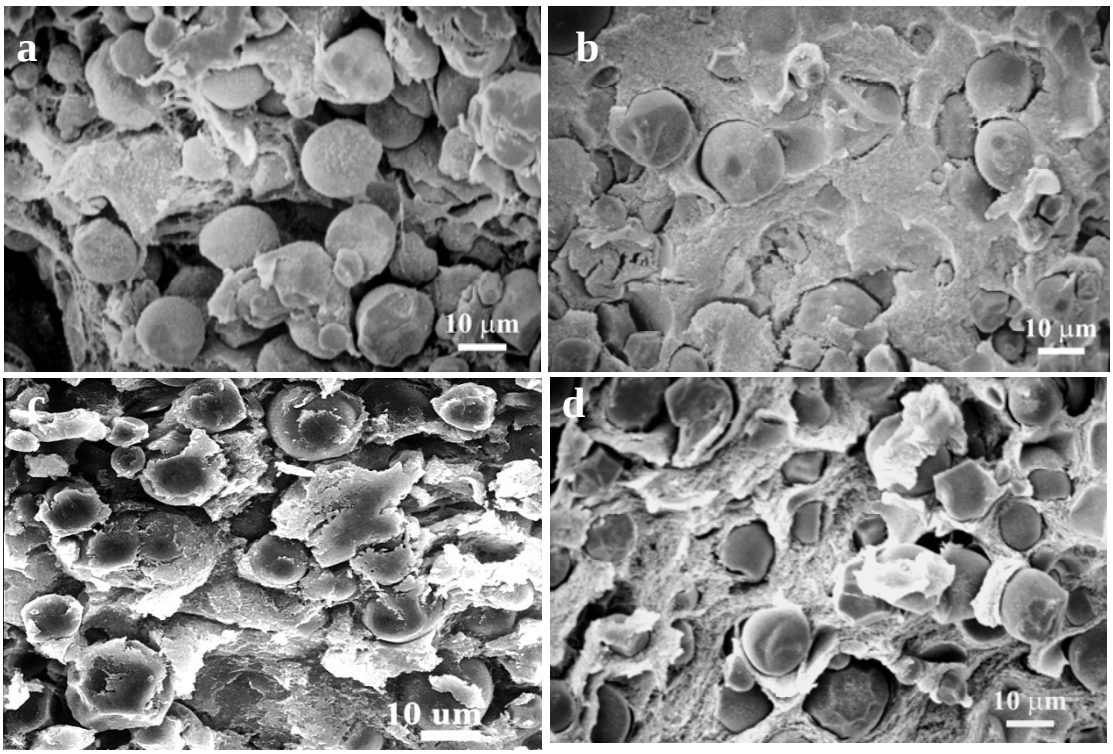

Figure 4.4 Scanning electron micrographs (SEM) of anionic tapioca starch in presence and absence of various M_w of chitosan in 0.5% acetic acid solution: (a) anionic tapioca starch alone, (b) anionic tapioca starch/ high M_w of chitosan mixture, (c) anionic tapioca starch/ Medium M_w of chitosan mixture, and (d) anionic tapioca starch/ low M_w of chitosan mixture (1000x, Bar = 10 μm).

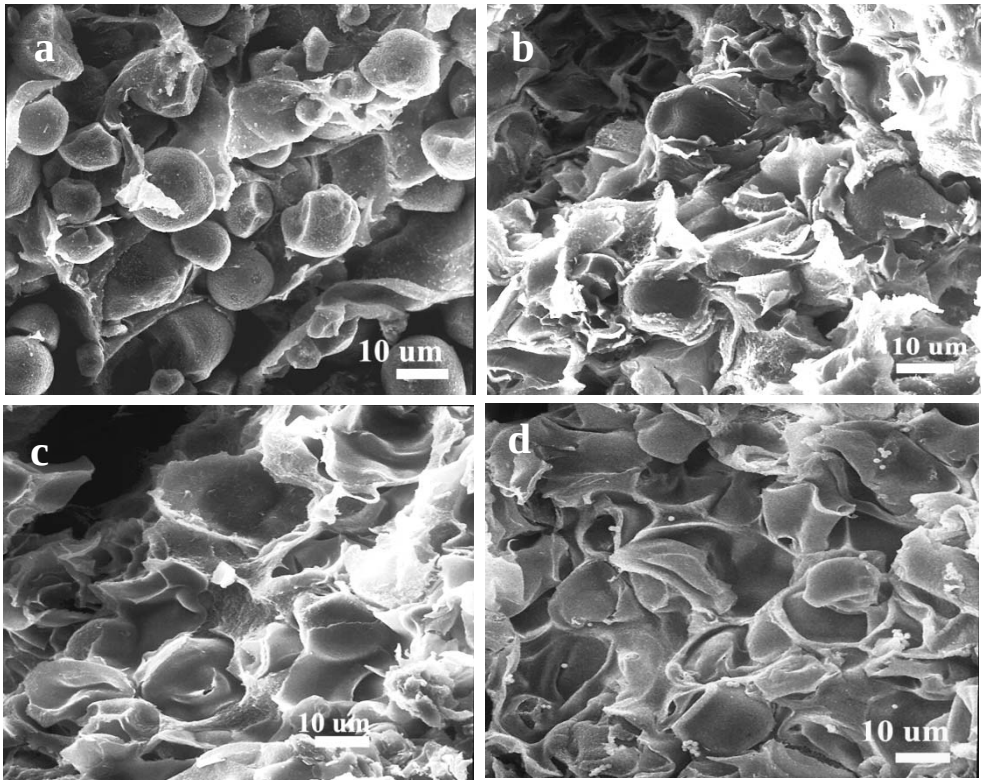

Figure 4.5 Scanning electron micrographs (SEM) of cationic tapioca starch in presence and absence of various M_w of chitosan in 0.5% acetic acid solution: (a) cationic tapioca starch alone, (b) cationic tapioca starch/ high M_w of chitosan mixture, (c) cationic tapioca starch/ medium M_w of chitosan mixture (d) cationic tapioca starch/ low M_w of chitosan mixture (1000x, Bar = 10 μm).

4.4 Thermal properties of starch and starch-chitosan mixtures

The gelatinization temperatures of 12% (w/w) NT, AT, and CT starches alone and starch/chitosan (5.5/0.5, w/w ratio) mixtures, determined by DSC, are summarized in Table 4.3. In the case of native and anionic tapioca starches, the onset (T_o), peak (T_p), and conclusion (T_c) gelatinization temperatures were significantly ($p<$ 0.05) increased by the addition of these chitosans regardless of their molecular weights. In contrast, the addition of chitosans to cationic starch did not affect the gelatinization temperatures, except for the low M_w chitosan which significantly increased T_c as compared with that of the control.

The addition of chitosans with various M_w significantly decreased the gelatinization temperature ranges (T_c-T_o) of native starch and seemed to be unaffected for anionic and cationic starches, except for the addition of CSL to CT which resulted in a significant increase in T_c-T_o.

The gelatinization enthalpies (ΔH_1) were significantly decreased for native starch, increased for anionic starch, and unaffected for cationic starch when chitosans were added to these starches. However the effects appeared to be independent of the M_w of chitosan tested.

Table 4.3 Gelatinization temperature and enthalpy ratio for 12% (w/w) starches alone and starch/chitosan mixtures at a ratio of 5.5/0.5 measured by the differential scanning calorimeter (DSC)[1,2].

Sample[3]	T_o (°C)	T_p (°C)	T_c (°C)	T_c -T_o (°C)	ΔH_1 (J/g)
NT	64.3 ± 0.13[b]	70.7 ± 0.24[b]	78.4 ± 0.19[b]	14.1 ± 0.32[a]	13.2 ± 0.32[a]
NT/CSL	65.9 ± 0.06[a]	72.5 ± 0.32[a]	79.2 ± 0.27[a]	13.2 ± 0.23[b]	6.9 ± 0.32[b]
NT/CSM	66.1 ± 0.40[a]	72.4 ± 0.19[a]	79.0 ± 0.15[a]	12.9 ± 0.19[b]	6.6 ± 0.10[b]
NT/CSH	66.0 ± 0.18[a]	72.4 ± 0.17[a]	79.1 ± 0.18[a]	13.0 ± 0.36[b]	7.5 ± 0.43[b]
AT	64.7 ± 0.03[b]	70.4 ± 0.44[b]	77.8 ± 0.13 [b]	13.1 ± 0.15[a]	12.7 ± 0.68[b]
AT/CSL	66.0 ± 0.12[a]	71.6 ± 0.11[a]	79.0 ± 0.13[a]	13.0 ± 0.10[a]	15.0 ± 0.50[a]
AT/CSM	65.7 ± 0.13[a]	71.6 ± 0.01[a]	78.7 ± 0.21[a]	12.9 ± 0.09[a]	14.1 ± 0.16[a]
AT/CSH	65.7 ± 0.90[a]	71.5 ± 0.01[a]	78.5 ± 0.09[a]	12.8 ± 0.17[a]	14.2 ± 0.87[a]
CT	55.4 ± 0.15[a]	62.4 ± 0.23[a]	71.8 ± 0.16[b]	16.4 ± 0.31[b]	12.1 ± 0.26[a]
CT/CSL	54.9 ± 0.03[b]	62.9 ± 0.09[a]	73.0 ± 0.13[a]	18.1 ± 0.11[a]	12.8 ± 0.23[a]
CT/CSM	55.5 ± 0.16[a]	63.0 ± 0.15[a]	71.9 ± 0.29[b]	16.5 ± 0.15[b]	11.9 ± 0.52[a]
CT/CSH	55.5 ± 0.33[a]	63.0 ± 0.29[a]	71.7 ± 0.15[b]	16.2 ± 0.22[b]	11.3 ± 0.27[b]

[1] Assays were performed in triplicate. Mean ± standard deviation values in the same column followed by the same superscripts are not significantly different ($p > 0.05$).

[2] T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH_1, gelatinization enthalpy.

[3] Refer of Table 4.1 for the sample codes.

4.5 Dynamic viscoelastic properties

Mechanical spectra of the starch gels in the presence and absence of chotosan with various M_w obtained immediately after gelatinization and cooling to room temperature are illustrated in Figure 4.6. These rheograms show that the storage modulus (G') was much larger than the loss modulus (G''), both moduli show only slight increases with increasing frequency (ω), and a cross over between these two moduli was not observed throughout the tested frequency range. This behavior may be classified rheologically as a typical weak gel structure, as proposed by Clark and Ross-Murphy (1987).

The addition of chitosan with various M_w showed an increase in G′ and G″ values of the starch gels as compared with those of the control gels. This effect appeared to be more pronounced for the NT/CS systems, in which G′ and G″ values increased with increasing M_w of chitosan. A comparison of G′ and G″ values measured at an angular frequency of 1 rad/s was also made in Figure 4.7.

The loss tangent (tan δ = G″/G′) values of the NT/CS and AT/CS mixed gels were generally higher than those of the control starch alone gels (Figure 4.8a and 4.8b), whereas the CT/CS gels exhibited a slightly lower tan δ values as compared with the CT alone gel (Figure 4.8c). The tan δ values of all samples measured at a frequency of 1 rad/s were compared in Figure 4.9.

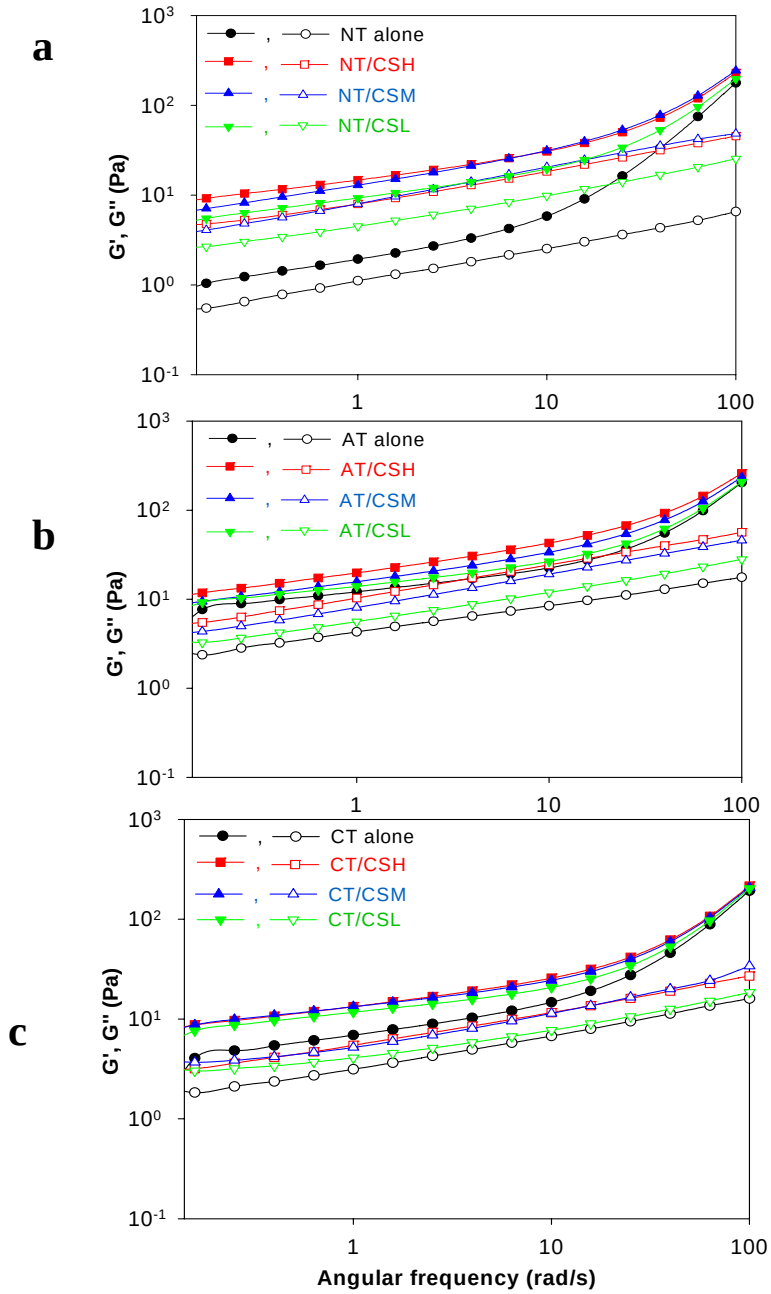

Figure 4.6 Frequency dependence of storage modulus, G' (closed symbol) and loss modulus, G'' (open symbol) of 6% w/w, starches, i.e. (a) native starch (b) anionic starch and (c) cationic starch alone and starch/chitosan mixture gels at a ratio of 5.5/0.5 immediately after gelatinization. Measurements were made at 0.5% strain and 25°C. Refer to Table 4.1 for the sample.

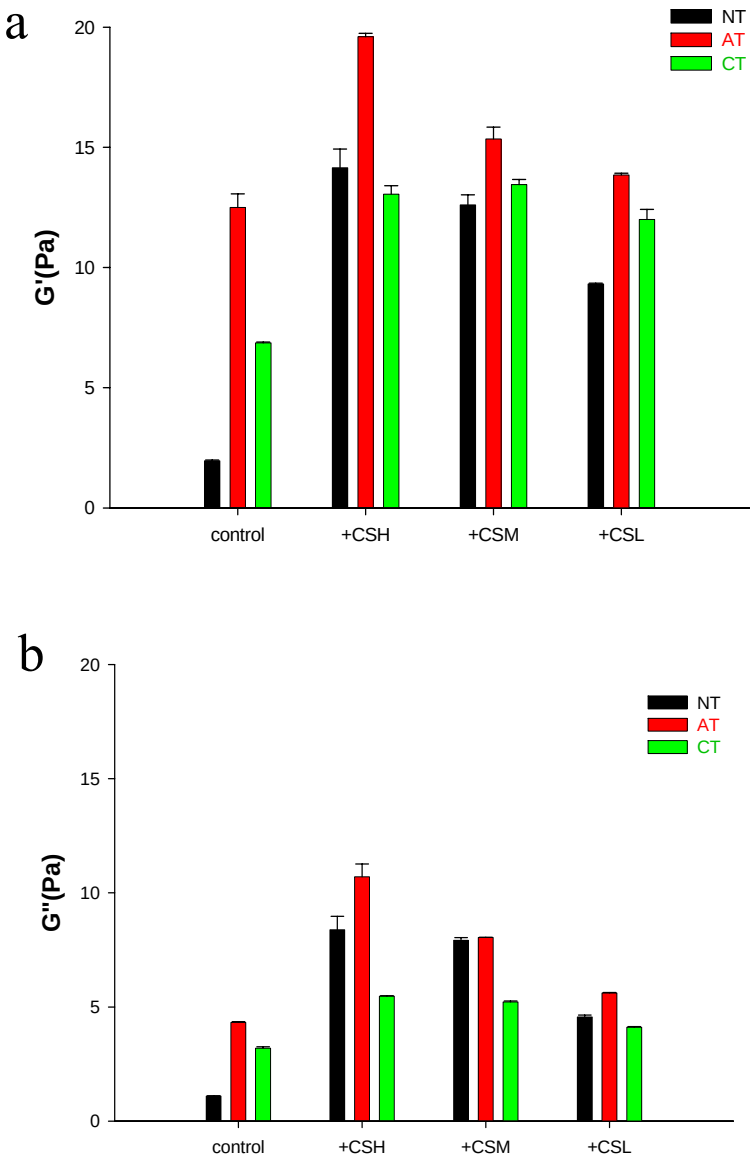

Figure 4.7 Histogram of Frequency dependence of storage modulus, G' (a) and loss modulus, G'' (b) at 1 angular frequency (rad/s) of 6% w/w, starch alone and starch/chitosan mixture gels at a ratio of 5.5/0.5 immediately after gelatinization. Measurements were made at 0.5% strain and 25°C. Refer to Table 4.1 for the sample.

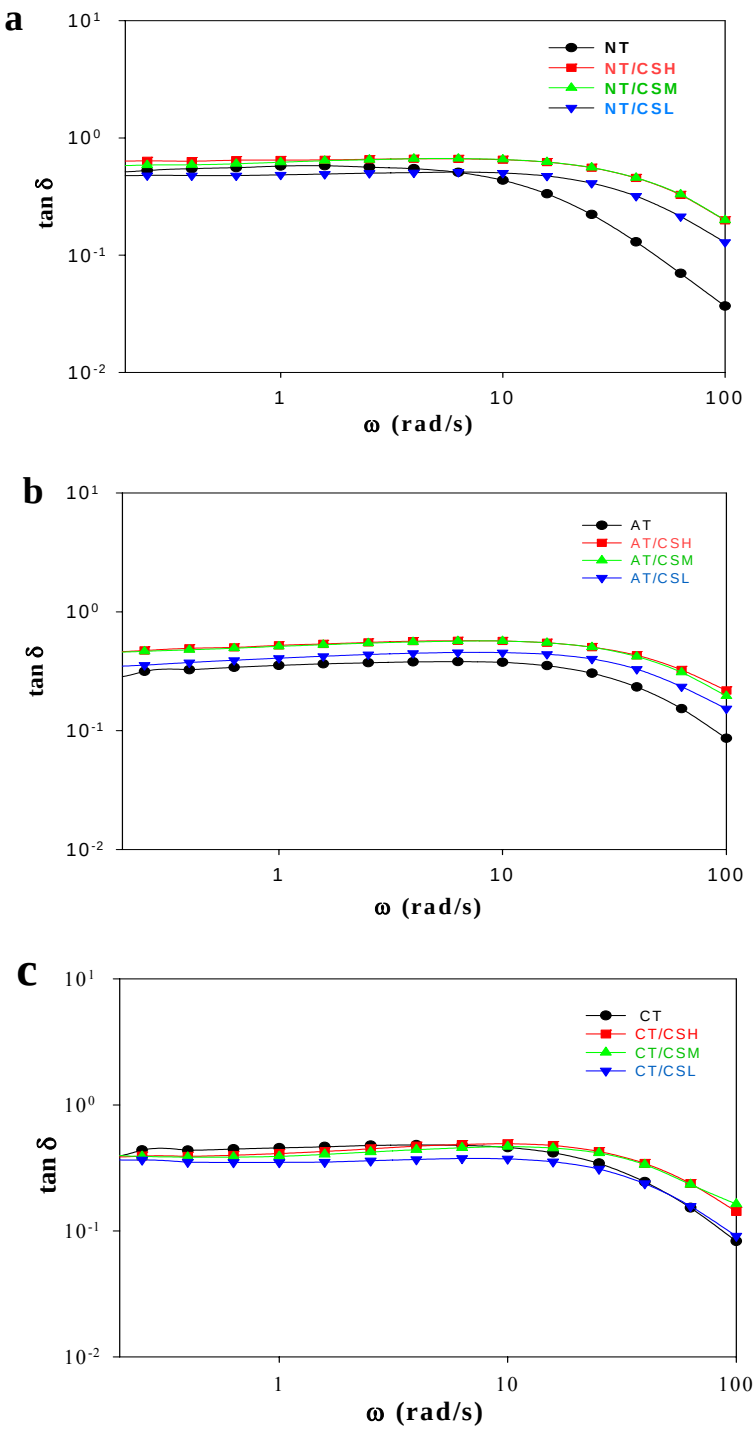

Figure 4.8 Dynamic mechanism loss tangents (tan δ) of 6% w/w, starches, i.e. (a) native starch (b) anionic starch and (c) cationic starch alone and starch/chitosan mixture gels at a ratio of 5.5/0.5 immediately after gelatinization. Measurements were made at 0.5% strain and 25°C. Refer to Table 4.1 for the sample codes.

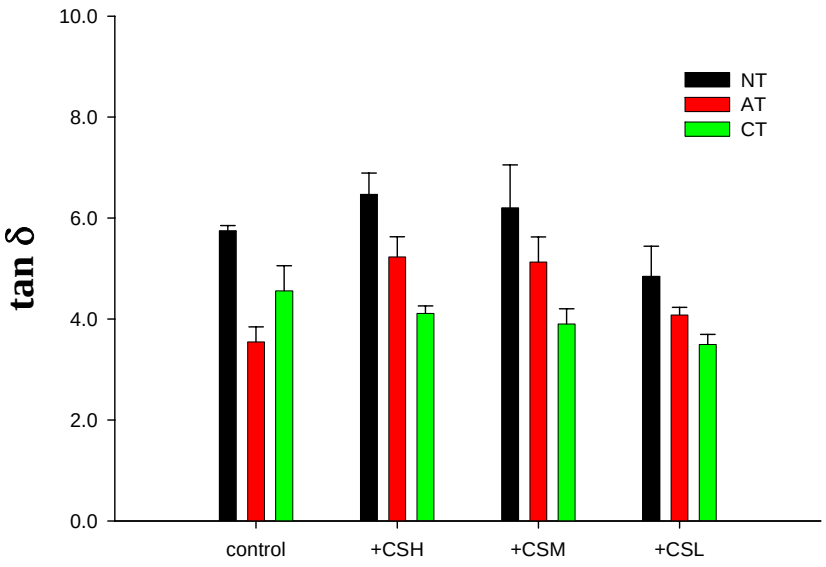

Figure 4.9 Dynamic mechanism loss tangents (tan δ) at 1 angular frequency (rad/s) of 6% w/w, starch alone and starch/chitosan mixture gels at a ratio of 5.5/0.5 immediately after gelatinization. Measurements were made at 0.5% strain and 25°C. Refer to Table 4.1 for the sample codes.

4.6 Steady shear rheological properties

The steady flow characteristics of the starches (6%) and starch/chitosan mixed gels (5.5%starch and 0.5%chitosan) are shown in Figure 4.10. For the range of shear rates from 0 to 300 s^{-1} (up curve) and then back to 0 (down curve), the power law model accurately described the flow behavior of each gel. A hysteresis loop area was seen and calculated from this ascending and descending curves as well. For the range of shear rate used in this study, all gels exhibited mainly time-dependent shear-thinning (thixotropic).

For the fresh gels, the consistency coefficients (K) and flow behavior indices (n) along with the coefficients of determination (R^2) for each upward or downward flow curve, and hysteresis loop areas between these two curves are summarized in Table 4.4. All the fitted curves illustrated classically pseudoplastic and shear-thinning behavior, for which $n < 1$.

The addition of CS to all starches resulted in a noticeable increase in K values and decrease in n values of the gels, in which the effect was more pronounced with increasing M_w of CS. Moreover, hysteresis loop areas of all NT/CS and AT/CS mixed gels, except for the NT/CSL sample, were lower than those of the starch alone gels. In contrast, the hysteresis loop areas of the CT/CS mixed gels were higher than that of the control CT alone gel. The effect was varied with the M_w of chitosan used, i.e. increased with increasing M_w for the NT/CS and AT/CS gels and decreased with increasing M_w for the CT/CS gels

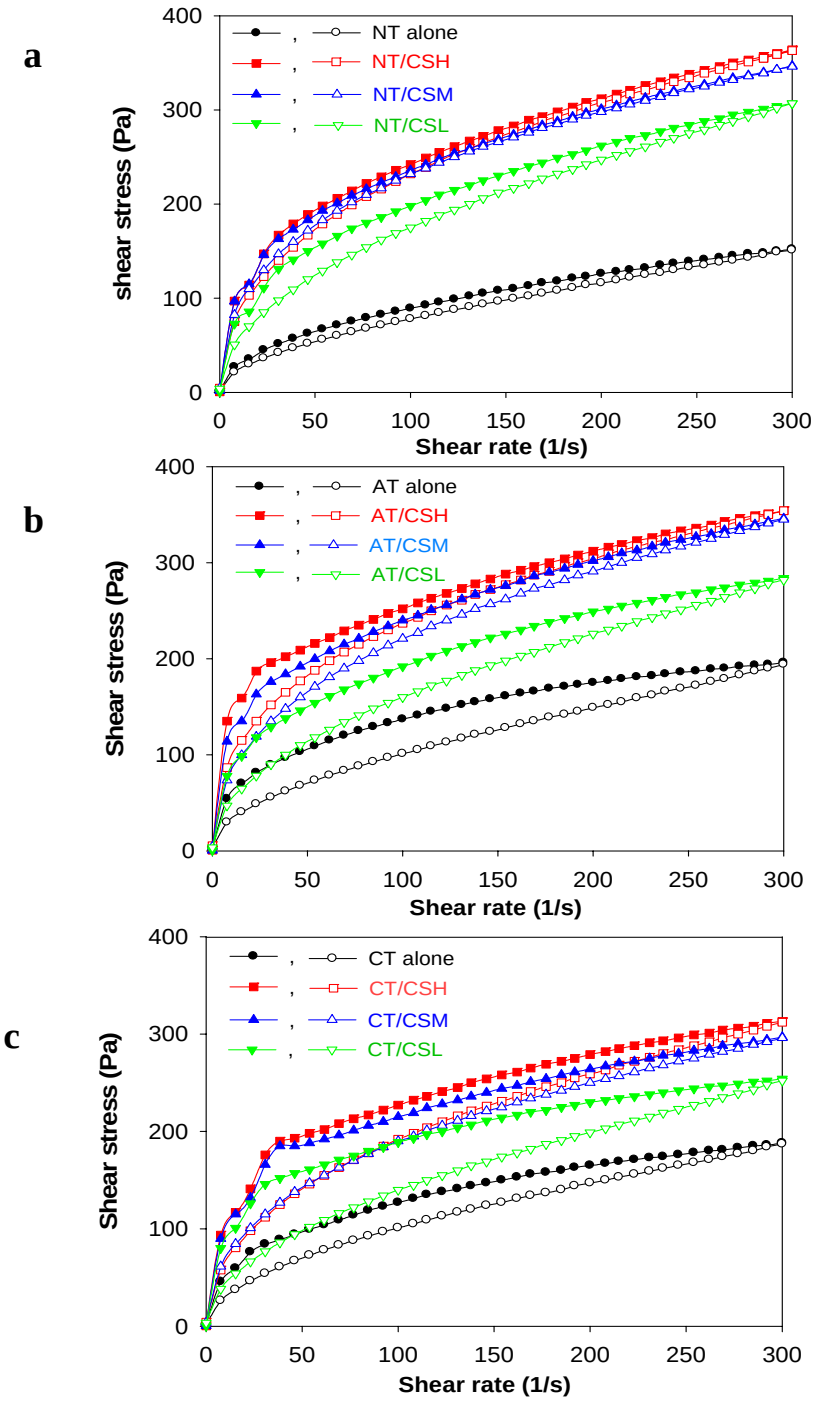

Figure 4.10 Flow curves of 6%, w/w, starches, i.e. (a) native starch (b) anionic starch and (c) cationic starch with and without various chitosan mixture gel at a ratio of 5.5/0.5 immediately after gelatinization. Measurements were made at 25°C. Closed symbols represent upward flow curves and open symbols represent downward flow curves. Refer to Table 4.1 for the sample.

Table 4.4 The power law parameters for 6% (w/w) starch alone and starch/chitosan mixture gels at a ratio of 5.5/0.5 immediately after gelatinization and cooling to room temperature (25°C)[1].

Samples[2]	Hyteresis loop area (Pa/s)	Upward curve			Downward curve		
		K	n	r^2	K	n	r^2
NT	2,020	9.39	0.47	0.998	14.09	0.54	0.996
NT/CSL	3,861	24.40	0.42	0.999	15.48	0.50	0.999
NT/CSM	1,770	32.26	0.38	0.999	24.42	0.42	0.999
NT/CSH	1,305	45.98	0.36	0.999	36.68	0.39	0.999
AT	8,068	26.91	0.35	0.999	9.31	0.53	0.999
AT/CSL	7,378	36.52	0.36	0.992	16.07	0.49	0.999
AT/CSM	5,011	60.05	0.31	0.997	32.36	0.42	0.999
AT/CSH	4,544	76.71	0.26	0.998	41.55	0.38	0.999
CT	5,821	22.05	0.38	0.991	8.62	0.52	0.999
CT/CSL	11,165	49.16	0.29	0.992	13.27	0.50	0.999
CT/CSM	7,311	52/05	0.29	0.992	21.27	0.42	0.999
CT/CSH	6,279	54.41	0.30	0.985	27.49	0.38	0.999

[1]Power law equation; K, consistency coefficient (Pa s^n); n, flow behavior index (dimensionless). Measurements were made at 25°C

[2] Refer of Table 4.1 for the sample codes.

CHAPTER V
DISCUSSIONS

5.1 Pasting characteristics

The results showed that cationic tapioca starch (CT) alone exhibited higher peak, breakdown, final, and setback viscosities and lower pasting temperature and peak time than the anionic (AT) and native (NT) starches (Table 4.1). This could be attributed to the fact that the cationic starch as a modified starch had higher swelling power than the anionic and native tapioca starches (see Section 4.2), leading to lower rigidity of the starch granular structure (Lii et al., 1996). It can be concluded that the pasting properties of starch depended mainly on interactions among the close-packed granules and their rigidity during the heating process.

In general, the results showed a significant increase in peak, breakdown, final, and setback viscosities, pasting temperatures and peak time of both NT and AT by addition of chitosans with various M_w used in this study. In addition, the effect was found to be more pronounced with increasing M_w of chitosan, except for pasting temperatures which seemed to be unaffected. The increase in viscosity reflects the ability of the starch granules to swell freely before their physical breakdown (Rojas et al., 1999). However, the addition of gums affected the starch concentration by immobilizing water molecules (Yoshimura et al., 1996). When the aqueous suspensions of the starch/gum mixtures were heated, the starch gelatinized in the gum medium, a volume of continuous phase accessible to the gum was reduced, yielding in an increase in gum concentration. This resulted in a dramatic increase in the viscosity of the continuous phase and in turn the overall viscosity of the suspension itself owing to the thickening properties of the gum (Alloncle et al., 1989). According to the results an increase in M_w of chitosan resulted in a higher viscosity of the mixtures during pasting due to the high viscosity of the high M_w chitosan. The increase in viscosity

would make the shear forces exerted on the swollen granules in the shear field much larger than those encountered in starch/water suspensions (Christianson, et al., 1981).

This results in the loss of granule integrity and subsequent disruption leading to a reduction in the paste viscosity, which is defined as breakdown viscosity, and release of more solubilised starch, primarily amylose. It appeared that interactions between certain leached starch molecules and gums could be responsible for an increase in final viscosities of starch/gum mixtures (Christianson et al., 1981; Shi & BeMiller, 2002). In the case of cationic starch, addition of chitosan resulted in a significant (p<0.05) reduction of peak and breakdown viscosities except for the cationic starch/high M_w chitosan mixture (CT/CSH) in which the peak viscosity was significantly increased. The repelling forces between the positively charged ammonium groups on the cationic starch granules and the positively charged amino groups on the chitosan molecules could be the cause. This hypothesis was proven in details by Shi and BeMiller (2002), who concluded that retardation of granule destruction and leaching of amylose seems to be the cause of the delay in pasting and reduction in peak viscosity when the starch and gum had the same charge. Chaisawang and Suphantharika (2006) also reported that the gelatinization of starch granules and gum molecules both having the same ionic charge was retarded as compared with the starch alone due to the repulsion of both molecules. In contrast, the CT/CSH sample exhibited a significantly higher peak viscosity than the control possibly due to the high viscosity of CSH counteracted the charge repulsion effects of peak viscosity reduction. A significant (p<0.05) shift in the gelatinization to a higher temperature in the presence of gums could be resulted from the decrease of water availability (Ferrero et al., 1996), which could make starch gelatinization more difficult (Kruger et al., 2000).

5.2 Swelling power

According to the swelling power (SP) measurement by method of Mandala and Bayas (2004), the assumption that the whole amount of gum is remained in the continuous phase is not exactly true due to sedimentation of gum with the starch granules. The degree of swelling and granule integrity are directly related to the viscosity of starch paste (Borwanker, 1992). In the case of native and anionic tapioca starches, chitosan exhibited a slight increase in the SP of these starches at high

temperature as compared with the control starch alone. Moreover, the SP profiles obtained from this study were similar to those previously reported by Li and Yeah (2001) and Chaisawang and Suphantharika (2006) for native tapioca starch. In addition, maximal swelling might also be related to the molecular weight of chitosan molecules. It suggested that most of native and anionic tapioca starch granules can swell freely in the gum solutions. For the cationic tapioca starch, its higher SP value as compared with that of the native starch could be attributed to the substitution of hydroxyl group in the starch molecules by the cationic ammonium groups which also caused a marked reduction in pasting temperature. The reduction of SP values of the CT starch in the presence of chitosan at higher temperatures was likely to be due to the electrostatic repulsion between the starch granules and chitosan molecules. It was suggested that these strong electrostatic interactions suppressed the swelling of starch granules. In general, the effect of chitosan on the SP of these starches was slightly varied with its M_w. The evidence in this study demonstrated that the presence of chitosan exhibited different effect depending on the chemical and electrostatic interactions between the starch granules and chitosan molecules.

5.3 Morphological structures of starch pastes in presence and absence of chitosan

From the morphological structure of starch pastes in the presence and absence of chitosan, the starch granules gelatinized in 0.5% acetic acid exhibited tiny coverage of agarose gel which was used for fixation of the pastes. Chitosan wrapped around the granules of NT and AT starches but formed a sheet structure in the continuous phase of the CT/CS systems indicating difference in the electrostatic interactions between starch granules and chitosan molecules. In the case of CT/CS systems, chitosan did not wrap the starch granules due to the repelling forces between the same charge of starch and chitosan molecules. Polymer chains of chitosan were incapable to penetrate the granule, and consequence, adsorbed only on the surface and stabilized granular shape.

5.4 Thermal properties of starch and starch-chitosan mixtures

Studies by Rao (1999) on corn starch, DSC data were compared with dynamic rheological data. The initial temperature of gelatinization from both methods was about the same. In our experiment the T_p from DSC (Table 4.3) was not much different with pasting temperature from RVA (Table 4.1). It was suggested that energy was required for reversible swelling of starch granules before the pasting temperature was reached. In the cases of native and anionic tapioca starches significant shift of T_o, T_p, and T_c (about 2°C) to higher values was observed due to chitosan additions. On the other hand, addition of chitosan did not affect the gelatinization temperatures of cationic tapioca starch. However, these gelatinization temperatures seemed to be unaffected by the molecular weight of chitosan. The increase in the gelatinization temperatures of NT and AT starches could be attributed to the different reasons; lower amount of free water to starch ratio due to immobilization of water molecules by gums (Biliaderis, Maurice, and Vose, 1980; Chungcharoen, 1987), lower heat transfer rates (Kruger, Ferrero, & Zaritzky, 2003), and mass transfer of water, which could make starch gelatinization more difficult in the presence of wrapping chitosan. In the case of CT/CS system where chitosan did not wrap the CT granules, the starch can gelatinize freely. Chitosan had an effect on the gelatinization enthalpy (ΔH) of native and anionic tapioca starches. In the case of NT starch, a decrease of two fold of ΔH values by addition of chitosan with various molecular weights suggested that such changes in limited amount of water might contribute to a decrease in endothermic size by reducing the energy difference between the granular starches with and without chitosans. In contrast, the anionic tapioca starch showed the opposite result, i.e. increasing of ΔH with addition of chitosan. For cationic tapioca starch, the enthalpy was not different from those of the control. The gelatinization temperature ranges (T_c-T_o) of all starches studied were slightly affected by chitosan addition. It is generally reported that the gum may interact with the starch to produce an increase (Lui, Eskin, & Cui, 2003) or decrease (Rojas, Rosell, & Benedito de Barber, 1999) in gelatinization temperature ranges (T_c-T_o), depending on the types of starch and gum used.

5.5 Dynamic viscoelastic properties

Based on dynamic shear data, native and modified tapioca starch pastes with and without chitosans were rheologically classified as weak gels (Figure 4.6). The main properties of weak gel are given by Clark and Ross-Murphy (1987) as G′ > G″ throughout the accessible frequency range; both G′ and G″ are slightly increased with increasing frequency and the separation of the two moduli (tan δ = G″/G′) is smaller than 0.1 for typical polysaccharide gels. The increase in G′ values was also related to the rate of amylose and amylose-gums associations occurred during cooling. The increase in G′ can not be attributed to the aggregations of amylopectin because amylopectin retrograded in long time periods (Biliaderis & Prokopowich, 1994). As suggested by Ferrero, Martino and Zarritzky (1994), gum - amylose association against amylose - amylose rearrangement essential for retrogradation. The loss tangent (tan δ = G″/G′) values of the NT/CS and AT/CS mixed gels were higher than those of the control (Figure 4.8), whereas those of the CT/CS gels were lower than the control (Figure 4.8). Chitosan decreased tan δ of starch paste, possibly through strong network formation with starch.

5.6 Steady shear rheological properties

The steady flow characteristics of NT, AT, and CT starches in the presence or absence of chitosan with various molecular weight are presented in Figure 4.10. For the range of shear rates used in this study, all gels exhibited mainly time-dependent shear-thinning (thixotropic) with enhanced pseudoplasticity of starches gels (Maria, Adriana, & Noemi, 2006; Wang, Qiu, Cosgrove, & Denbow, 2009).

CHAPTER VI
CONCLUSION

This study clarified that the pasting and rheological properties of the native (NT) and modified (anionic; AT, and cationic; CT) tapioca starches were largely affected by the addition of chitosan with various molecular weights. Results of RVA indicated the increases in peak, breakdown, final, and setback viscosities, pasting temperatures, and peak times of the NT and AT starch dispersions during pasting as compared with the control. In contrast, addition of chitosan, except for the one with high molecular weight, resulted in a significant decrease in peak viscosities. These results can be attributed to the difference in ionic interactions between various starches and chitosans as well as the difference in molecular weights and in turn viscosities of chitosan itself. Viscosity of the starch pastes with an addition of chitosan was synergistically increased due to interaction between leached components of starch and chitosan. However, increasing molecular weight of chitosan used in the continuous phase resulted in intermolecular associations that play an important role in the viscoelastic behavior of the composite. Morphological study revealed that chitosan wrapped the NT and AT starch granules but did not wrap the CT granules due to the repelling forces between the positively charged starch and chitosan. This result was supported by the DSC data which demonstrated that the gelatinization temperatures (T_o, T_p and T_c) of NT and AT starches increased by addition of chitosan with various molecular weights wheras those of CT starch seemed to be unaffected. Dynamic viscoelastic measurements on the fresh starch/chitosan mixed gels indicated that the G' and G'' were increased by the addition of chitosan with various molecular weights. A slight increase in tan δ values of the starch/chitosan mixed gels, except for the CT/CS gels, as compared with those of the starch alone gels, indicating the

development of weaker gel structures. Steady flow tests showed that all gels exhibited mainly time-dependent shear-thinning (thixotropic) with no yield stress behavior.

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Chawanthayatham, S., & Suphantharika, M. (2009, February 12-13). *Effect of Chitosan Additions on Physical and Rheological Properties of Native and*

Modified Tapioca Starch. Oral presented at the The 12th National Graduate Research Conference, Khonkhan University, Thailand.

PUBLICATION

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