# GELATINIZATION AND RETROGRADATION OF RICE STARCH-XANTHAN MIXTURES: EFFECTS OF MOLECULAR WEIGHT OF XANTHAN AND DIFFERENT SALTS

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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**
**2008**

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Thesis
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was submitted to the Faculty of Graduate Studies, Mahidol University
for the degree of Master of Science (Biotechnology)
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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.

My sincerely gratitude is also given to all members of the advisory committee, Dr. Pairoj Luangpituksa, Dr. Jirarut Wongkongkatep and Asst. Prof. Dr Jirarat Tattiyakul for their valuable suggestion, expert comments, generosity in contributing time and criticism with kindness about the work of my thesis.

I am thankful to Cho Heng Rice Vermicelli Factory Co. Ltd. I am grateful to Mettler-Toledo (Thailand) Ltd. for kindly providing the differential scanning calorimeter (DSC) and all technician staffs for their supporting and suggesting me during this research. Metrohm Siam Ltd., Mr. Passakorn, for kindly providing the rheometer used in these experiments, and for valuable comments.

I am also particularly grateful to all members of the room BT204, BT208 and BT210 for their helps, friendship and encouragement and also to “Mae-Bann” group and my all fellows in the Department of Biotechnology, Faculty of Science for their helps and providing facilities during my study.

I would like to specially express my gratitude and deepest appreciation to my father, mother and my sisters for their love, kindness, understanding, patience and encouragement throughout my study.

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

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## ABSTRACT

Effects of molecular weight ($M_w$) of xanthan (XG) and salts (0.1 M NaCl or $CaCl_2$) on the gelatinization and retrogradation of rice starch (RS) were studied. A series of five XG samples, having various $M_w$, was prepared by homogenization of native XG solutions in the presence or absence of salts. The presence of salts greatly reduced the intrinsic viscosities, [$\eta$], of all XG solutions. Rapid visco-analysis (RVA) data showed that XG addition increased the peak, breakdown, final, and setback viscosities of RS, either in the presence or absence of salts, whereas the pasting temperatures were unaffected. Differential scanning calorimetry (DSC) data demonstrated that the onset ($T_o$), peak ($T_p$), and conclusion ($T_c$) gelatinization temperatures of RS were unaffected by XG addition but slightly increased by $CaCl_2$ addition, whereas the gelatinization enthalpies ($\Delta H_1$) were significantly decreased by addition of XG and salts. Dynamic shear data revealed weak gel-like behavior in all paste samples in which their rigidity was decreased by XG addition. Flow tests showed that all pastes exhibited time-dependent shear-thinning (thixotropic) with yield stress behavior in which the hysteresis loop areas were significantly decreased by XG addition, whereas the other rheological parameters varied differently among the samples, with and without added salts. Storage of the mixed gels, with or without added salts, at 4°C resulted in a decrease in $T_o$, $T_p$, $T_c$, and melting enthalpies ($\Delta H_2$) and an increase in the phase transition temperature ranges ($T_c$-$T_o$) compared to those obtained from the first run. The addition of XG increased the retrogradation ratio ($\Delta H_2/\Delta H_1$) of RS but did not affect the ($T_c$-$T_o$) and these results seemed to be unaffected by added salts. The syneresis and viscoelastic characteristics of the mixed gels, either in the presence or absence of salts, increased with storage time, but this effect was reduced by XG addition. In general, the effects of XG addition on the gelatinization and retrogradation behavior of RS were more pronounced with increasing $M_w$ of XG and these effects depended on salts added.

KEYWORDS: RICE STARCH/ XANTHAN/ SALT/ MOLECULAR WEIGHT/ GELATINIZATION/ RETROGRADATION

p. 122

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เจลาติไนเซชั่นและเรโทรเกรเดชั่นของของผสมแป้งข้าวจ้าวและแซนแทนกัม: ผลของขนาดโมเลกุลของแซนแทนและเกลือชนิดต่างๆ
(GELATINIZATION AND RETROGRADATION OF RICE STARCH-XANTHAN MIXTURES: EFFECTS OF MOLECULAR WEIGHT OF XANTHAN AND DIFFERENT SALTS)

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## บทคัดย่อ

การศึกษาผลของขนาดโมเลกุลของแซนแทนกัมและเกลือชนิดต่างๆ (0.1 M NaCl หรือ $CaCl_2$) ต่อการเกิดเจลาติไนเซชั่นและเรโทรเกรเดชั่นของแป้งข้าวจ้าว แซนแทนกัมที่มีขนาดโมเลกุลต่างๆกัน 5 ตัวอย่าง เตรียมโดยการ homogenization ของสารละลายแซนแทนที่มีเกลือหรือไม่มีเกลือ เกลือในสารละลายจะทำให้ค่า intrinsic viscosity [$\eta$] ของแซนแทนลดลงอย่างมาก ผลของ Rapid Visco-Analysis (RVA) แสดงให้เห็นว่าการเติมแซนแทนจะทำให้ค่าความหนืดสูงสุด ค่าความหนืดที่ลดลงในช่วงให้ความร้อน ค่าความหนืดที่เพิ่มขึ้นในช่วงลดอุณหภูมิ และค่าความหนืดสุดท้ายของแป้งข้าวเพิ่มขึ้นทั้งในสารละลายที่มีเกลือและไม่มีเกลือในขณะที่อุณหภูมิที่เกิดการเจลาติไนเซชั่นไม่ได้รับผลกระทบดังกล่าว ผลของ differential scanning calorimetry (DSC) แสดงให้เห็นว่าอุณหภูมิที่เริ่มเกิดเจลาติไนเซชั่น ($T_o$) อุณหภูมิสูงสุด ($T_p$) และอุณหภูมิเมื่อสิ้นสุดการเกิดเจลาติไนเซชั่น ($T_c$) ของแป้งข้าวไม่ได้รับผลกระทบจากการเติมแซนแทน แต่มีค่าเพิ่มขึ้นเล็กน้อยจากการเติม $CaCl_2$ ในขณะที่ค่าพลังงานความร้อนที่ใช้ในการเจลาติไนเซชั่น($\Delta H_1$) ลดลงอย่างมีนัยสำคัญจากการเติมแซนแทนและเกลือ ผลของการวัดรีโอโลยีแบบไดนามิคแสดงให้เห็นว่า เจลของตัวอย่างมีพฤติกรรมเป็นเจลชนิดอ่อน โดยมีค่าความแข็งของเจลจะลดลงเมื่อเติมแซนแทน การทดสอบการไหลแสดงให้เห็นว่าเจลมีพฤติกรรมการไหลแบบ time-dependent shear-thinning (thixotropic) และมีค่า yield stress ด้วย โดยที่ค่า hysteresis loop area ลดลงอย่างมีนัยสำคัญเมื่อเติมแซนแทน ในขณะที่ค่า rheological parameter อื่นๆมีค่าที่เปลี่ยนแปลงแตกต่างกันในแต่ละตัวอย่างทั้งที่มีและไม่มีเกลือ การเก็บเจลที่มีและไม่มีเกลือไว้ที่อุณหภูมิ 4 องศาเซลเซียส ทำให้ค่า $T_o$, $T_p$, $T_c$, และค่าพลังงานความร้อนที่ใช้ในการหลอมเหลว ($\Delta H_2$) ลดลง และช่วงอุณหภูมิที่เปลี่ยนแปลง ($T_c$-$T_o$) ของเจลของผสมเพิ่มขึ้นเมื่อเทียบกับค่าที่ได้จากการทดสอบครั้งแรก การเติมแซนแทนทำให้ค่าอัตราการเกิดเรโทรเกรเดชั่น ($\Delta H_2/\Delta H_1$) ของแป้งข้าวเพิ่มขึ้นแต่ไม่มีผลต่อค่า $T_c$-$T_o$ และการเติมเกลือไม่มีผลกระทบต่อค่าดังกล่าว ค่า syneresis และคุณสมบัติของวัตถุกึ่งแข็งกึ่งเหลวของเจลทั้งที่มีและไม่มีเกลือเพิ่มขึ้นตามระยะเวลาของการเก็บ แต่การเติมแซนแทนทำให้ผลกระทบดังกล่าวลดลง กล่าวโดยทั่วไปผลกระทบของการเติมแซนแทนต่อการเจลาติไนเซชั่นและเรโทรเกรเดชั่นของแป้งข้าวจะมากขึ้นเมื่อขนาดของโมเลกุลของแซนแทนเพิ่มขึ้นและผลกระทบดังกล่าวจะขึ้นอยู่กับชนิดของเกลือที่เติมลงไปด้วย
122 หน้า

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# CONTENTS



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# CONTENTS

(continued)



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# CONTENTS

(continued)



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# CONTENTS

(continued)



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# LIST OF TABLES



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# LIST OF TABLES

(continued)

**TABLE** **Page**



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# LIST OF FIGURES



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# LIST OF FIGURES

(continued)

**FIGURE** **Page**



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# LIST OF FIGURES

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**FIGURE** **Page**



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# LIST OF FIGURES

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**FIGURE** **Page**



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**FIGURE** **Page**



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(continued)

**FIGURE** **Page**



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# LIST OF FIGURES

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**FIGURE** **Page**



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# LIST OF ABBREVATIONS

| | |
|---|---|
| & | And |
| ANOVA | Analysis of variance |
| C | Concentration |
| DP | Degree of polymerization |
| DSC | Differential scanning calorimeter |
| ed. | Edition |
| 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 |
| GPC | Gel permeation chromatography |
| h | Hour |
| $\Delta H$ | Enthalpy, J/g |
| i.e. | Id est (Latin), that is |
| J | Joule |
| $K$ | Consistency coefficient, Pa $s^n$ |
| mg | Milligram |
| ml | Milliliter |
| min | Minute |
| M | Molarity |
| $M_w$ | Weight-average molecular weight |
| $n$ | Flow behavior index, dimensionless |
| NMR | Nuclear Magnetic Resonance |
| p.(pp.) | Page (pages) |
| Pa | Pascal |
| rad | Radian |

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# LIST OF ABBREVATIONS

(continued)

| | |
|---|---|
| RS | Rice starch |
| RVA | Rapid Visco Analyzer |
| RVU | Rapid Viscosity Units |
| $R^2$ | Coefficient of determination |
| s | Second |
| SEM | Scanning electron micrographs |
| SD | Standard deviation |
| SPSS | Statistical Package for the Social Science |
| 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 |
| μm | Micrometer |
| w/w | Weight by weight |
| XG | Xanthan gum |
| / | Per |
| % | Percent |
| °C | Degree Celsius |
| $\dot{\gamma}$ | Shear rate, 1/s |
| $\sigma$ | Shear stress, Pa |
| $\sigma_0$ | Yield stress, Pa |
| $\omega$ | Angular frequency, rad/s |
| $C^*$ | Critical polymer concentration |
| $\eta$ | Intrinsic viscosity |
| $\eta_{red}$ | Reduced viscosity |
| $\eta_{rel}$ | Relative viscosity |
| $\eta_{sp}$ | Specific viscosity |

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# CHAPTER I
# INTRODUCTION

Rice (*Oryza sativa* L.) starch has many unique attributes that make it one of the most interesting starches in the food industry. Rice starch is hypoallergenic for many, bland in taste, white in color and, as a gel, is smooth in texture (Bao & Bergman, 2004). However, rice starch, in common with other cereal starches, has negative aspects, such as gel syneresis, retrogradation, and tendency to exhibit breakdown, either from extended cooking, high shear or acidic conditions, producing weak-bodied, cohesive, rubbery pastes and undesirable gels (BeMiller, 2007). These short comings can be overcome by the blending of native starches with polysaccharide hydrocolloids as reviewed by Appelqvist and Debet (1997). The mixtures of starch and hydrocolloids have been used widely in food products to modify and control rheological and textural properties, improve moisture retention, control water mobility, and maintain overall product quality during storage.

Xanthan gum is a non-linear anionic microbial heteropolysaccharide produced by aerobic fermentation of the bacterium *Xanthomonas campestris*. Xanthan is widely and more extensively used as a food gum than any polysaccharide other than starch because of its unique and useful properties. Xanthan is soluble in hot or cold water and solutions exhibit high viscosities at low concentrations and are highly pseudoplastic. These characteristics have excellent stability over a wide pH and temperature range and the polysaccharide is resistant to action of enzymes found in food systems (BeMiller, 2007; Sworn, 2000).

Addition of xanthan gum to rice starch is known to modify and control the pasting (Shi & BeMiller, 2002; Song, Kwon, Choi, Kim, & Shin, 2006) and rheological properties (Kim & Yoo, 2006) of starch. In general, however, starch and hydrocolloids usually co-exist with other ingredients in many food formulations. One such ingredient is salt. Salts have been shown to have a significant effect on the gelatinization (Ahmad & Williams, 1999, 2002; Chungcharoen & Lund, 1987; Jane, 1993; Jyothi, Sasikiran, Sajeev, Revamma, & Moorthy, 2005; Maaurf, Che Man, Asbi,

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Junainah, & Kennedy, 2001; Oosten, 1982, 1983, 1990), retrogradation (Alloncle & Doublier, 1991; Alloncle, Lefebvre, Llamas, & Doublier, 1989; Christianson, Hodge, Osborne, & Detroy, 1981; Fanta & Christianson, 1996; Gudmundsson, Eliasson, Bengtsson, & Aman, 1991; Kim & D'Appolonia, 1977; Kohyama & Nishinari, 1992; Sajjan & Rao, 1987; Tye, 1988; Williams & Ahmad, 2000; Yoshimura, Takaya, & Nishinari, 1996, 1999; Yousria & William, 1994) and rheological properties (Ahmad & Williams, 1999) of various starches. Eliasson and Gudmundsson (2006) have reviewed the effect of salts on these properties of starch and found that it depends on the type of salt as well as on the concentration. The effect of salts on xanthan conformation and the solution rheology have been studied by many researchers (Carrington, Odell, Fisher, Mitchell, & Hartley, 1996; Ma & Barbosa–Cánovas, 1997; Meyer, Fuller, Clark, & Kulicke, 1993; Rochefort & Middleman, 1987; Sato, Norisuye, & Fujita, 1984). In the presence of salts, xanthan undergoes a disorder to order conformational transition from a random coil to a helix which affects its solution rheology. Additionally, the molecular weight of hydrocolloids such as guar gum have been found to affect the gelatinization (Funami, Kataoka, Omoto, Goto, Asai, & Nishinari, 2005) and retrogradation (Funami et al., 2004a) behavior of starch. However, there are very few reports in the literature concerning the effect of salts on the starch/hydrocolloid mixtures such as corn starch/xanthan (Sudhakar, Singhal, & Kulkarni, 1995), wheat starch/xanthan (Mandala, Michon, & Launay, 2004), and corn starch/iota-carrageenan (Funami, Noda, Hiroe, Asai, Ikeda, & Nishinari, 2007) combinations. In particular, no attempt has been made to study the effect of molecular weight of hydrocolloids on these combinations. Therefore, the main objective of this study was to investigate the effect of molecular weight of xanthan and salts on the gelatinization, retrogradation and rheological properties of rice starch/xanthan mixtures.

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# CHAPTER II
# LITERATURE REVIEW

## 1. Starch

Starch's unique chemical and physical characteristics and nutritional attributes set it apart from all other carbohydrates. Starch, products derived from starch, and sucrose constitute most of the digestible carbohydrate in the adult human diet, lactose being the only other carbohydrate digestible by humans. Starch provides 70–80% of the calories consumed by human worldwide. It is the predominant food-reserve substance in plants, and the majority of starch consumed by humans occurs naturally within grains (much of it after the grains have been reduced to flours) and in vegetables. Of the edible dry matter in the world's food supply, 70% is in the form of cereals (corn/maize, 22%; wheat, 21%; rice, 16%; other cereal, 11%); 7% is in the form of roots and tubers (primarily potatoes, sweet potatoes, and cassava, all of which are starchy commodities); 6% is in the form of commodities that are not primarily composed of carbohydrates (animal products, 8%; legumes and oilseeds, 4%) (BeMiller, 2007).

Commercial isolated starches are obtained from seeds (particularly from corn, waxy corn (waxy maize), high-amylose corn, wheat, and various rices) and from tubers or roots, particularly potato and cassava (manioc/yucca). Different starches have different properties and are applied by the food industry. The thickening and gelling properties of starch have very positive influences on the sensory character of food products and also have important technological/functional effects in industrial processing and in the kitchen preparation of foods. The amount of starch used in the preparation of food products (which greatly exceeds the amount of all food hydrocolloids combined) does not include the starch in flours used to make bread and other bakery products, that naturally occurring in grains used to make breakfast cereals, or in fruits and vegetables, such as potatoes (BeMiller, 2007).

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### 1.1 Rice starch

Rice (*Oryza sativa* L.) is consumed as cooked milled rice, but a small portion if ground into flour or separated into a starch fraction is used to make pharmaceutical, food and animal feed products. Rice starch is hypo-allergenic and bland in flavor, including a small granule size and soft gel formation. From these properties, rice starch have made it desirable as a fat replacer in foods such as flavored milk base beverage, ice cream, yogurt and salad dressing. From being smooth in texture and less filling than fat, rice starch is finding increasing applications in pastry cream. Rice starch is reported to have a rapid hot-set after such processing techniques as extrusion. This characteristic makes it desirable for use in breakfast cereals that need to remain crisp after exposure to milk. It is also used to generate maltodextrins that are incorporated into food as a filler, flavor carrier, texture modifier or sweetness reducer (Bao & Bergman, 2004).

### 1.2 Starch structure and chemical composition

Rice starch granules are the smallest known to exist in cereal grains, with the size reported to range from 3 to 8 μm (Figure 2.1). Starch is partially crystalline, as typical cereal starches, rice starch has the A-type X-ray diffraction pattern (Figure 2.2). Starch granules are composed of a mixture of two polymers: an essentially linear polysaccharide called amylose and a highly branched polysaccharide called amylopectin (Figure 2.3).

Amylose is essentially a linear chain of (1→4)-linked α-D-glucopyranosyl units. However, some amylose molecules have a few α-D-(1→6) branches (0.3–0.5% of the total linkages). The branches are generally either very long or very short and separated by large distances, allowing the molecules to act essentially as linear molecules, forming strong films and fibers and retrograding easily. The average molecular weight of amylose molecules of different commercial starches is reported to be in the approximate range $1.3 \times 10^5$ to $5 \times 10^5$ (DP ~800–3,000), with wide ranges of polydispersity in all preparations. The axial→equatorial position coupling of the (1→4)-linked α-D-glucopyranosyl units in amylose chains gives the molecules a right-handed spiral or helical shape. A consequence of helix formation is

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that films and fibers from amylose are more elastic than are films and fibers from cellulose molecules. The hydroxyl groups are positioned on the exterior of the coil so that the interior of the helix is lined with hydrogen atoms and is lipophilic (hydrophobic). Most starches contain about 25% amylose.

Amylopectin is a very large, highly branched molecule. Starch molecules are believed to grow from a single β-D-glucopyranosyl unit attached to a self-glucosylating initiator protein molecule called "amylogenin." Other D-glucopyranosyl units are added sequentially, being donated by adenosine diphosphate glucose molecules to produce a chain of α-D-glucopyranosyl units joined by (1→4) linkages. However, in addition to the chain-lengthening enzyme, a branching enzyme is active. After a linear chain reaches 40–50 units, the branching enzyme transfers a portion of the chain near its nonreducing end to a unit toward the reducing end, where the chain portion becomes an α-D (1→6)-linked branch, whereupon both nonreducing ends may continue to be elongated. The branch point linkages constitute 4–5% of the total linkages. An amylopectin molecule consists of a chain, called the C chain, which carries the one reducing end-group and numerous branches, termed B chains, to which A chains are attached. Pairs of nonreducing chain ends in clusters are entwined around each other in parallel double helices. Average structures, average molecular weights, molecular weight ranges, and, perhaps, shape of amylopectin molecules vary with the botanical source. However, most amylopectin molecules have a trimodal distribution of branch chain lengths. The smallest fraction consists of the outermost chains, that is, the A chains. The other two fractions are generally referred to as "short B chains" and "long B chains." Average molecular weights of amylopectin molecules are reported to be as $2 \times 10^9$ (DP $\sim 10^7$), making them among the largest molecules in nature (Bao & Bergman, 2004).

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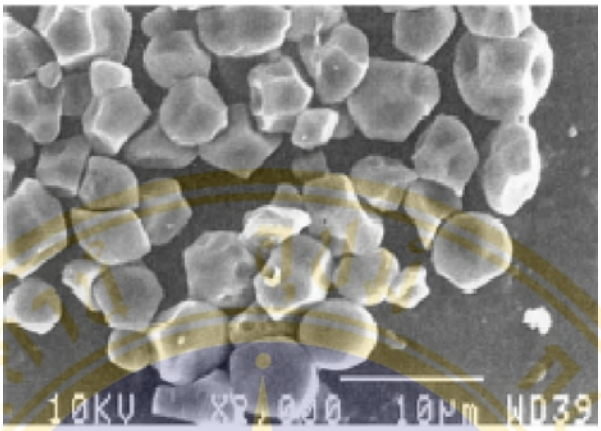


**Figure 2.1** Scanning electron micrographs (SEM) of rice starch (bar = 10 μm) (Bao & Bergman, 2004).

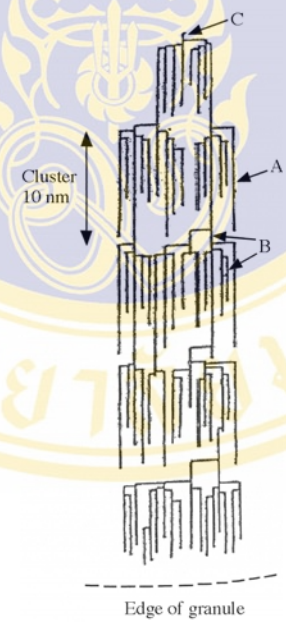


**Figure 1.2** Cluster structure showing linear chains of amylopectin. The C chain has the only free reducing group in the molecule (Bao & Bergman, 2004).

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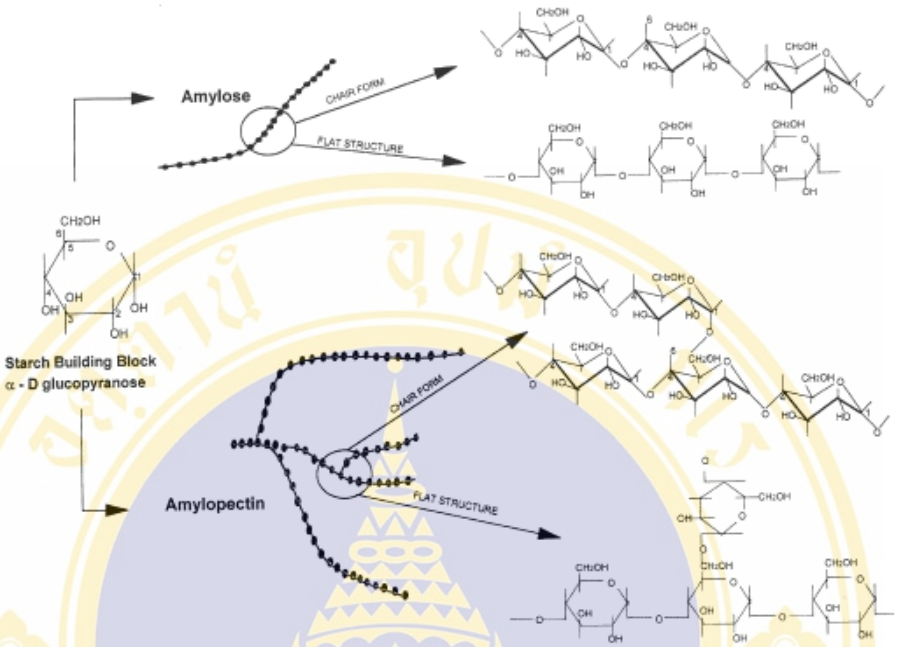


**Figure 2.2** Amylose and amylopectin models (Murphy, 2000).

### 1.3 Other components of granules

The granules of all starches contain nonstarch components, including ash, lipid, and protein (Table 2.1). Cereal starches either do not have phosphate ester groups or have very much smaller amounts (Muhrbeck, Svensson & Eliasson, 1991).

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**Table 2.1** Composition of some selected starch granules (Muhrbeck, Svensson & Eliasson, 1991)

| Starch | Amylose (%) | Protein (g/100 g) | Lipids (g/100 g) |
|---|---|---|---|
| Cassava | 17 | 0.1 | 0.1 |
| Potato | 21 | 0.06 | 0.05 |
| Rice | 12.2–28.6 | — | 0.63–1.11 |
| Waxy rice | 0–2.32 | — | — |
| Wheat | 28 | 0.3 | 0.8 |
| | 29.2 | — | 0.85 |
| A-granules | 28.4–27.8 | — | 0.67–0.73 |
| B-granules | 27.5–24.5 | — | 0.73–0.91 |
| Barley, waxy | 2.1–8.3 | — | 0.30–0.49 |
| Barley, normal | 25.3–30.1 | — | 0.68–1.28 |
| Barley, high amylose | 38.4–44.1 | — | 1.05–1.69 |
| Oat | 25.2–29.4 | — | 1.35–1.52 |
| Maize, normal | 28.7 | 0.3 | 0.8 |
| | 25.8–32.5 | — | 0.61–0.82 |
| Maize, waxy | 1.4–2.7 | — | 0.02–0.14 |
| Maize, high amylose | 42.6–67.8 | — | 1.01–1.09 |
| Fava bean | 33 | 0.9 | 0.1 |
| Pea | 33 | 0.7 | 0.1 |

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### 1.4 Major commercial applications

The main reasons for using starch in manufactured foods are as follows: to provide thickening, to influence the texture, to influence the appearance and to act as a filler. For assessing the suitability of starches for this purpose, it is convenient to consider manufactured foods in two broad categories: powdered products, i.e. dry mixes, and processed products, i.e. liquid, gel or paste forms (Rapaille., & Vanhemelrijck, 1999).

#### 1.4.1 Baked goods

Wheat flour is the basis of many baked goods. It is an economic commodity but is overstretched in respect of modern products such as frozen, chilled, low-fat and gluten-free foods, where aesthetics and processing can be limiting without recourse to starch co-ingredients. Whilst formulations will vary significantly depending on the desired final product, typical ingredients, apart from starch, could include wheat flour (8–16% protein, 71–79% carbohydrate), fats, sugars, eggs, emulsifiers, milk and/or water. Processing conditions will also vary with the formulations. The effect of these ingredients and processes on the use of starch or modified starch can be exacerbated since baked goods have a limited amount of moisture. Gelatinization of the starch (in the wheat flour as well as in the added starch) is critical to building structure and texture in bakery products. Since wheat starch will only thicken during baking, pregelatinized starches can be used to bind the limited water available early on. This creates a host of benefits: suspension of particulates (as in muffin mixes); reduction in the stickiness of doughs; improved handling and machinability; increased cake volume; improved water binding for increased moistness; and softer textures. A common problem in bakery products is staling caused by retrogradation. Stabilized starches, in particular hydroxypropylated, pregelatinized starches bind water more effectively thereby providing baked goods with enhanced shelf-life through extension of the perception of freshness. Native maize, waxy maize or tapioca starches are alternatives to wheat flour for gluten-free products for celiac sufferers who are intolerant to wheat gluten.

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Glazes and icings are used to enhance aesthetics and add value to baked goods. A glaze is typically a thin wash containing sugar, water and/or milk, whilst an icing may be applied as a thickened layer and usually contains fats. Pregelatinized starches and, especially dextrins, find application here where the main functions are viscosity control, colouring, softening and texturing (Murphy, 2000).

### 1.4.2 Batters and breadings

The concept of a breaded or battered coating is to create additional value for meat, poultry, seafood and vegetables. Flours are a major component in batters and breadings but a common problem for food manufacturers is fluctuation of the quality of commodity native flours. Specialty starches are used to solve this and extend the features of the native base flour. The wide array of substrates for coatings means that the accompanying wide range of storage and reconstitution methods become key considerations, in addition to textural attributes, in the selection of a starch.

Starches in batters provide viscosity control which, in turn, controls the quantity and thickness of the batter layer; the adhesion efficiency; visual effects (smooth to blistered); texture; storage; and reconstitution stability. Pregelatinized starches are used to control the cold viscosity. These can be cross-linked and stabilized to provide shear and freeze-thaw stability for batter slurries which are to be recycled or used in chilled or frozen products. The textural attributes can be enhanced by high amylose starches for fried or oven-baked poultry and meat products. High amylose starches need higher temperatures, as in frying, to functionalize them. Stabilization lowers the gelatinization temperatures and therefore makes the starches suitable for applications with lower cooking temperatures. Dextrins are used for vegetable coatings where they will enhance color and, at high dosage rates (~30%), will create blistered effects to transform the surface of a coating. Very high levels of dextrins or incompletely gelatinized, high amylose starches may cause stickiness. To achieve a smooth, uniform coating at lower cooking temperatures, a modified high amylose starch is recommended. Since amylose has excellent film-forming properties, the incorporation of high amylose starches in batters can reduce oil pick-up in fried, battered products (Murphy, 2000).

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### 1.4.3 Beverage emulsions and flavor encapsulation

Lipophilic starches have replaced gum arabic, the traditional emulsion stabilizer, in concentrated flavor emulsion for soft drinks and in encapsulated ingredients, e.g., spray-dried flavours and creamers. Indeed, lipophilic starches can be extended beyond gum Arabic applications to high-load encapsulation of flavor oils. Generally these starches provide improved oxidation resistance and low temperature emulsion stability (Murphy, 2000).

### 1.4.4 Confectionery

Starches are found in a wide range of confectionery products, contributing from soft through to hard gels, and from brittle through to chewy textures. Starch is also active as the structure builder in coatings and even as the moulding medium to support the shaping of confections. Starches are selected primarily on their ease of cooking in high-sugar environments and their ease of handling during production.

The majority of confections are high in sugar, sugar syrups or polyols, with solids in the region of 68–72%. In these products there will be considerable competition for water, with the starch suffering in that gelatinization temperature is increased and the product more difficult to functionalize under 100°C.

Converting amylose and high amylose starches to different degrees of hydrolysis creates a suitable range of acid-thinned or oxidized starches for confections. These starches have a range of low hot viscosities and consequently they allow rapid and efficient cooking of starch solutions in the presence of concentrated sugar syrups. A further useful modification is stabilization where the reduced starch gelatinization temperature allows easier cooking, especially of high amylose starches, so that stronger gels can be achieved with increased clarity and extended shelf life for the confections. Converted starches are also used in confections on the exterior of products, as in pan-coating. Dextrins, with their good film-forming properties are used with high-sugar solutions to create stable, flexible coatings as in jelly beans or shell-coated chocolates. Texture can be further modified by using starches in combination with other hydrocolloids.

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Starches also serve as a process aid rather than an ingredient in confections. The shaping of confectionery pastes normally occurs in starch moulds where the desired shape or design has been imprinted into trays of moulding starch. The moulding starch is also treated with a small percentage of mineral oil which enables it to hold the mould imprint and to minimize dusting the manufacture process. The moulding starch has two key functions, its shape and the absorption of moisture. The moisture content of the moulding starch is critical in obtaining a high-quality confection. Above 9% moisture, the drying time is extended – this reduces production rates – whilst below 6% moisture, the confection has a crust of hardened exterior as the rate of moisture loss is too fast (Murphy, 2000).

**1.4.5 Canned and bottled foods**

This group comprises a wide range of food types including vegetables, pet foods, fish, meats, fruits, soups, baked beans, baby foods, pasta, sauces, desserts and pie fillings (Rapaille & Vanhemelrijck, 1999).

Properties of starches for use in canned and aseptically filled foods

1.4.5.1 Water binding

The starch must have adequate water-binding properties to minimize syneresis.

1.4.5.2. Thickening

The characteristics/product image dictate the amount of thickening required. In addition to viscosity, texture and mouthfeel are critical.

1.4.5.3. Heat penetration

Heat penetration rates vary between the unmodified starch types (tapioca and waxy maize are the fastest, maize the slowest). With modified starches, the higher cross-bonded starches allow faster heat penetration.

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1.4.5.4. Milk protein protection/compatibility

With low levels of milk/cream (e.g. creamed soups), the starch-thickened system can protect the protein, giving a smooth texture. In custards, however, hydrolysis of the acetyl group in acetylated starches leads to reaction with the proteins, resulting in granularity. Hydroxypropyl starches overcome this problem.

1.4.5.5. Stability during sterilization

The correct choice of starch will ensure viscosity stability under the heat and shear conditions found in the can.

1.4.5.6. Shelf-life stability

In addition to microbiological stability, the physical and textural properties of the production must not adversely change during storage. Correct starch usage can ensure this does not happen.

1.4.5.7. For bottled foods

The choice of starch is also governed by its ability to withstand the acidity of the system and the shear during either hot or cold processing.

Multiplicities of starches are used. These range from unmodified maize and waxy maize to all classes of modified starches. A summary of the use of these starches in canned and bottled foods is given in Table 2.2 and 2.3.

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**Table 2.2** Canned foods (Rapaille & Vanhemelrijck, 1999)

| Application | Starch type | Properties/purpose |
|---|---|---|
| Soups, sauces | (Acetylated) di-waxy maize starch phosphates | Viscosity stability aginst heat/shear/acid |
| Gravies | Acetylated di-waxy maize starch adipates | Provides texture/gloss/transparency<br>Imparts mouthfeel |
| | Pregelatinised native waxy maize starch | Mechanical dosing of particulates |
| | Oxidised waxy maize starch | Body/transparency/no viscosity |
| Baked beans, pasta | Di-waxy maize starch phosphates | Viscosity stability against heat/shear<br>Provides texture<br>Imparts mouthfeel |
| Ready meals | Acetylated di-waxy maize starch adipates and phosphates | Binding and thickening agent<br>Texture and mouthfeel |
| Pie fillings | Acetylated di-waxy maize starch phosphates | Viscosity stability against heat/acid<br>Provides texture/gloss/transparency<br>Good-to-excellent freeze-thaw stability |
| UHT custards and puddings | Hydroxypropylated di-waxy maize starch phosphates | Thickening agent, good shelf-life, creamy body, gloss |

**Table 2.3** Bottled foods (Rapaille & Vanhemelrijck, 1999)

| Application | Starch type | Properties/purpose |
|---|---|---|
| Salad dressings, salad creams, mayonnaise, hot preparation | Native maize starches and/or acetylated di-waxy maize starch adipates<br>Di-waxy maize starch phosphates<br>Hydroxypropylated di-waxy maize phosphate | Viscosity stability against heat/shear/acid |
| Cold preparation | Pregelatinised native starches and/or pregelatinised acetylated di-waxy maize starch adipates, pregelatinised di-waxy maize starch phosphates | Provides texture/ flowability<br>Mouthfeel and gloss<br>Viscosity stability against heat/shear/acid Body/transparency/no viscosity |
| Pickles | Di-waxy maize starch phosphates | Thickening |
| Relishes | Acetylated di-waxy maize starch adipates | Suspension of particulates |
| Baby foods | Acetylated di-waxy maize starch adipates | Viscosity stability against heat/shear/acid<br>Excellent freeze-thaw stability<br>Long shelf-life |

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### 1.4.6 Frozen foods

A significant sector of the food industry today involves product storage under frozen or chilled conditions. In products which contain starch-thickened sauces or gravy, an additional demand is made upon the functionality of the starch.

Retention of starch gel systems under cold storage hastens the retrogradation mechanism, with the concomitant dangers of syneresis and destabilization. Although systems based on waxy maize starch (amylopectin) are superior to amylose-containing starches, such as wheat-flour and corn-flour, they are still subject to retrogradation on cold storage. In its mildest form this could be manifest as the development of a cloudy appearance in a fruit pie filling, where good clarity is sought.

A common method of avoiding such problems is to superimpose a further modification in addition to the cross-linking. This second reaction may be an esterification, for example the introduction of acetyl groups onto the starch molecule, or etherification by reacting it with propylene oxide. It would appear that the insertion of substituent groups on the starch chain effectively interferes with the aggregation of molecules, which normally causes retrogradation.

Frozen products place special demands on starches: they must not only wthistand processing (heat, shear, acid conditions) but also retain the desired paste or gel characteristics (clarity, absence of retrogradation) during the storage life of the food product (Rapaille & Vanhemelrijck, 1999).

### 1.4.7 Dairy desserts

These milk-based foods are produced in many forms with a range of consistencies, from firm-textured products through thick, spoonable products to thin, pourable toppings. Water binding and thickening are the main functional properties required of all types of milk dessert. The starch must have adequate water-binding properties to minimize syneresis. The characteristics of the end-product dictate the amount of thickening required. In addition to viscosity, texture and mouthfeel are critical. A whole range of starch-thickened milk desserts is available to the consumer (Rapaille & Vanhemelrijck, 1999).

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### 1.4.8 Domestic preparations

The earliest attempts at convenience puddings were the dry mix, cook-up starch puddings for domestic preparation. The classical starch thickening and gelling agent is maize starch, although combinations of maize and other starches are possible, and products based on other starches such as wheat, arrowroot, rice, etc. are known. The maize starch product is simple but versatile and can be eaten as a mouldable pudding or a thick pouring sauce can be eaten cold or warn. This product can also be used as a cake filling. Two attributes are probably responsible for this dessert's popularity: its good eating quality and its potentially low cost. The lowest cost products contain only starch, color and flavor, and are sold in simple packages. The cook, in this case, has to add both sugar and milk.

Puddings of this type have a short, but heavy, almost sticky texture, and the use of milk in their preparation gives them a good nutritional image. They can be unmoulded, but with some difficulty. The products thicken rapidly on cooling but a relatively long time is needed before true gelling occurs as a result of retrogradation of the starch amylose fraction. At this point products can be removed from the mould.

When starch is combined with carrageenan, desserts become less pudding-like and have more flow-type properties (Rapaille & Vanhemelrijck, 1999).

### 1.4.9 Ready-to-eat desserts

Starches and carrageenan have found wide application in the area of ready-to-eat milk desserts; they are inevitably the hydrocolloids of choice for these products. Therefore, after describing the various processes used to produce milk desserts on the industrial scale, the effects of the applied heat and mechanical and filling treatments on these texturing agents will be discussed and also the extent to which they affect the texture of the final product.

The process of manufacturing milk desserts involves heat and mechanical treatments. Various heat treatments can be applied: pasteurization or sterilization by either rotary retorting or an ultra high-temperature short-time process. The objective of such heat treatments, apart from ensuring food safety, is to extend the shelf-life of the milk desserts by reduction/destruction of micro-organisms and by

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inactivation of enzymes, at the same time preserving as much as possible the sensory properties and nutritional value.

In addition to water binding and thickening, starches control the following properties in ready-to-eat desserts:

Heat penetration: Heat penetration rates vary between the unmodified starch types (tapioca and waxy maize are the fastest, maize the slowest). With modified starches, the higher cross-bonded starches allow faster heat penetration.

Stability during sterilization: The correct choice of starch will ensure viscosity stability under the heat and shear conditions found in the can.

Shelf-life stability: In addition to microbiological stability, the physical and textural properties of the production must not adversely change during storage. Correct starch usage can ensure this does not happen (Rapaille & Vanhemelrijck, 1999).

### 1.4.10 Food powders

These range from the traditional custard and blancmange powder, dry soup and sauce mixes to the more recent adjunct mixes for stews and casseroles. Usually, the starch is present in its granular form and, hence, poses no stability problems during storage. Occasionally, a reduced starch moisture content is desirable if other sensitive ingredients are included. These food products are reconstituted just prior to consumption using mild conditions of heat and shear, and therefore, even in the swollen state, the process presents no serious technical problems. For this reason, unmodified native starches are widely used in the formulation of these powdered, dry mix products.

These are a group of products within this category in which reconstitution and thickening is carried out by a cold process. Typical of these is the ‘instant dessert’ prepared by the addition of cold milk or water. In these circumstances it is necessary to use a ‘pregelatinized’ or ‘instant’ starch. Pregelatinization is a physical modification in which the starch slurry is passed between heated rollers. The process has the effect of gelatinizing and drying the starch simultaneously. In this way the starch is capable of reconstitution and thickening without further heating. As stated

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above, the desired functionality of starch does not always involve thickening. Sometimes the opposite is needed: bulk and volume in the powder mix without undue thickening power. In this case the starch acts as a filler and inert bulking agent. In this role it frequently serves an additional function of aiding dispersion of the powdered mix upon reconstitution (Rapaille & Vanhemelrijck, 1999).

**1.5 Gelatinization properties of starches**

When the starch granule is heated up to the gelatinization temperature in excess water, heat transfer and moisture transfer phenomena occur. The granule swells to several times its initial size as result of the loss of the crystalline order and the absorption of water inside the granular structure. The swelling behavior of starch is primarily the property of its amylopectin content and amylose acts as both a diluents and an inhibitor of swelling, especially in the presence of lipid (Tester & Morrison, 1990a). The maximal swelling might also be related to the molecular weight and the shape of amylopectin molecules (Tester & Morrison, 1990b).

When starch granules swell, the amylose inside the granules leaches out simultaneously. The leached-out amylose forms a three-dimensional network (Eliasson, 1986; Tester & Morrison, 1990a). The swollen granules are embedded in such a continuous matrix (Richardson, Robinson, Ross-Murphy & Todd, 1981; Wong & Lelievre, 1981; Ring & Stainsby, 1982; Ring, 1985). The paste is formed by gelatinizing the aqueous suspension of starch. When starch concentration is high enough, the paste can convert into gel during cooling. The paste and the gel may be considered as a composite material with the swollen starch granules filling the polymer solution, or polymer gel network (Ring & Stainsby, 1982; Miles, Morris, Orford, & Ring, 1985). The initial stage of gelation of starch is dominated by the gelation of the solubilized amylose (Miles et al., 1985). This may imply that the solubilized amylose plays the key role in the gelation of starch. However, Svegmark, Kidman, and Hermanson (1993) found that the inherent amylose of potato starch did not contribute to the gel formation, and suggested that the starch granules caused the rheological behavior of the hot paste. The swollen starch granules formed a close-packed gel structure which possessed high shear resistance (Svegmark & Hermansson

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1990). Evans and Haisman (1979) indicated that the material outside the swollen granules (e.g., amylose) had little effect on the rheology of the starch suspensions.

Gelatinized starch dispersion may be regarded as a composite material consisting of swollen granules and granular fragments dispersed in a continuous biopolymer matrix (Evans & Haisman, 1979; Eliasson, 1986; Morris, 1990; Noel, Ring, & Whittam, 1993). Therefore, the properties of the dispersed phase, the continuous phase, and interactions between the components are three important considerations for insights into the rheology of gelatinized starch suspensions.

### 1.6 Effect of salts on the gelatinization of starch

Salts have been shown to have a significant effect on the gelatinization and rheological properties of starches. Eliasson and Gudmundsson (2006) have reviewed the effect of salts on these properties of starch and found that it depends on the type of salt as well as on the concentration. Generally, it has been found that they can cause an elevation or depression of the gelatinization temperature, $T_p$, and gelatinization enthalpy, $\Delta H$ (Wooton & Bamunuarachchi, 1980; Evans & Haisman, 1982; Chuncharoen & Lund, 1987; Paredes-Lopez & Hernandez-Lopez, 1991; Jane, 1993) and similarly might increase or decrease the rate and degree of gelation and retrogradation (Ciacco & Fernandes, 1979; Chang & Lui, 1991).

### 1.7 Rheological properties of gelatinized starch dispersions

When an aqueous suspension of starch is heated isothermally above the gelatinization onset temperature, the granules absorb large amounts of water and swell considerably to impart substantial increase to the viscosity of the suspension. As granule swelling progresses, the suspension is transformed into a paste that exhibits rheological properties very different from the initial suspension. The swelling of granules is also accompanied by leaching or solubilization of granular constituents, mainly amylose, into the continuous suspending matrix. Granules swell to a maximum value at elevated temperatures, followed by granular disruption and exudation of granule contents into the suspension matrix.

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### 1.8 Rheology of starch dispersions with intact granules

Starch gelatinization up to peak viscosity studied by various techniques, such as viscometry and particle size measurement by laser diffraction, is essentially a first-order kinetic process whose extent is determined principally by pasting temperature and time (Kubota, Hosakawa, & Hosaka, 1979; Okechukwu, Rao, Ngoddy, & McWatters, 1991).

Mild thixotropy has been observed in gelatinized wheat starch dispersions characterized at low temperatures such as 25°C but not at 60°C and above (Doublier, 1981). The time-dependent rheological behavior shown by starch is attributed to structure formation by amylose leached into the suspending matrix on cooling to low gelling temperatures (Ellis, Ring, & Whittam, 1989).

Gelatinized starch suspensions often exhibit yield stresses at low shear rates (Evans & Haisman, 1979; Kubota et al., 1979; Doublier, 1981). At high shear rates, the shear responses follow the power law relations, prompting the use of the Hershel-Bulkey and the power law models for the analysis and description of the rheological behavior of starch suspensions.

At low starch concentration, most authors have used the simple power law model without a yield stress for describing shear responses over a wide range of shear. The flow behavior index ($n$) and the consistency coefficient ($K$) are useful parameters for describing the flow behavior of starch suspensions. Gelatinized starch pastes preheated to temperatures of about 90°C are generally reported to be shear-thinning (pseudoplastic) fluids with values of $n$ considerably less than 1.0 (Evans & Haisman, 1979; Doublier, 1981; Ellis et al., 1989; Noel et al., 1993).

### 1.9 Retrogradation properties of gelatinized starch

Retrogradation is the process by which starch returns to a more ordered state after gelatinization (Atwell, Hood, Lineback, Varriano-Martson, & Zobel, 1988). During storage of starch aqueous system after gelatinization, both amylose and amylopectin molecules rearrange and the rigidity increases. This is the retrogradation process, which may proceed in two different stages, including short-term gelation of amylose within the continuous phase, which occurs at the first step of retrogradation completing within a few hours, and subsequent long-term crystallization of

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amylopectin, which occurs at a much slower rate (requiring several weeks) than amylose gelation (Ring, Colonna, I'Anson, Kalichevsky, Miles, & Orford, 1987) being reversed thermally upon heating to 100 °C (Ring et al., 1987; Yoshimura et al., 1999).

Retrograded starch can be examined by differential scanning calorimetry (DSC), which measures both the temperature and the enthalpies of retrogradation and the range in transition temperature that occur during the melting of recrystallized amylopectin are measured (Karim, Norziah & Seow, 2000). The retrogradation enthalpy is usually 60 to70% smaller than gelatinization enthalpy and the transition temperatures are 10 to 26 °C lower than those for gelatinization granules (Baker & Rayas-Duarte, 1998; White, Abbas, & Johnson, 1989; Yuan, Thompson & Boyer, 1993). Retrograded starches show lower gelatinization and enthalpy than native starches because they have weaker starch crystallinity (Sasaki, Yasui & Matsuki, 2000).

The retrograded gel is a three-dimensional network of swollen granules embedded in an entangled matrix of solubilized macromolecular chains (Hsu, Lu, & Huang, 2000). Starch gels thus obtained can be also regarded as composite materials, in which the continuous phase of amylose gel matrix is interspersed with filler particles (Keetels, van Vliet, & Walstra, 1996b; Keetels, van Vliet, & Walstra, 1996c). That is, swollen gelatinized granules, which are constituted of amylopectin during storage reinforces the interpenetrating amylose gel matrix due to increase in the rigidity of the granules. A number of factors, including rheological properties of the continuous phase, volume fraction, shape, and deformability of the dispersed phase, and the interaction between these two phases are associated with the mechanical properties of starch gels (Keetels, van Vliet, & Walstra, 1996a; Leloup, Colonna, & Buleon, 1991). Dynamic viscoelastic measurement was used to examine the structural changes of the three-dimensional network of swollen granules embedded in an entangled matrix of solubilized macromolecular chains as a function of storage times. A feature of cooked rice grains or pastes is the amount of residual granule structure remaining and therefore the matrix is not homogenous. Models of the starch paste are of a discontinuous phase (the remaining swollen granules) and a continuous phase of amylose. Additional phase separation can occur during the retrogradation process.

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Therefore granule size, amylose-amylopectin ratio, macromolecular organization of granules, starch concentration and temperature-time regimes are all important for the viscoelasticity of starch gel (Biliaderis & Zawistowski, 1990). Retrogradation of gelatinized starch materials involves formation and subsequent aggregation of double helices of amylose and amylopectin chains governing elasticity, firmness, and textural staling of starch-containing systems (Atwell et al., 1988). Basically, amylose retrogradation occurs on cooling and very short-term aging (Doublier & Choplin, 1989; Biliaderis & Zawistowiski, 1990; Silverio, Svensson, Eliasson, & Olofsson, 1996), while amylopectin retrogradation proceeds slowly during aging and requires several weeks or months of storage for equilibrium (Miles et al., 1985; Biliaderis & Zawistowiski, 1990). Amylose content bestows critical influences on the elastic property of freshly retrograded starch dispersions (Radhika Reddy, Subramanian, Zakiuddin Ali, & Bhattacharya, 1994) and on the hardness of freshly cooked rice (Bett-Garber, Champagne, McClung, Moldenhauer, Linscombe, & McKenzie, 2001). And amylopectin tend to have additional effects on the extent of retrogradation of starches (Yuan et al., 1993) or on the hardness of short-term staled cooked rice through the proportion of extra-long and long-B chains (Lai, Shen, Yeh, Juliano, & Lii, 2001). Using rheology and DSC, starch gels were observed to retrograde faster at low temperatures indicating that retrogradation follows nucleation type kinetics ( Biliaderis & Zawistowski, 1990). Several methods have been used to study starch retrogradation and have recently been reviewed by Karim et al. (2000).

Starch gels are thermodynamically unstable and undergo structure changes during storage (Ferrero, Martino, & Zaritzky, 1994). Upon cooling, starch molecules reassociate through intermolecular association; the hydroxyl groups of starch chains become bound and starch losing their ability to remain hydrated and thus releasing water. The phenomenon of the starch gel is called syneresis. Measurement of syneresis may be used to study starch retrogradation by using centrifugation method, which is the ability of starch to withstand the undesirable physical changes during storage at low temperature can be used as an indicator of the tendency of starch to retrograde. This is usually viewed unfavorably as product deterioration. The amount of syneresis is directly related to the tendency of a starch to retrograde.

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The extent of retrogradation may be affected by starch source (Orford, Ring, Carroll, Miles & Morris, 1987), concentration (Zeleznak & Hoseney, 1987; Orford et al, 1987), storage temperature, time and other ingredients such as acids, salts, lipids and surfactants, sugar.

### 1.10 Effects of salts on retrogradation properties of gelatinized starch

Generally, starch granules in sufficient moisture undergo gelatinization on heating, associating with granular swelling, disintegration and crystalline melting, and retrogradation or setback on cooling or aging (Eliasson, 1986). The moisture content of a starch gel determines the extent to which that starch will retrograde (Zeleznak & Hoseney, 1987). Retrogradation in wheat starch gels was controlled by the amount of water present during aging, regardless of the amount present during gelatinization (Zeleznak & Hoseney, 1987). Thermal analysis shows that storage time, storage temperature, and reheating after storage, which are known to influence the rate of starch retrogradation. Zeleznak and Hoseney (1987) postulated that the agents that retard retrogradation by altering moisture relationships during aging and thereby decrease the rate of recrystallization.

DSC has been used to study the retrogradation of gelatinized starch (Eliasson, 1985; Miles et al, 1985; Nakazawa, Noguchi, Takahashi, & Takada, 1985; Chang & Liu, 1991). Salt has differing effects on the retrogradation of starch. Sodium chloride reportedly increases retrogradation (Ciacco & Fernandes, 1979), decreases retrogradation, or has no effect on retrogradation (Chang & Liu, 1991). The concentration of salt in the three reports was similar (2% and 2.33%). Chang and Liu (1991) reported that the rate of retrogradation of rice starch decreased by adding NaCl.

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## 2. Hydrocolloids

The term 'hydrocolloids' refers to a range of polysaccharides and proteins that are nowadays widely used in a variety of industrial sectors to perform a number of functions including thickening and gelling aqueous solution, stabilizing foams, emulsions and dispersions, inhibiting ice and sugar crystal formation and the controlled release of flavors, etc. The commercially important hydrocolloids and their origin are given in Table 2.4.

**Table 2.4** Source of commercially important hydrocolloids (Williams & Phillips, 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 |

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**Figure 2.4** Examples of food products containing hydrocolloids (Williams & Phillips, 2000).

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The food industry, in particular, has seen a large increase in the use of these materials in recent years. Even though they are often present only at concentration of less than 1% they can have a significant influence on the textural and organoleptic properties of food products. Some typical examples of foods containing hydrocolloids are shown in Figure 2.4. The baked beans and hoi-sin sauce contain modified corn starch as a thickener while guar gum is used to thicken the sweet and sour sauce. The Sunny Delight fruit drink contains modified starch as an emulsifier with carboxymethyl cellulose (CMC), and xanthan gum as thickeners. The Italian dressing includes xanthan gum as a thickener and the 'Light' mayonnaise contains guar gum and xanthan gum as fat replacers to enhance viscosity. The yoghurt incorporates gelatin as a thickener rather than a gelling agent while the mousse contains modified maize starch as a thickener with guar gum, carrageenan and pectin present as 'stabilizers'. The Bramley apple pies contain modified maize starch with sodium alginate as a gelling agent. The fruit pie bars contain gellan gum and the blackcurrant preserve and redcurrant jelly contain pectin as gelling agents. The trifle contains xanthan gum, sodium alginate and locust bean gum as 'stabilizers', modified maize starch as a thickener and pectin as a gelling agent (Williams & Phillips, 2000).

The changes in modern lifestyle, the growing awareness of the link between diet and health and new processing technologies have led to a rapid rise in the consumption of ready-made meals, novelty foods and the development of high fiber and low-fat food products. In particular, numerous hydrocolloid products have been developed specifically for use as fat replacers in food. This has consequently led to an increased demand for hydrocolloids. Today the world hydrocolloids market is valued at around $4.4 billions p.a. with a total volume of about 260,000 tonnes. Growth through the 1990s has been at the rate of 2–3% (Williams & Phillips, 2000).

Hydrocolloid selection is dictated by the functional characteristics required but is inevitably influenced by price (Table 2.5) and security of supply. It is for these reasons that starches are the most commonly used thickening agents. It is interesting to note here, however, that xanthan gum, since its introduction in the early 1970s, has become the thickener of choice in many applications despite its high price. This is due to the fact that xanthan gum has unique rheological behavior. It forms highly viscous, highly shear thinning solutions at very low concentrations and the

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viscosity is not influenced to any great extent by changes in pH, the presence of salts and temperature. The high viscosity at low shear enables the gum to prevent particle sedimentation and droplet creaming and the shear thinning characteristics ensure that product readily flows from the bottle after shaking, hence its widespread application in sauces and salad dressings. An overview of the hydrocolloids market is given in Figure 2.5 (a) and (b).

**Table 2.5** Price of the major hydrocolloids (Williams & Phillips, 2000)

| Hydrocolloid | Principal function | Cost $/kg in 1983* | Cost $/kg in 1993** | Cost $/kg in 1999*** |
|---|---|---|---|---|
| Agar | Gelling agent | 15.0–15.4 | 19.72 | |
| Alginate | Gelling agent | 7.7–9.9 | 6.58 | |
| Arabic | Emulsifier | 2.64 | 3.69 | |
| Carrageenan | Gelling agent | 5.5–13.2 | 7.35 | 8–17.6 |
| Processed euchema seaweed | Gelling agent | | | 8 |
| Carboxymethyl cellulose | Thickener | 3.5–4.4 | 3.18 | 4.8–8 |
| Hydroxypropyl cellulose | Thickener and emulsifier | 6.6–8.3 | | |
| Methyl cellulose | Thickener, emulsifier and gelling agent | 6.6 | | 9.6–11.2 |
| Microcrystalline cellulose | Thickener and gelling agent | 3.9–4.3 | | |
| Gelatin | Gelling agent | 4.4 | 4.04 | 5.72–9.02 |
| Guar gum | Thickener | 1.0–1.1 | 0.77 | 2.86 |
| Karaya | Thickener | 4.6 | 2.89 | |
| Locust bean gum | Thickener | 4.6 | 6.40 | |
| Pectin | Gelling agent | 7.6 | 9.19 | 11.2–16 |
| Pectin (low methoxy) | Gelling agent | 10.6 | | |
| Propylene glycol alginate | Emulsifier and foam stabiliser | 9.1 | | |
| Starch | Thickener and gelling agent | 0.5 | | |
| Starch (modified) | Thickener and gelling agent | 1.3 | | |
| Tragacanth | Thickener | 26.4–35.2 | 9.60 | |
| Xanthan gum | Thickener | 13.4 | | 13.64 |

*Sources*:
* R. G. Morley, in *Gums and Stabilisers for the Food Industry 2* eds G. O. Phillips, D. J. Wedlock and P. A. Williams, Pergamon Press (1984) p. 211.
** US Department of Commerce.
*** Suppliers.

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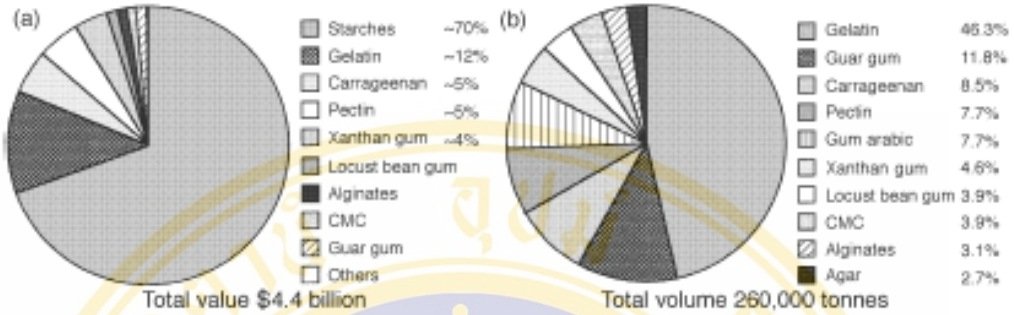


**Figure 2.5** (a) Value of world market for individual hydrocolloids. (b) Volume of world market for individual hydrocolloids (Williams & Phillips, 2000).

### 2.1 Thickening characteristics

Hydrocolloids are widely used to thicken food systems and a much clearer understanding of their rheological behavior has been gained over the last twenty years or so particularly through the development of controlled stress and strain rheometers capable of measuring to very low shear rates ($< 10^{-3}$ $s^{-1}$). The viscosity of polymer solutions shows a marked increase at a critical polymer concentration, commonly referred to as $C^*$, corresponding to the transition from the so-called 'dilute region', where the polymer molecules are free to move independently in solution without interpenetration, to the 'semi-dilute region' where molecular crowding gives rise to the overlap of polymer coils and interpenetration occurs.

Polysaccharide solutions normally exhibit Newtonian behavior at concentrations well below $C^*$, i.e., their viscosity is independent of the rate of shear. However, above $C^*$ non-Newtonian behavior is usually observed. A typical viscosity–shear rate profile for a polymer solution above $C^*$ is given in Figure 2.6 and shows three distinct regions: (a) a low-shear Newtonian plateau; (b) a shear-thinning region, and (c) a high-shear Newtonian plateau. At low shear rates the rate of disruption of entanglements is less than the rate of re-entanglement and hence viscosity is independent of shear. Above a critical shear rate, disentanglement predominates and the viscosity drops to a minimum plateau value at infinite shear rate.

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The viscosity of polymer solutions is influenced significantly by the polymer molecular mass as illustrated in Figure 2.7 which gives the shear viscosity of a series of guar gum samples as a function of shear rate. The viscosity shear rate dependency increases with increasing molecular mass and the shear at which shear thinning occurs shifts to lower values

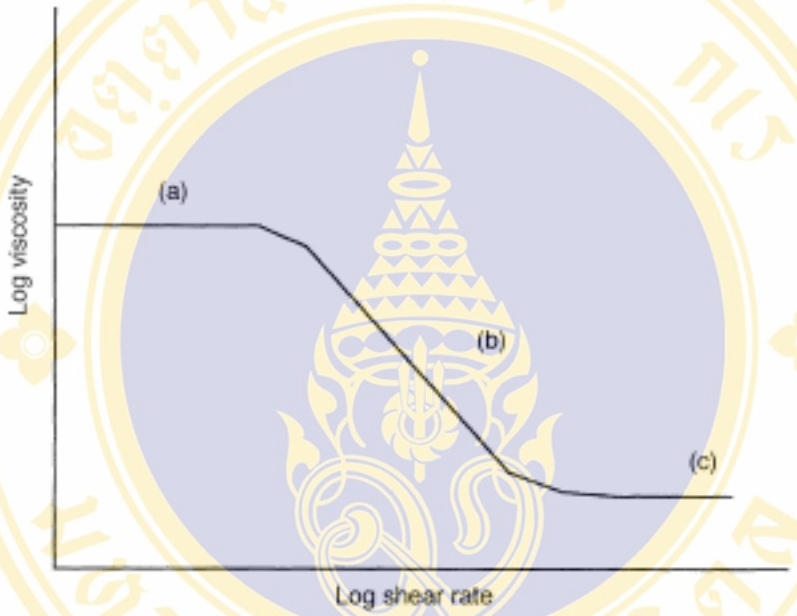


**Figure 2.6** Typical viscosity-shear rate profile for a polymer solution above $C^*$ (Williams & Phillips, 2000).

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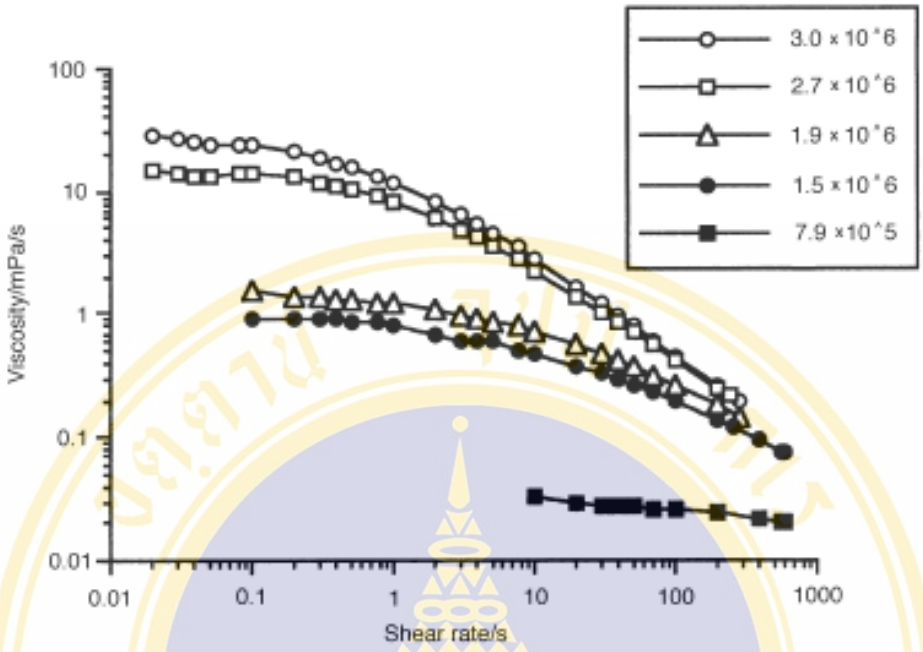


**Figure 2.7** Viscosity-shear rate profiles for 1% solutions of guar gum of varying molecular mass (Williams & Phillips, 2000).

In addition to molecular mass effects, the hydrodynamic size of polymer molecules in solution is significantly influenced by molecular structure. Linear stiff molecules have a larger hydrodynamic size than highly branched, highly flexible polymers of the same molecular mass and hence give rise to a much higher viscosity. This is illustrated in Figure 2.8 which shows the viscosity shear rate profiles for carboxymethylcellulose (CMC), hydroxyethylcellulose (HEC), dextran and gum arabic. The cellulosic polymers are relatively stiff molecules and at 1% are close to or above $C^*$. Consequently they have a high viscosity at low shear rates with the viscosity decreasing with increasing shear to a low minimum value. Dextran is slightly branched and has a very flexible structure due to the (1→6) glycosidic linkages while gum arabic is very highly branched. Both, therefore, are compact and have relatively small radii of gyration for their molecular mass compared to the linear cellulosic polymer. Significant interpenetration does not occur even at concentrations of 20–30% and hence their viscosity–shear rate profiles exhibit Newtonian characteristics. It should be noted, however, that although the viscosity of these concentrated solutions

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is much less than the viscosity of the 1% cellulosic solutions at low shear, they are greater at high shear rates.

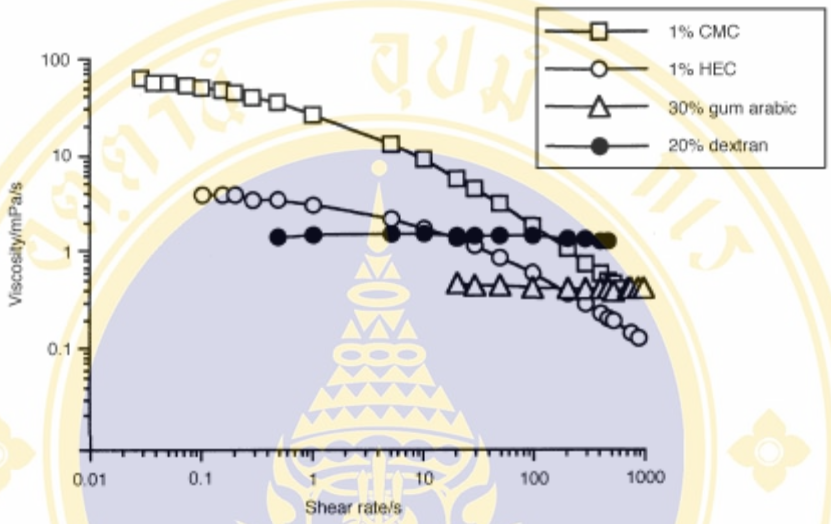


**Figure 2.8** Viscosity-shear rate profiles for 1% CMC, 1% HEC, 20% dextran and 30% gum arabic (Williams & Phillips, 2000).

The stiffness of the polysaccharide chains also has a very pronounced influence on the shear thinning characteristics of the polysaccharide solutions as illustrated in Figure 2.9 which gives the viscosity-shear rate profile for 1% xanthan gum persistence length, $q$, ($q$ > 100nm), 1% CMC ($q$ ~10–30nm), and 5% sodium polyacrylate ($q$ < 10nm). The slopes of the shear thinning section of the curves decrease in the order xanthan > CMC > sodium polyacrylate indicating that the extent of shear thinning increases dramatically with increasing polymer persistence length.

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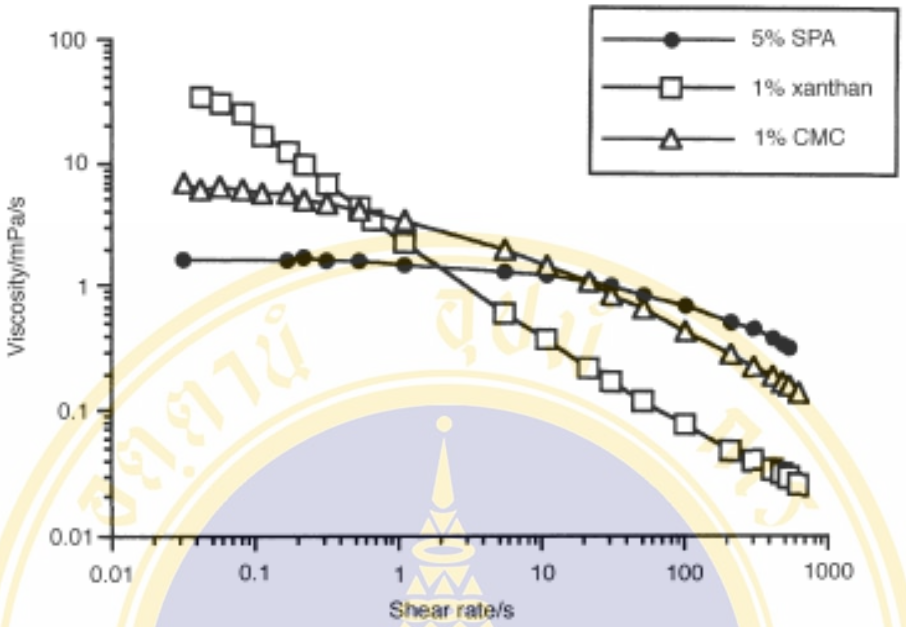


**Figure 2.9** Viscosity-shear rate profiles for solutions of 1% xanthan gum, 1% CMC and 5% sodium polyacrylate (Williams & Phillips, 2000).

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Charged polymers have a higher viscosity than non-ionic polymers of similar molecular mass due to the fact that their molecular coils are expanded due to intramolecular charge repulsions. Addition of electrolyte or adjustment of the pH to reduce the degree of dissociation of the charged groups normally lead to compaction of the coils and a significant drop in viscosity. Interestingly, CMC and xanthan gum show atypical behavior with viscosity actually showing an increase on addition of electrolyte. The main hydrocolloid thickeners used in food products are listed in Table 2.6.

**Table 2.6** Main hydrocolloid thickeners (Williams & Phillips, 2000).

| |
|---|
| **Xanthan gum**<br>Very high low-shear viscosity (yield stress), highly shear thinning, maintains viscosity in the presence of electrolyte, over a broad pH range and at high temperatures. |
| **Carboxymethyl cellulose**<br>High viscosity but reduced by the addition of electrolyte and at low pH. |
| **Methyl cellulose and hydroxypropyl methyl cellulose**<br>Viscosity increases with temperature (gelation may occur) not influenced by the addition of electrolytes or pH. |
| **Galactomannans (guar and locust bean gum)**<br>Very high low-shear viscosity and strongly shear thinning. Not influenced by the presence of electrolyte but can degrade and lose viscosity at high and low pH and when subjected to high temperatures. |

### 2.2 Xanthan gum

Xanthan gum is an extracellular polysaccharide secreted by the micro-organism *Xanthomonas campestris*. Xanthan gum is soluble in cold water and solution exhibit highly pseudoplastic flow. Its viscosity has excellent stability over a wide pH and temperature range and the polysaccharide is resistant to enzymatic degradation. Xanthan gum exhibits a synergistic interaction with the galactomannans, guar gum and locust bean gum (LBG) and the glucomannan, konjac mannan. This results in enhanced viscosity with guar gum and at low concentrations with LBG. At higher concentrations soft, elastic, thermally reversible gels are formed with locust bean gum and konjac mannan.

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### 2.2.1 Manufacture

The bacterium *Xanthomonas campestris* produces the polysaccharide at the cell wall surface during its normal life cycle by a complex enzymatic process. In nature the bacteria are found on the leaves of the *Brassica* vegetables such as cabbage. Commercially, xanthan gum is produced from a pure culture of the bacterium by an aerobic, submerged fermentation process. The bacteria are cultured in a well-areated medium containing glucose, a nitrogen source and various trace elements. To provide seed for the final fermentation stage, the process of inoculums build-up is carried out in several stages. When the final fermentation has finished the broth is pasteurized to kill the bacteria and the xanthan gum is recovered by precipitation with isopropyl alcohol. Finally, the product is dried, milled and packaged (Sworn, 2000).

### 2.2.2 Structure

The primary structure of xanthan gum shown in Figure 2.10, is a linear (1→4) linked $\beta$-D-glucose backbone (as in cellulose) with a trisaccharide side chain on every other glucose at C-3, containing a glucuronic acid residue linked (1→4) to a terminal mannose unit and (1→2) to a second mannose that connects to the backbone. Approximately 50% of the terminal mannose residues are pyruvated and the non-terminal residue usually carries an acetyl group at C-6. X-ray diffraction studies on orientated xanthan gum fibres identified the molecular conformation as a right-handed, five-fold helix with a rise per backbone disaccharide residue of 0.94 nm, i.e., a five-flod helix with a pitch of 4.7 nm. In this conformation the trisaccharide side chain is aligned with the backbone and stabilizes the overall conformation by non-covalent interactions, principally hydrogen bonding. In solution the side chains wrap around the backbone thereby protecting the labile $\beta$-(1→4) linkages from attack. It is thought that this protection is responsible for the stability of the gum under adverse conditions (Sworn, 2000).

Xanthan gum solutions at low ionic strength undergo a thermal transition. This was first observed as a sigmoidal change in viscosity of 1% salt-free solution. Subsequent studies using optical rotation and circular dichroism show transitions coincident with the viscosity change. These results are consistent with a

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helix coil transition. It has been proposed that the xanthan helix in solution should be considered as a rigid rod. The transition is thermally reversible with the structure returning to its original state upon cooling. The transition temperature increases with increasing salt concentration as shown in Figure 2.11. Xanthan gum in solution is also able to form intermolecular associations that result in the formation of a complex network of weakly bound molecules.

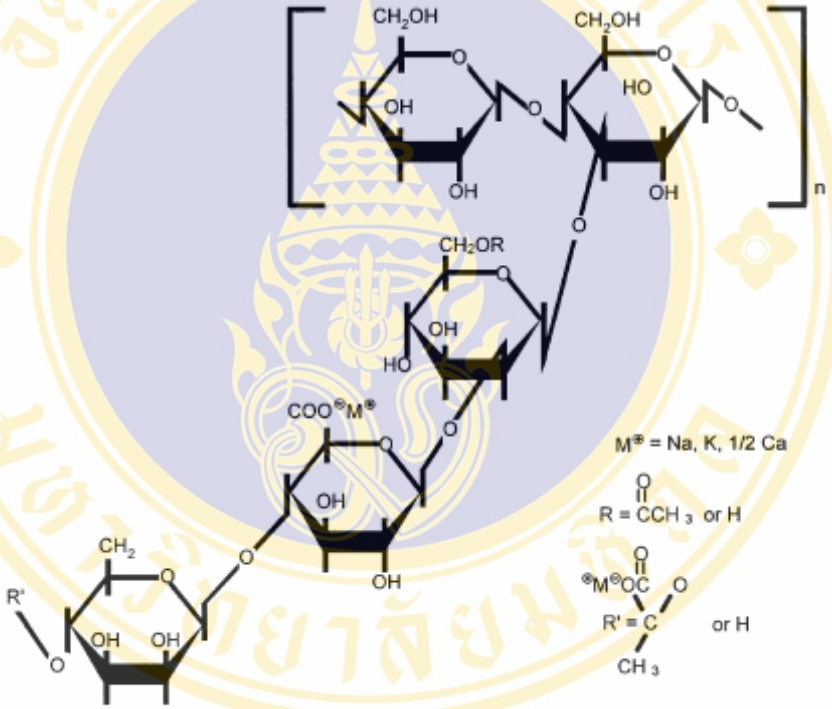


**Figure 3** Primary structure of xanthan gum (Sworn, 2000).

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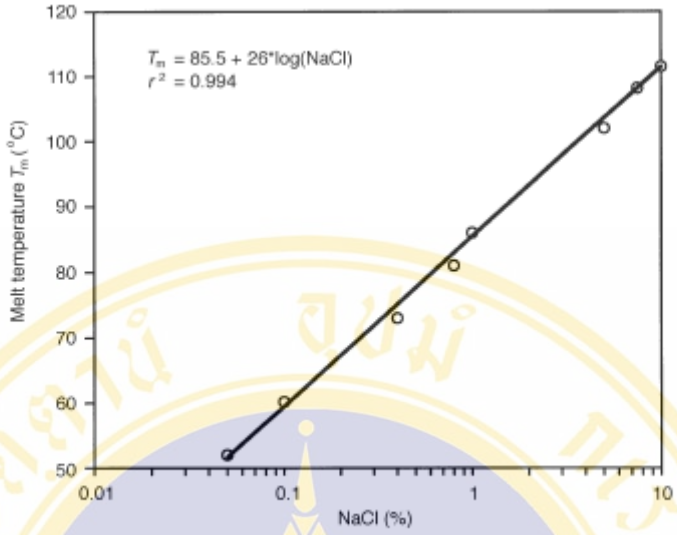


**Figure 2.11** Melting temperature of 1.0% xanthan gum solutions as a function of sodium chloride concentration (Sworn, 2000).

### 2.2.3 Rheology of xanthan gum solutions

Present knowledge of the structure and conformation of xanthan gum explains many of its unique solution properties. The relationship between the structure of xanthan gum and its properties is summarized in Table 2.7. Xanthan gum solutions are highly pseudoplastic. When shear stress is increased, viscosity is progressively reduced. Upon the removal of shear, the initial viscosity is recovered almost instantaneously. This behavior results from the ability of xanthan molecules, in solution, to form aggregates through hydrogen bonding and polymer entanglement. This highly ordered network of entangled, stiff molecules results in high viscosity at low shear rates, and in practical terms, accounts for the outstanding suspending properties of xanthan gum solutions. These aggregates are progressively disrupted under the influence of applied shear, hence the highly pseudoplastic flow characteristics of xanthan gum solution. Figure 2.12 shows the effect of shear rate on a 0.5% xanthan gum solution. Eleven orders of magnitude are covered, and xanthan gum shows pseudoplastic properties over most of the range. This solution varies in viscosity from 1 million mPa.s at low rates of shear to about 1.7 mPa.s at the highest rates of shear. At both the highest and lowest shear rates there is evidence of a leveling

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off of the viscosity. These regions are known respectively as the upper and lower Newtonian regions (Sworn, 2000).

Solutions of xanthan gum at 1% or higher concentration appear almost gel-like at rest yet these same solutions pour readily and have low resistance to mixing and pumping. These same qualities are observed at typical use levels of about 0.1–0.3%. The high viscosity of xanthan gum solutions at low shear rates accounts for their ability to provide long-term stability to colloidal systems. The reduction in viscosity in response to increasing shear is important to the pouring properties of suspensions and emulsions and to the efficacy of xanthan gum as a processing aid (Sworn, 2000).

In Figure 2.13, the viscosity of some common gums is compared over a range of shear rates relating to specific functions or processes. At low shear rate, solution of xanthan gum have approximately 15 times the viscosity of guar gum and significantly more viscosity than carboxymethylcellulose (CMC) or sodium alginate which accounts for its superior performance in stabilizing suspensions. At a shear rate of approximately 100 $s^{-1}$, however, the viscosity of xanthan gum solutions drops sharply compared to the other gums making it easy to pour, pump or spray.

**Table 2.7** Structure/property relationship for xanthan gum (Sworn, 2000).

| Structural features | Properties |
|---|---|
| Complex aggregates, with weak intermolecular forces | High viscosity at low shear rates (suspension stabilising properties)<br>High viscosity at low concentrations<br>High elastic modulus<br>Pseudoplastic rheology |
| Rigid helical conformation, hydrogen bonded complexes, anionic charge on side chains | Temperature insensitivity and salt compatibility |
| Backbone protected by large overlapping side chains | Stability to acids, alkalis and enzymes |

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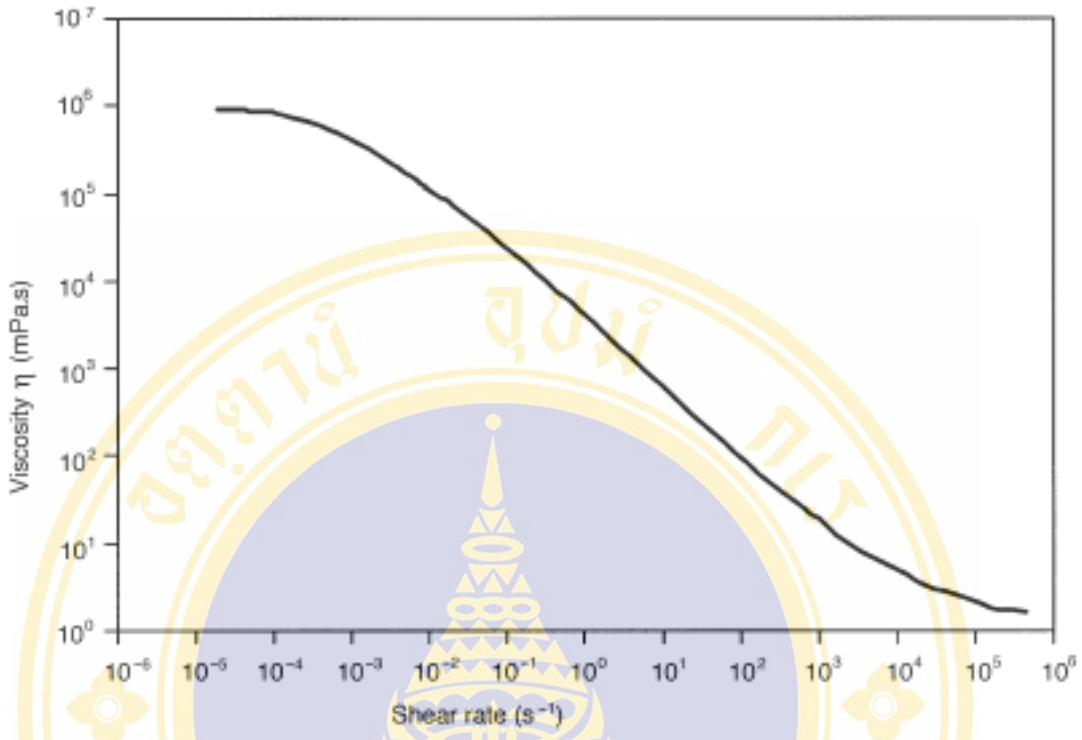


**Figure 2.12** Flow curve of 0.5% xanthan gum solution in standardised tap water. Standardised tap water is prepared by dissolving 1.00 g NaCl and 0.15 g $CaCl_2.2H_2O$ in 1 liter of deionised water (Sworn, 2000).

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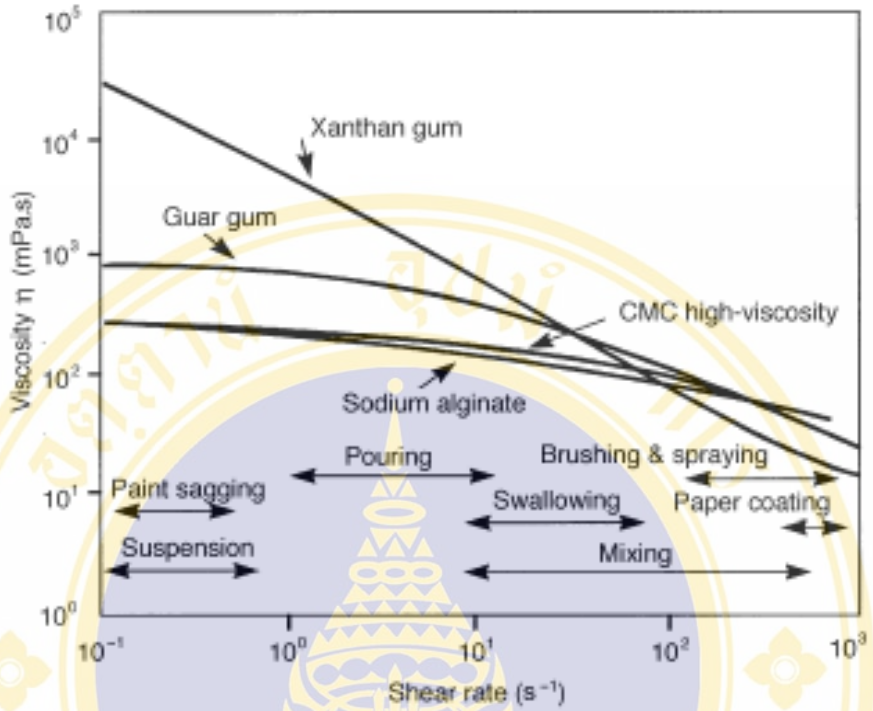


**Figure 2.13** Comparison of the flow behavior of xanthan gum to other hydrocolloid solutions (0.5% concentration) (Sworn, 2000).

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#### 2.2.3.1 Intrinsic viscosity

When the polysaccharide has been solubilized in water, viscosity measurements in dilute conditions provide an easy and interesting means to characterize the behavior of the polysaccharide in aqueous solution. If the concentration is low enough to yield a Newtonian behavior, determination of the intrinsic viscosity through extrapolation to zero concentration provides an indirect estimate of the molecular size of the macromolecule (Doublier & Cuvelier, 2006). From the viscosity value, generally obtained using a capillary viscometer, the relative viscosity is given by:

$$\eta_{rel} = \frac{\eta}{\eta_s} \quad (1)$$

where η is the viscosity of the solution, and $\eta_s$ is the viscosity of the solvent. From the relative viscosity, the specific viscosity ($\eta_{sp}$) can defined as:

$$\eta_{sp} = \eta_{rel} - 1 \quad (2)$$

and the reduced viscosity as:

$$\eta_{red} = \eta_{sp} / c \quad (3)$$

where c is the polysaccharide concentration (expressed in g/dl or in g/ml). By definition, the intrinsic viscosity [η] is given by:

$$[\eta] = \lim_{c \to 0} \eta_{sp} / c \quad (4)$$

This parameter corresponds to the volume occupied by the mass unit in infinite dilute conditions. It is directly related to the molecular weight of the macromolecule.

The intrinsic viscosity can be calculated by plotting specific viscosity ($\eta_{sp}$) divided by concentration ($\eta_{sp} / c$) against concentration (c, %), they followed Huggins's equation (Equation 5).

$$\frac{\eta_{sp}}{c} = [\eta] + k_1[\eta]^2 c \quad (5)$$

#### 2.2.3.2 Effect of salts on viscosity

How salts affect viscosity depends on the concentration of xanthan gum in solution. At or below about 0.25% gum concentration, monovalent salts such as

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ssodium chloride cause a slight decrease in viscosity. At higher gum concentration, vicosity increases with added salt. At a sodium chloride level of 0.1%, a viscosity plateau is reached, and further addition of salt has little effect on viscosity. Many divalent metal salts, including those of calcium and magnesium, have a similar impact on viscosity. To develop optimal rheological and uniform solution properties, some type of salt should be present; usually the salts found naturally in tap water are sufficient to generate these effects. Salt concentrations of greater than 1–2% in the water can slow down the hydration of xanthan gum and it is therefore recommended to hydrate the gum in the absence of excess salt. Once hydrated, additional salt can be added without adverse effects (Doublier & Cuvelier, 2006).

#### 2.2.3.3 Effect of pH on viscosity

Generally, pH has little effect on the viscosity of xanthan gum solution over the range encountered in food systems. Uniform and high viscosity is maintained over the pH range 2–12, with some reduction at extreme pH values. However, differences in viscosity are more evident at low concentrations of xanthan gum. The solutions have excellent stability at low pH over long time periods.

Xanthan gum hydrates in many acidic solutions. For example, it is directly soluble in 5% acetic acid, 5% sulfuric acid, 5% nitric acid and 25% phosphoric acid. Additionally, xanthan gum will hydrate in up to 5% sodium hydroxide. Hydration rate, however, is improved when it is dissolved in water before adding the acid or alkali. In the presence of most organic acids, stability is excellent. At elevated temperatures, however, acid hydrolysis of the polysaccharide is accelerated and a reduction in viscosity will occur (Doublier & Cuvelier, 2006).

#### 2.2.3.4 Effect of temperature on viscosity

Xanthan gum solutions are unique in their ability to retain their viscosity until a definite ‘melting temperature’ is reached. At this temperature, the viscosity drops sharply due to a reversible molecular conformation change. The melting temperature is dependent on the ionic strength of the solution as shown in Figure 2.11. Above approximately 5% NaCl the melting temperature is greater than

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100°C. The viscosity loss is reversible and upon cooling the original high viscosity is recovered (Doublier & Cuvelier, 2006).

### 2.2.4 Uses and applications

The following is a summary of the many applications for xanthan gum or blend of xanthan gum and galactomannans and their related functions and benefits.

#### 2.2.4.1 Batters

In wet prepared batters, xanthan gum reduces flour sedimentation; improves gas retention; imparts enzyme, shear and freeze-thaw stability; and provides uniform coating and good cling. In pre-dusts, xanthan gum improves adhesion and controls moisture migration during frying. In pancake batters, xanthan gum improves spread control, volume and air retention.

Xanthan gum is used in batter coatings for onion rings where inconsistent adhesion, caused by the waxy coating found in onion rings, can be eliminated. Variations in waxiness between varieties and seasons occur and can increase this problem. Cling properties associated with the high at-rest viscosity of xanthan gum solutions contribute to the increased adhesion of the batter. Use levels of approximately 0.15% of the batter weight are effective in this application. Batters for fish can be stabilized with 0.06% xanthan gum whereas 0.1–0.15% is recommended for thin batters such as those used with shrimps (tempura). Xanthan can also be used in batters for frozen products such as chicken, shrimp or fish (Sworn, 2000).

#### 2.2.4.2 Baked goods, bakery and pie fillings

Xanthan gum contributes smoothness, air incorporation and retention, and recipe tolerance to batters for cakes, muffins, biscuits and bread mixes. Baked goods have increased volume and moisture, higher crumb strength, less crumbling and greater resistance to shipping damage. Xanthan gum improves volume, texture and moisture retention in refrigerated dough, reduced calorie baked goods and gluten-free breads

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Moisture control is essential at all stages of cake production and also when formulating a dry cake mix. Improper moisture control can result in lumpy cakes batters and uneven mixing, giving poor structure which results in collapsed cakes during or after baking. The overall quality of the finished cake, particularly after storage, can be affected by poor hydration characteristics of the dry ingredients. For example, volume can be reduced and texture may be non-uniform or fragile when moisture is not evenly distributed throughout the cake. Xanthan gum blended with the other dry cake ingredients, hydrates rapidly and evenly to aid in the uniform distribution of moisture in the batters, which in turn helps stabilize the fine air cells formed during the mixing process. The stabilization of air cells improves volume and symmetry in the finished cake. Xanthan can be added to the cake batter at 0.05% (total batter weight) without the need for any other formulation change.

Adding xanthan gum to either cold or hot processed bakery and fruit pie fillings improves texture and flavor release. The added benefits in cream and fruit fillings are extended shelf stability, freeze-thaw stability and syneresis control (Sworn, 2000).

2.2.4.3 Dairy products

Blends of xanthan gum, carrageenan and galactomannans are excellent stabilizers for a range of frozen and chilled dairy products such as ice cream, sherbet, sour cream, sterile whipping cream and recombined milk. These economical blends are available prepared and provide optimal viscosity, long-term stability, improved heat transfer during processing, heat shock protection and ice crystal control (Sworn, 2000).

2.2.4.4 Dressings

This is arguably the largest single application of xanthan gum in the food industry. Xanthan gum's stability to acid and salt, effectiveness at low concentrations and highly pseudoplastic rheology make it the ideal stabilizer for pourable, no-oil, low-oil and regular salad dressings. Dressings with xanthan gum have excellent long-term stability and a relatively constant viscosity over a wide

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temperature range. They pour easily but cling well to the salad. Use level is typically between 0.2–0.4% xanthan depending on the oil content. Generally, as the oil content of the dressing increases less xanthan gum is required for stabilization and a guide to use levels is given in Table 2.8.

**Table 2.8** Suggested stabilizer level for salad dressing formulation (Sworn, 2000).

| % Oil used | 10 | 20 | 30 | 40 |
|---|---|---|---|---|
| % Starch | 2.0 | 2.0 | 1.5 | 1.5 |
| % Xanthan gum | 0.35 | 0.3 | 0.25 | 0.25 |

2.2.4.5 Dry mixes

Fine particle size xanthan gum provides rapid, high viscosity development in cold or hot systems and yields excellent texture and flavor release. It also permits easy preparation of desserts, salad dressings, dips, soups, milkshakes, sauces, gravies and beverages. In dry mix beverages, xanthan gum provides enhanced body and quality to the reconstituted drink. In addition, it uniformly suspends fruit pulp in prepared drinks to improve product appearance and texture (Sworn, 2000) .

2.2.4.6 Frozen foods

Stability, syneresis control and consistent viscosity during freeze-thaw cycles and heating are achieved by adding xanthan gum to a variety of frozen products such as whipped toppings, sauces, gravies, batters, entrees and soufflés (Sworn, 2000).

2.2.4.7 Retorted products

Although xanthan gum provides stable, high viscosity over a range of temperatures, this viscosity is temporarily reduced at retort temperatures, ensuring good thermal penetration in retorted foods. At the same time the ability of xanthan gum to recover its viscosity upon cooling, provides a uniform, high-quality product. In retort pouch products, xanthan gum also improves filling and reduces splashing and fouling of the critical heat-seal area of the pouch. Xanthan gum can be used to

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partially replace starch in this application. This results in improved heat stability and a cleaner, less pasty mouthfeel. Typically, xanthan is used at 0.1–0.2% concentrations (Sworn, 2000).

2.2.4.8 Sauces and gravies

Low levels of xanthan gum provide high viscosity in sauces and gravies at both acid and neutral pH. Viscosity is also stable to temperature changes and is maintained under a variety of long-term storage conditions. Sauces and gravies containing xanthan gum cling to hot foods (Sworn, 2000).

2.2.4.9 Syrups and toppings

Xanthan gum promotes ease of pouring and excellent cling to ice cream, fruits and pancakes. Under refrigerated storage, syrups and toppings retain uniform consistency. Cocoa powder in chocolate syrups remains suspended. Frozen non-dairy whipped toppings and frozen whipped topping concentrates have firm texture, high overrun and excellent freeze-thaw stability (Sworn, 2000).

**2.2.5 Regulatory status**

Xanthan gum is recognized as a food additive under the provisions of the US Food and Drug Administration regulations (21 CFT 172.695) for use as a stabilizer, thickener or emulsifier. Xanthan gum is designated by the European Union as E415 with a non-specified acceptable daily intake (ADI). KELTROL® and KELTROL® F xanthan gum are approved for Kosher use.

Gums can help thicken products without the associated starchy mouthfeel or flavor-masking that starches sometimes create. Gelling agents such as pectin, gellan, alginate, gelatin, and carrageenan tend to provide better flavor release and less flavor masking than an equally thick or gelled system based on starch. This is party because gums are used at lower levels than starches. Gums are generally used in the range of 0.01 to 1.0%, while starches are usually used in the 0.75 to 10.0% range. Because starches are used at higher concentrations, they tend to encapsulate, or

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capture, flavor molecules more readily than gums, requiring that flavors be used at higher levels in order to achieve equal flavor impact (Sworn, 2000).

### 2.3. Interactions between starch and hydrocolloids

These days, the food industry often uses mixtures of starch and hydrocolloids to modify and control the texture, improve moisture retention, and control water mobility and "eating quality" of food products. Changes in starch structure, such as melting, gelatinization, fragmentation, and retrogradation can all the affected by the presence of hydrocolloids (Donovan, 1979; Lund, 1984). During the gelatinization process, the extent of granule swelling, disintegration, and release of amylose depend on the type of starch, its concentration, the temperature, the processing applied, and the presence of other solutes and hydrocolloids. These factors will in turn affect the final product properties.

From a rheological point of view, it is well known that gelatinization cereal starch dispersions in the presence of a hydrocolloid (e.g., guar gum, locust bean gum, xanthan gum, carrageenans, carboxymethylcellulose) strongly influences the viscosity of the hot starch paste and has been observed by various workers (Christianson et al., 1981; Alloncle et al., 1989; Fanta & Christianson, 1996). The origin of this "synergistic" effect has been explained in terms of complex formation between starch molecules and hydrocolloid (Christianson et al., 1981; Sajjan & Rao, 1987).

In order to understand more fully the interactions between wheat starch and gums during pasting and gelation, Christianson et al. (1981) studied the changes that occur in the granule structure and in the viscosity of the exudates, when wheat starch is pasted in xanthan, guar, and cellulose dispersions. These authors found that the viscosity of wheat starch pastes was significantly increased by the addition of small amounts of these gums. The initial onset of paste viscosity was also detected earlier than in the control. This is attributed to the detection of the first stage of swelling due to the increased viscosity of the external media. Media isolated from starch-guar and starch-xanthan dispersions displayed synergistic viscosity, which remained stable at room temperature. These authors postulated that this viscosity

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stability suggests that strong associations of soluble starch with these gums are developed during pasting.

However, synergistic interaction is not always observed between starch and hydrocolloids (Tye, 1988) and it seems unlikely that hydrocolloids in general will form complexes with starch molecules either in molecular dispersion or in intact granules. A more likely explanation for the synergistic effect can be ascribed to phase separation phenomena (Alloncle & Doublier, 1991; Appelqvist et al., 1997).

Alloncle et al. (1989) and Alloncle and Doublier (1991) investigated the rheological characteristics of cereal starch-galactomannans mixed systems by means of a coaxial cylinders viscometer and the gelation process by means of a controlled stress rheometer. Flow curves of these starch-galactomannans mixtures were compared with the curves obtained from starch or galactomannan alone. A strong synergistic effect was observed, resulting in a large increase in the viscosity of the mixtures compared with starch or galactomannan alone.

In contrast to Christianson et al. (1981), Alloncle et al. (1989) have proposed a simple model based on phase separation of the polymers to account for the synergistic effect. They suggest that during heating of the starch-galactomannan system, the galactomannan molecules form the continuous phase while the starch granules swell and occupy a greater phase volume at the expense of the galactomannan molecules. It is likely that these molecules remain in the continuous phase, because their diffusion to inside the granules would be impossible due to hydrodynamic reasons. As a result, the effective concentration of the galactomannans within the continuous phase is increased, which will lead to a substantial increase in viscosity of this phase since it increases considerably more than linearly with concentration.

These effects, due to the exclusion of hydrocolloid from the gelatinized starch granule can go some way to explain the starch-hydrocolloid rheology observed. However, the structural (e.g., conformation, molecular weight) and rheological properties (e.g., shear-thinning) of the hydrocolloid are also relevant, particularly in rationalizing the differences in synergistic effects shown by different hydrocolloids.

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Brabender viscograms have shown that starch-guar gum systems yield a much higher viscosity after heating and cooling than starch-locust bean gum. This has been partly attributed to the lower molecular weight of locust bean gum with respect to guar gum and to the difference in chemical structure of the two galactomannans (mannose/galactose ratio). According to Sajjan and Rao (1987), an increase in the degree of pseudoplasticity of starch-gum pastes may be explained on a molecular level.

### 2.3.1 Effect of xanthan gum on gelatinization of starches

Addition of xanthan gum to rice starch is known to modify and control the pasting (Shi & BeMiller, 2002) and rheological properties (Kim & Yoo, 2006) of starch. It is well known that hydrocolloids such as xanthan gum improve viscosity of starch pastes (measured by RVA) although they might not affect gelatinization temperatures ($T_o$, $T_p$, and $T_c$) and transition temperature ($T_c - T_o$) (measured by DSC).

Christianson et al. (1981) attributed the increase in viscosity to interaction between exudates from the starch granule (solubilized amylose and low-molecular-weight amylopectin) and gums. A second explanation given was that addition of thickening gums enhanced the forces being exerted on the starch granules in the shear field compared to the starch-water suspension with equal starch concentrations. Sudhakar et al. (1995) suggested that gums are located within the continuous phase and the volume of phase accessible to the gum is reduced, which caused a dramatic increase in concentration in the continuous phase resulted in a very high viscosity. BeMiller (2007) reported that the presence of xanthan gum retarded granule swelling and protected the resulting starch paste against breakdown resulting in a subsequent increase in the final viscosity indicating cross-linking had occurred. Funami et al. (2005a) hypothesized the increment in the viscosity of the continuous phase should prevent the diffusion of amylose form the starch granules, leading to the decrease in amount of leached amylose.

The gelatinization process is typically followed using differential scanning calorimetry (DSC). Hydrocolloids have been reported to elevate the starch gelatinization temperatures. The effect that any particular hydrocolloid has on the gelatinization process differs with the type of starch employed (Gudmundsson et al., 1991).

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Adding another biopolymer, like xanthan, in starch dispersion modifies the structure of the continuous phase, resulting in a marked change in the rheological properties of the mixture. Starch pastes and amylose gels are dominantly elastic and their storage modulus is strongly dependent on their concentration (Evans & Lips, 1992). On the other hand, xanthan solutions can be considered as highly structured liquids with much longer relaxation times compared to classical entangled macromolecular solutions (Doublier & Cuvelier, 1996). Moreover, phase separation phenomena as interactions between chain segments of the same type can be favored energetically and exclusion effects may occur (Closs, Conde-Petit, Roberts, Tolstoguzov, & Escher, 1999; Mandala & Palogou, 2003).

**2.3.2 Effect of salt and molecular weights of xanthan gum on retrogradation of gelatinized starch**

The addition of polysaccharide gums decreased retrogradation rate as well as syneresis of a starch-based system (Ferreo et al., 1994).

The molecular weight of hydrocolloids have been found to affect the retrogradation (Funami et al., 2005b) behavior of starchHowever, there are very few reports in the literature concerning the effect of salts on the starch/hydrocolloid mixtures such as corn starch/xanthan (Sudhakar et al., 1995), wheat starch/xanthan, and corn starch/iota-carrageenan (Funami et al, 2007) combinations. In particular, no attempt has been made to study the effect of molecular weight of hydrocolloids on these combinations.

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# CHAPTER III
# MATERIAL AND MATHODS

## 1. Materials

Commercial normal rice starch (RS) was supplied by Cho Heng Rice Vermicelli Factory Co. Ltd., Nakhon Pathom, Thailand. Moisture and amylose contents for the RS sample were 12.7% and 33.0%, respectively. Xanthan (XG) used in this study was a food grade sample and obtained from Jungbunzlauer Austria AG, Wulzeshofen, Austria. All salts used, namely sodium chloride (NaCl) and calcium chloride ($CaCl_2$), were of reagent grade and purchased from Merck KGaA, Darmstadt, Germany.

## 2. Methods

### 2.1 Preparation of xanthan with different molecular weights

Xanthan prepatations with different average molecular weights were prepared by shearing solutions of native XG in distilled water or in salt solutions using a high-pressure homogenizer (Type Panda, Niro-Soavi S. p. A., Parma, Italy). Xanthan solutions (1% w/w) were obtained by dispersing a weighed amount of XG powder in distilled water or an aqueous salt (0.1 M NaCl or $CaCl_2$) solution while stirring at room temperature (~25°C). Sodium azide (0.02%) was used as a preservative. The solutions were heated for 20 min at 80 °C in a water bath to completely solubilize the gum and then were cooled to room temperature. These solutions were then passed through the homogenizer at a pressure of 700 bar for 1, 2, 4, or 12 passes which were denoted as XG1, XG2, XG4, or XG12, respectively. The non-homogenized XG solutions (XG0) were used as controls. The average molecular weights and intrinsic viscosities of the xanthan preparations were determined.

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**2.2 Molecular weight determination**

Molecular weights of XG in distilled water and salt solutions were determined by gel permeation chromatography (GPC) using a Waters 600E instrument (Waters Corporation, Milford, MA, USA) equipped with a 7.8×300 mm Ultrahydrogel linear column packed with hydroxylated polymethacrylate-based gel, a 6×40 mm guard column, and a refractive index detector. Pullulan standards, ranging from 5800 to $1.6\times10^6$ Da of peak molecular weight (Showa Denko K. K., Tokyo, Japan), were used for calibration. For XG solutions containing salts, the salts were removed from the samples by dialysis at 4 °C using a cellulose dialysis membrane (Spectra/Por®1, Spectrum Laboratories Inc., CA, USA) with a molecular weight cut off 6000-8000 Da. An aqueous sodium azide solution (0.02%) was used as a dialysis solution and was changed every 3-5 h for four days and finally followed by distilled water. Xanthan solution (0.02% w/v) in pH 11 buffer (0.1 M NaOH and 0.05 M $NaHCO_3$) were prepared and filtered using a 0.45 μm nylon filter membrane before injection into the GPC column. The conditions were set as follows; injection volume 20 μl, flow rate 0.6 ml/min, and temperature 30°C. Buffer pH 11 was used as an eluent.

**2.3 Intrinsic viscosity determination**

The intrinsic viscosity, $[\eta]$, of XG solutions was measured by using an Ubbelohde dilution capillary viscometer (Size 50, Cannon Instrument Co., State College, PA, USA) immersed in water bath maintained at 25 ± 0.1 °C. The efflux times of solvents and of gum solutions ranging in concentrations from 0.01% to 0.05% were measured in triplicate and averaged. Additional dilutions of the 1% XG solutions were made with the same solvents used in the stock solutions, i.e. either distilled water or an aqueous salt (0.1 M NaCl or $CaCl_2$) solution.

The concentration-dependence of the viscosity of XG solutions was analyzed by using the classic Huggins equation:

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$$\frac{\eta_{sp}}{c} = [\eta] + k_1[\eta]^2 c \quad (6)$$

where $\eta_{sp}$ is the specific viscosity and $k_1$ is the Huggins constant. For each concentration, the specific viscosity was determined using the equation:

$$\eta_{sp} = \frac{(\eta - \eta_s)}{\eta_s} \quad (7)$$

where $\eta$ is the solution viscosity and $\eta_{sp}$ is the solvent viscosity. The plots of $\eta_{sp}/c$ versus $c$ would result in straight lines in which the $[\eta]$ was determined as the zero concentration-limit of the $\eta_{sp}/c$ value.

### 2.4 Determination of pasting properties

Pasting properties of RS alone and RS/XG mixtures suspended in distilled water or salt solutions were determined by a rapid visco-analyzer (Model RVA-4C, Newport Scientific Pty. Ltd., Warriewood, Australia). Rice starch slurry alone (6% w/w) was prepared by dispersing weighed amount of RS (dry basis) in distilled water or salt solutions. In the case of mixtures (5.7% RS and 0.3% XG), RS was slurried in the weighed amounts of dilute homogenized or non-homogenized XG in distilled water or salt solutions. In both cases, the slurries were stirred for 15 min at room temperature to avoid lump formation. The slurries (28 g) were then poured into aluminium canisters and stirred manually using plastic paddles for 20-30 s before insertion into the RVA machine. The heating and cooling cycles were programmed, following the general pasting method (STD 1). The slurry was held at 50°C for 1 min, heated to 95°C within 3 min 42 s and then held at 95°C for 2 min 30 s. It was subsequently cooled to 50°C within 3 min 48 s and held at 50°C for 2 min, while maintain a rotation speed of 160 rpm.

### 2.5 Determination of thermal properties

Thermal properties, namely gelatinization temperatures and enthalpy, of RS alone, RS/salt, RS/XG, and RS/XG/salt systems, were measured by a differential scanning calorimeter (DSC 822$^e$, Mettler-Toledo GmbH, Schwerzenbach, Switzerland). The total solids content of samples was selected to be 12% w/w (dry

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basis), while keeping the RS/XG mixing ratio constant at 5.7/0.3. The samples were prepared by the procedure described above. After hydration for 1 h at room temperature, 10-15 mg of the well-stirred sample suspensions were exactly weighed into 40 μl aluminium crucibles and immediately hermetically sealed to prevent moisture loss. Scans were performed from 25 to 100°C at a controlled constant rate of 10°C/min. A sealed empty crucible was used as a reference, and the DSC was calibrated by using indium. The enthalpy and transition temperatures, namely the onset temperature ($T_o$), peak temperature ($T_p$), and conclusion temperatures ($T_c$), were determined, based on the DSC heating 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. After the fist-run heating, the gelatinized samples were cooled down and kept at 4 °C for 1, 3, 7, 14, 21, 35, 49, and 63 days. The stored samples were heated again to study the effect of $M_w$ of XG and salts on retrogradation of RS. The retrogradation ratio was calculated by dividing the re-gelatinization enthalpy ($\Delta H_2$) in the second-run heating by the gelatinization enthalpy ($\Delta H_1$) in the first-run heating (Kohyama & Nishimari, 1992)

**2.6 Syneresis determination**

Batches of 500 g RS suspensions (6%, w/w) or RS/XG (5.7/0.3, w/w ratio) mixtures were prepared by dispersing the weighed amount of RS and XG in distilled water or salt solutions (containing 0.02% sodium azide) using a motorized stirrer for 1 h at room temperature (~25°C). The suspensions were then gelatinized in a Brabender Viscoamylograph Type E (Duisburg, Germany). The heating temperature started from 25 to 95°C, held at 95°C for 20 min and then cooled to 50°C. The heating and cooling rates were 1.5°C/min. The gelatinized mixtures (25 g) were transferred into 50 ml screw-cap plastic centrifuge tubes of 27 mm internal diameter and 115 mm height. All the sample tubes were kept at 4°C for 1, 3, 7, 14, 21, 35, 49, and 63 days. At each storage time, three tubes of each stored sample were randomly selected and adjusted to room temperature in a water bath at 25°C fro 20 min. Syneresis of the samples was estimated using a centrifugation method. The extent of syneresis (%) was

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determined as the weight percentage of liquid released from the gel due to centrifugation at 1180g for 15 min to the original weight of gel.

### 2.7 Determination of rheological properties

Dynamic viscoelastic and steady flow properties of the freshly prepared pastes of RS (6%, w/w) alone and RS/XG (5.7/0.3, w/w ratio) mixtures, in the presence or absence of salts obtained from pasting in the RVA and keeping at room temperature (~25 °C) for 1 h, were determined by using a rheometer (Physica MCR 301, Anton Paar GmbH, Graz, Austria) with a cone and plate geometry sensor (1° cone angle, 50 mm diameter, and 0.05 mm gap). The sample was placed into the rheometer which was equilibrated to 25 °C. For dynamic viscoelastic determination, two steps of rheological measurements were performed: (1) deformation sweeps at a constant frequency (10 rad/s) to determine the maximum deformation attainable by a sample in the linear viscoelastic range and (2) frequency sweeps over a range of 0.1 to 100 rad/s at a constant deformation (0.5% strain) within the linear viscoelastic range. The storage modulus ($G'$), loss modulus ($G''$), and loss tangent ($\tan\delta = G''/G'$) as a function of frequency ($\omega$) were obtained.

Steady flow tests were also performed on the freshly prepared paste samples at 25 °C to obtain shear rate versus shear stress (flow curves) data. The cone was programmed to increase the shear rate from 0 to 300 $s^{-1}$ in 3 min (upward flow curve), followed immediately by a reduction from 300 to 0 $s^{-1}$ in the next 3 min (downward flow curve). Using the equipment software, the areas of the hysteresis loops were found and the Herschel-Bulkley rheological model expressed by Equation (8) was fitted to the experimental data.

$$\sigma = \sigma_0 + K\dot{\gamma}^n \qquad (8)$$

where $\sigma$ is the shear stress (Pa), $\sigma_0$ is the yield stress (Pa), $\dot{\gamma}$ is the shear rate ($s^{-1}$), $K$ is the consistency coefficient (Pa $s^n$), and n is the flow behavior index (dimensionless).

In the case of retrogradation study the fresh RS (3.5%, w/w) or RS/XG (5.7/0.3, w/w ratio) pastes obtained from the RVA were cooled to room temperature (~25°C) and then kept at 4°C for 0, 1, 3, 7, 14, 21, 35, 49, and 63 days. At each

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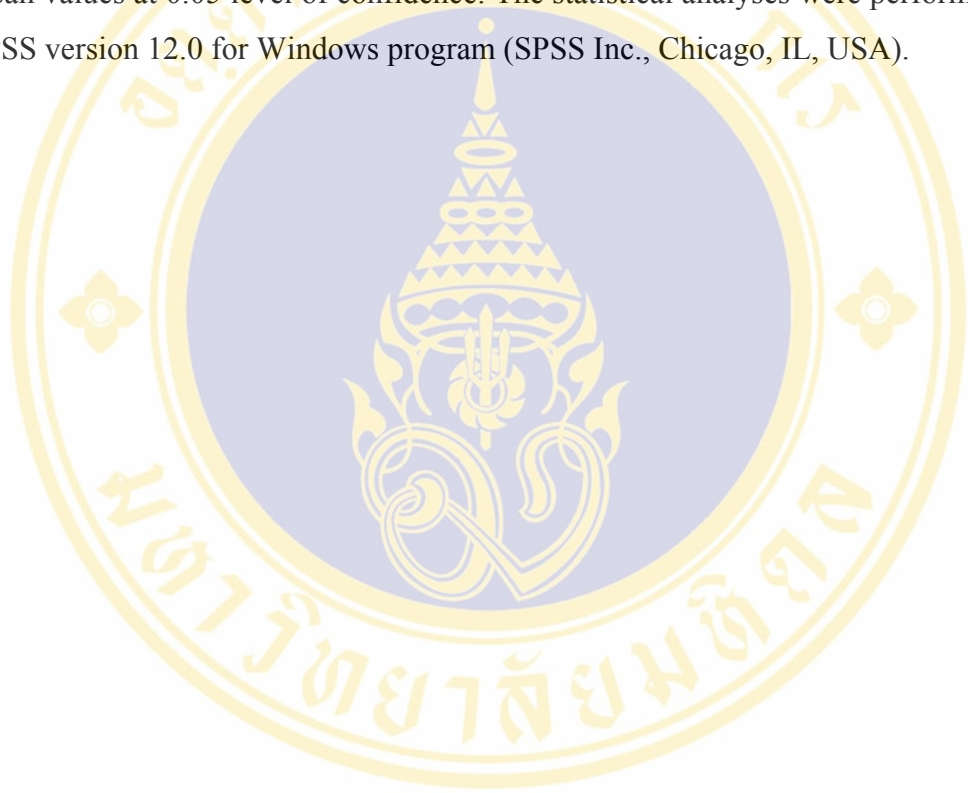
storage time the samples were determined for viscoelastic properties by using the rheometer.

**2.8 Statistical analysis**

All measurements were made in triplicate for each sample. Results are expressed as mean ± standard deviations. A one-way analysis of variance (ANOVA) and Tukey's test were used to establish the significance of differences among the mean values at 0.05 level of confidence. The statistical analyses were performed using SPSS version 12.0 for Windows program (SPSS Inc., Chicago, IL, USA).



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# CHAPTER IV
# RESULTS

## 1. Molecular weight and intrinsic viscosity of xanthan

The effect of high-pressure homogenization of 1% XG solutions in the presence or absence of salts on weight-average molecular weight ($M_w$) and intrinsic viscosity [$\eta$] of XG is shown in Table 4.1. The homogenization resulted in a decrease in $M_w$ of XG with increasing number of passes either in the presence or absence of salts. The presence of salts caused about a double increase in $M_w$ of XG in the non-homogenized solutions.

The intrinsic viscosity of XG solutions with and without added salts significantly ($P \leq 0.05$) decreased with decreasing $M_w$ of XG. The addition of salts was found to greatly reduce (5-10 times) the [$\eta$] of XG.

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**Table 4.1** Effect of high pressure homogenization on weight-average molecular weight ($M_w$) and intrinsic viscosity ([$\eta$]) of xanthan gum (XG) in aqueous solutions with or without salts

| Homogenization[1] | Molecular weight (Da) $\times 10^{-6}$ | | | Intrinsic viscosity[2] (dl/g) | | |
|---|---|---|---|---|---|---|
| (Number of passes) | No salt | 0.1M NaCl | 0.1M $CaCl_2$ | No salt | 0.1M NaCl | 0.1M $CaCl_2$ |
| 0 (XG0) | 4.05 | 8.96 | 8.17 | $110.34 \pm 3.59^{a}$ | $21.22 \pm 1.21^{a}$ | $18.92 \pm 0.68^{a}$ |
| 1 (XG1) | 3.33 | 5.11 | 4.99 | $96.84 \pm 3.19^{b}$ | $12.56 \pm 0.12^{b}$ | $13.93 \pm 1.08^{b}$ |
| 2 (XG2) | 3.10 | 3.67 | 3.64 | $94.46 \pm 5.27^{b}$ | $10.10 \pm 0.43^{c}$ | $10.59 \pm 0.71^{c}$ |
| 4 (XG4) | 2.70 | 2.83 | 2.73 | $78.53 \pm 3.50^{c}$ | $8.30 \pm 0.71^{c}$ | $9.03 \pm 0.39^{cd}$ |
| 12 (XG12) | 2.01 | 2.06 | 1.99 | $65.10 \pm 5.16^{d}$ | $6.14 \pm 0.80^{d}$ | $7.22 \pm 0.38^{d}$ |

[1] The sample codes are denoted in parentheses.

[2] Assays were performed in triplicate at 25°C. Mean ± standard deviation values in the same column followed by different superscripts are significantly different ($P \leq 0.05$).

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**2. Pasting properties**

The pasting and paste characteristics of RS alone and RS/XG mixtures, with various $M_w$ of XG suspended in distilled water or aqueous salt solutions, determined by RVA analysis, are summarized in Table 4.2.

Addition of 0.1 M NaCl or $CaCl_2$ to RS alone suspensions resulted in a significant ($P \leq 0.05$) increase in peak and breakdown viscosities and decrease in final and setback viscosities as compared with the control, with the exception of $CaCl_2$ which increased final viscosity and did not affect setback viscosity of RS alone. There was no significant change in pasting temperatures of RS alone in the presence of either NaCl or $CaCl_2$ as compared with the control.

In the case of RS/XG mixtures in the presence or absence of salts, addition of XG generally resulted in a significant ($P \leq 0.05$) increase in peak, breakdown, final, and setback viscosities, whereas pasting temperatures were not significantly affected.

Addition of salts significantly increased peak, breakdown, final, and setback viscosities of RS/XG mixtures with various $M_w$ of XG. Within each RS/XG blend, divalent cations from $CaCl_2$ showed a more pronounced effect on the peak and final viscosities compared with monovalent cations from NaCl.

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**Table 4.2** Pasting properties of 6% rice starch (RS) alone and RS(5.7%)/xanthan gum (XG; 0.3%) mixtures dispersed in aqueous solutions with or without salts at 0.1 M concentration[1]

| Salt | Hydrocolloid[2] | Peak viscosity (RVU) | Breakdown (RVU) | Final viscosity (RVU) | Setback (RVU) | Pasting temperature (°C) |
|---|---|---|---|---|---|---|
| None | None | 43.9 ± 1.1$^{c}$ | 2.7 ± 1.3$^{d}$ | 62.2±0.3$^{c}$ | 21.2 ± 0.3$^{d}$ | 94.7 ± 1.0$^{a}$ |
| | XG0 | 69.4 ± 1.8$^{a}$ | 15.4 ± 0.9$^{a}$ | 83.7±2.3$^{a}$ | 29.7 ± 2.3$^{ab}$ | 86.4 ± 1.4$^{b}$ |
| | XG1 | 49.7 ± 0.6$^{b}$ | 9.7 ± 0.9$^{b}$ | 71.8±1.5$^{b}$ | 32.0 ± 0.4$^{a}$ | 95.0 ± 0.4$^{a}$ |
| | XG2 | 42.8 ± 0.2$^{c}$ | 7.8 ± 0.8$^{bc}$ | 62.1±1.3$^{c}$ | 27.1 ± 2.1$^{bc}$ | 94.3 ± 1.2$^{a}$ |
| | XG4 | 38.6 ± 1.8$^{e}$ | 7.5 ± 0.3$^{bc}$ | 57.0±4.0$^{cd}$ | 25.9 ± 2.1$^{bc}$ | 92.5 ± 3.4$^{a}$ |
| | XG12 | 37.5 ± 0.4$^{e}$ | 6.1 ± 0.7$^{c}$ | 55.8±1.3$^{d}$ | 24.4 ± 0.6$^{cd}$ | 94.4 ± 0.1$^{a}$ |
| NaCl | None | 48.0 ± 0.4$^{d}$ | 9.9 ± 0.4$^{bc}$ | 45.7 ± 0.4$^{f}$ | 7.6 ± 0.6$^{e}$ | 95.1 ± 0.2$^{a}$ |
| | XG0 | 86.4 ± 0.8$^{a}$ | 27.2 ± 0.7$^{a}$ | 98.3 ± 1.7$^{a}$ | 39.3 ± 2.1$^{a}$ | 92.2 ± 0.4$^{a}$ |
| | XG1 | 60.0 ± 0.9$^{b}$ | 12.2 ± 2.6$^{b}$ | 73.2 ± 1.0$^{b}$ | 25.5 ± 2.3$^{b}$ | 94.9 ± 0.4$^{a}$ |
| | XG2 | 53.1 ± 0.2$^{bc}$ | 9.6 ± 0.9$^{bc}$ | 65.7 ± 0.7$^{c}$ | 22.2 ± 0.5$^{bc}$ | 95.1 ± 0.2$^{a}$ |
| | XG4 | 49.1 ± 0.3$^{cd}$ | 8.2 ± 0.8$^{c}$ | 59.9 ± 0.4$^{d}$ | 19.0 ± 1.0$^{cd}$ | 95.0 ± 0.1$^{a}$ |
| | XG12 | 43.9 ± 1.9$^{d}$ | 6.8 ± 0.5$^{c}$ | 53.8 ± 4.3$^{e}$ | 16.7 ± 0.8$^{d}$ | 94.9 ± 0.3$^{a}$ |
| $CaCl_2$ | None | 52.1 ± 1.2$^{f}$ | 5.9 ± 0.1$^{e}$ | 67.3 ± 1.6$^{f}$ | 21.1 ± 1.7$^{d}$ | 94.9 ± 0.5$^{a}$ |
| | XG0 | 118.6 ± 0.3$^{a}$ | 21.8 ± 0.2$^{a}$ | 124.7 ± 0.2$^{a}$ | 28.0 ± 0.5$^{bc}$ | 93.7 ± 1.4$^{a}$ |
| | XG1 | 79.0 ± 0.8$^{b}$ | 16.3 ± 0.6$^{d}$ | 100.0 ± 1.4$^{b}$ | 37.2 ± 0.3$^{a}$ | 91.2 ± 1.5$^{a}$ |
| | XG2 | 73.6 ± 0.5$^{c}$ | 19.6 ± 0.7$^{b}$ | 88.5 ± 0.5$^{c}$ | 34.5 ± 0.7$^{a}$ | 93.0 ± 1.9$^{a}$ |
| | XG4 | 68.2 ± 1.0$^{d}$ | 17.8 ± 0.4$^{c}$ | 80.7 ± 1.5$^{d}$ | 30.4 ± 1.1$^{b}$ | 91.2 ± 1.4$^{a}$ |
| | XG12 | 64.1 ± 2.3$^{e}$ | 17.1 ± 0.3$^{cd}$ | 72.8 ± 1.2$^{e}$ | 26.7 ± 1.1$^{c}$ | 92.5 ± 2.0$^{a}$ |

[1] Assays were performed in triplicate. Mean ± standard deviation values in the same column for each solution followed by different superscripts are significantly different ($P \leq 0.05$).

[2] Refer to Table 4.1 for the sample codes of XG (XG0 –XG12) having various $M_w$ values.

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**3. Thermal properties**

The onset ($T_o$), peak ($T_p$), and conclusion ($T_c$) gelatinization temperatures, and the gelatinization temperature range ($\Delta T$) and enthalpy ($\Delta H$) of RS alone, and RS/XG aqueous suspensions in the presence or absence of salts, determined by the DSC, are summarized in Table 4.3.

For RS alone, addition of 0.1 M NaCl did not significantly ($P \leq 0.05$) affect the magnitudes of $T_o$, $T_p$, $T_c$ and $\Delta T$, whereas 0.1 M $CaCl_2$ addition significantly increased $T_o$ and $T_p$ values without any effect on $T_c$ and $\Delta T$ as compared with those of the control. The $\Delta H$ was found to be significantly decreased by the addition of both salts used in this study. The presence of $CaCl_2$ at the concentration used in this study seemed to exhibit a more pronounced effect on gelatinization of RS than did NaCl.

Addition of XG with various $M_w$, at a concentration used in this study, did not significantly ($P \leq 0.05$) affect the $T_o$, $T_p$, $T_c$ and $\Delta T$ values of RS/XG blends as compared with those of the corresponding control samples without XG. The magnitude of $\Delta H$ values, in contrast, was significantly decreased by XG addition except for the systems with added $CaCl_2$, and this effect seemed to be independent of $M_w$ of XG.

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**Table 4.3** Thermal properties of 12% rice starch (RS) and RS/xanthan gum (XG; 5.7/0.3, w/w ratio) mixtures dispersed in aqueous solutions with or without salts at 0.1 M concentration[1]

| Salt | Hydrocolloid[2] | $T_o$ (°C) | $T_p$ (°C) | $T_c$ (°C) | $\Delta T$ ($T_c$-$T_o$) | $\Delta H$ (J/g) |
|---|---|---|---|---|---|---|
| None | None | 75.5±0.6$^a$ | 80.7±0.3$^a$ | 87.1±0.8$^a$ | 11.6±1.2$^a$ | 15.3±0.2$^a$ |
| | XG0 | 75.5±0.6$^a$ | 81.3±0.4$^a$ | 87.7±0.7$^a$ | 12.3±1.3$^a$ | 10.1±0.7$^b$ |
| | XG1 | 75.5±1.3$^a$ | 81.0±0.2$^a$ | 87.3±0.3$^a$ | 11.7±1.2$^a$ | 9.9±0.2$^b$ |
| | XG2 | 74.7±0.3$^a$ | 81.0±0.1$^a$ | 87.4±0.2$^a$ | 12.7±0.3$^a$ | 8.2±0.5$^c$ |
| | XG4 | 76.2±0.2$^a$ | 81.0±0.3$^a$ | 86.7±0.3$^a$ | 10.6±0.4$^a$ | 7.6±0.4$^c$ |
| | XG12 | 75.9±1.2$^a$ | 81.3±0.3$^a$ | 87.1±0.6$^a$ | 11.2±1.5$^a$ | 8.5±0.4$^c$ |
| NaCl | None | 77.0±2.1$^a$ | 81.9±2.1$^a$ | 86.5±2.8$^a$ | 9.5±1.1$^a$ | 12.2±2.1$^a$ |
| | XG0 | 77.3±1.2$^a$ | 82.2±2.4$^a$ | 86.6±3.4$^a$ | 9.3±2.3$^a$ | 7.4±0.3$^b$ |
| | XG1 | 78.4±0.1$^a$ | 83.8±0.1$^a$ | 89.3±0.3$^a$ | 10.8±0.3$^a$ | 7.4±0.2$^b$ |
| | XG2 | 78.6±0.1$^a$ | 84.0±0.3$^a$ | 89.5±0.1$^a$ | 10.8±0.2$^a$ | 7.5±0.1$^b$ |
| | XG4 | 79.2±0.5$^a$ | 84.3±0.1$^a$ | 89.5±0.7$^a$ | 10.4±1.2$^a$ | 8.0±0.5$^b$ |
| | XG12 | 78.5±0.6$^a$ | 83.8±0.2$^a$ | 89.1±0.1$^a$ | 10.5±0.7$^a$ | 7.5±0.4$^b$ |
| $CaCl_2$ | None | 79.5±0.5$^a$ | 85.0±0.2$^b$ | 90.6±0.2$^a$ | 11.1±0.6$^a$ | 8.8±0.3$^a$ |
| | XG0 | 80.3±1.1$^a$ | 85.1±0.1$^{ab}$ | 90.5±0.7$^a$ | 10.2±1.8$^a$ | 7.5±0.8$^a$ |
| | XG1 | 79.6±0.6$^a$ | 85.1±0.1$^{ab}$ | 91.0±0.4$^a$ | 11.5±0.9$^a$ | 8.8±1.6$^a$ |
| | XG2 | 80.1±0.7$^a$ | 85.4±0.2$^a$ | 90.9±0.1$^a$ | 10.8±0.8$^a$ | 8.6±1.6$^a$ |
| | XG4 | 79.5±0.3$^a$ | 85.0±0.1$^b$ | 90.4±0.4$^a$ | 10.9±0.7$^a$ | 7.5±0.5$^a$ |
| | XG12 | 79.7±0.2$^a$ | 85.0±0.0$^b$ | 90.6±0.1$^a$ | 10.9±0.9$^a$ | 7.3±0.6$^a$ |

[1] Assays were performed in triplicate. Mean ± standard deviation values in the same column for each solution followed by different superscripts are significantly different ($P \leq 0.05$).

[2] Refer to Table 4.1 for the sample codes of XG (XG0 –XG12) having various $M_w$ values.

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**4. Dynamic viscoelastic properties**

Small amplitude oscillatory shear measurements of RS alone and RS blended with XG having different $M_w$, both in the presence and absence of salts, were compared and demonstrated similar dynamic mechanical spectra (Figure 4.1 a-c). These rheograms show that the storage modulus ($G'$) was much larger than the loss modulus ($G''$) and both moduli show only slight variation with frequency ($\omega$), moreover, a crossover between these two moduli was not observed throughout the measured frequency range. The dynamic mechanical loss tangent ($\tan\delta = G''/G'$) values for all the gels tested were much smaller than unity indicates predominantly elastic behavior (Figure 4.2 a-c).

The magnitudes of $G'$ of RS/XG0 gels were comparable to those of RS alone gels, whereas those of $G''$ were about 2.4-4.6 times higher at a $\omega$ of 1 rad/s, indicating that RS/XG0 gels were more viscous than RS alone gels. This result is consistent with a substantial increase in $\tan\delta$ observed for all RS/XG gels as compared with RS alone gels. Although, the effects appeared to be less pronounced when $M_w$ of XG was decreased but the gel strength of the pure RS pastes having the lowest $\tan\delta$ values are never attained.

There were almost no differences in the viscoelastic properties of RS alone and RS/XG gels in distilled water when compared to those with the addition of either NaCl or $CaCl_2$.

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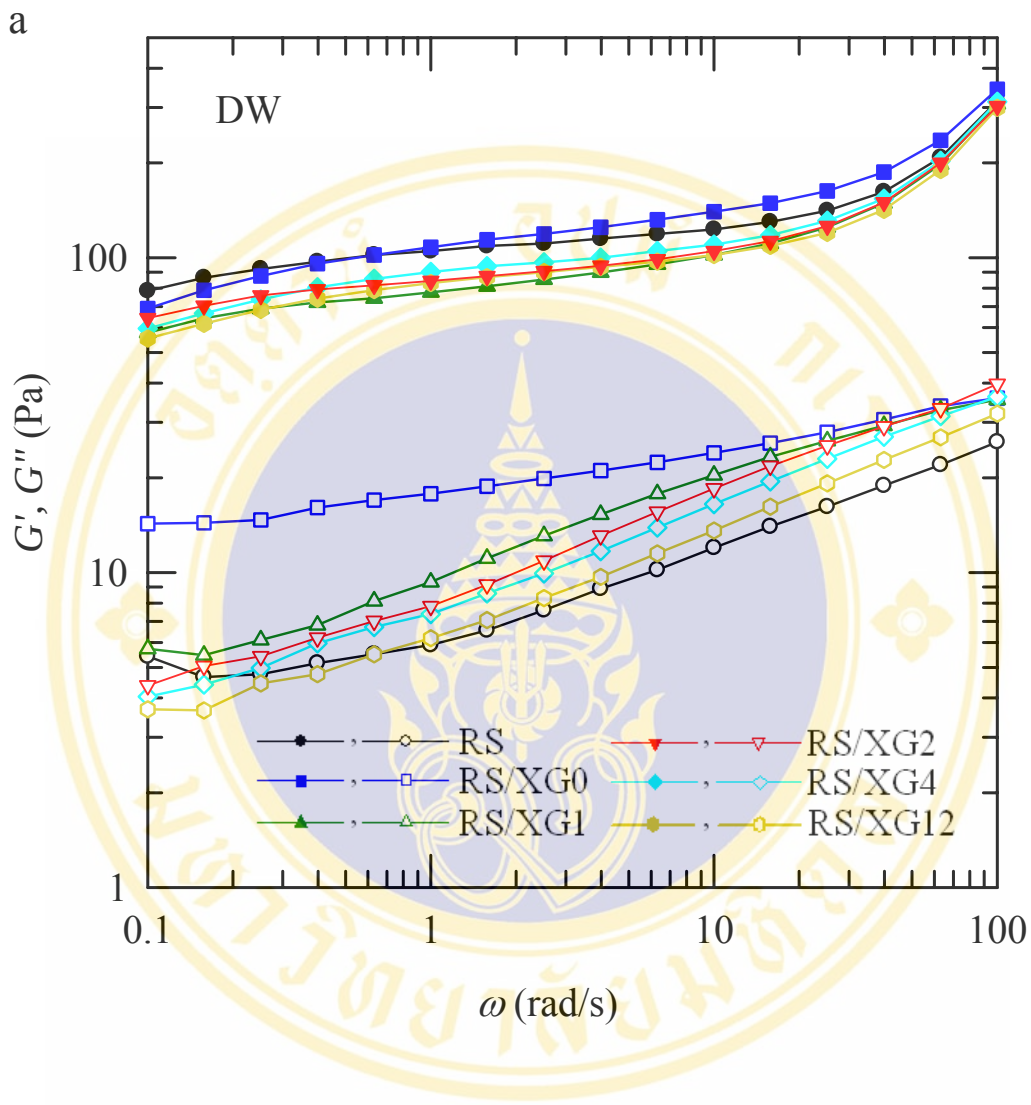


**Figure 4.1** Dynamic mechanical spectra of 6% rice starch (RS) and rice starch (5.7%)/xanthan gum (XG; 0.3%) pastes in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions. Measurements were made at 0.5% strain and 25°C. Closed symbols represent storage modulus ($G'$) and open symbols represent loss modulus ($G''$). Refer to Table 4.1 for the sample codes of XG (XG0 – XG12) having various $M_w$ values.

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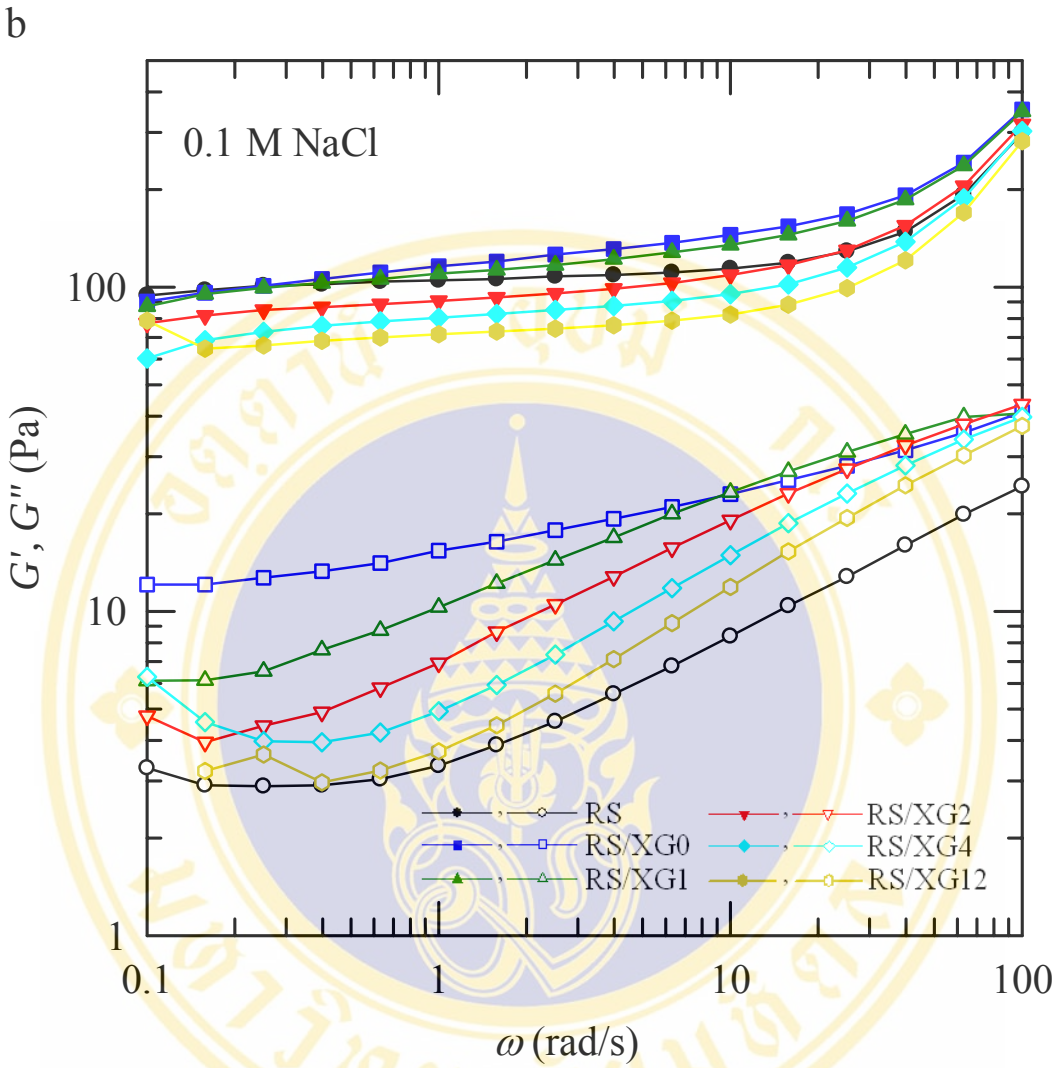


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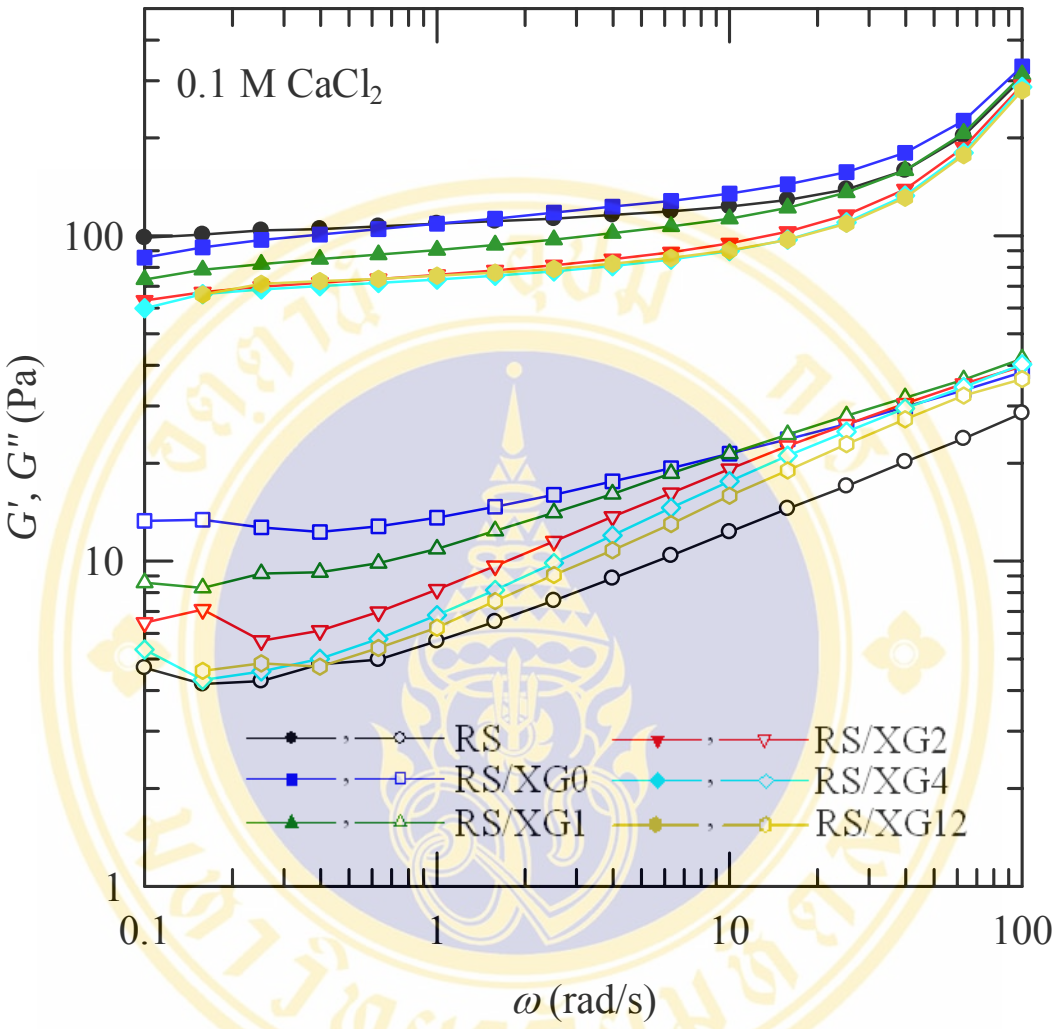


**Figure 4.1** Dynamic mechanical spectra of 6% rice starch (RS) and rice starch (5.7%)/xanthan gum (XG; 0.3%) pastes in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions. Measurements were made at 0.5% strain and 25°C. Closed symbols represent storage modulus ($G'$) and open symbols represent loss modulus ($G''$). Refer to Table 4.1 for the sample codes of XG (XG0 – XG12) having various $M_w$ values. (continued)

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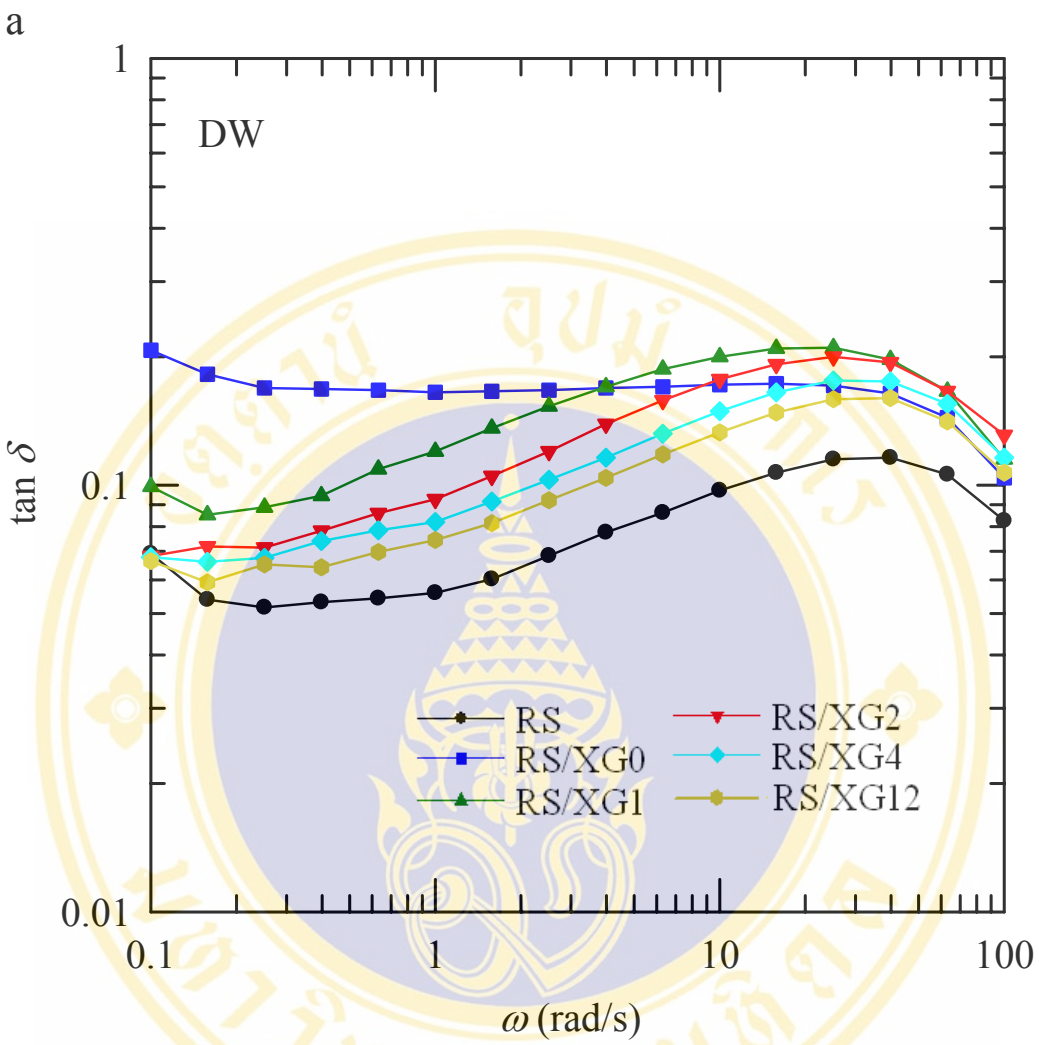


**Figure 4.2** Dynamic mechanical loss tangents ($\tan\delta$) of 6% rice starch (RS) and rice starch (5.7%)/xanthan gum (XG; 0.3%) pastes in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions. Measurements were made at 0.5% strain and 25°C. Refer to Table 4.1 for the sample codes of XG (XG0 – XG12) having various $M_w$ values.

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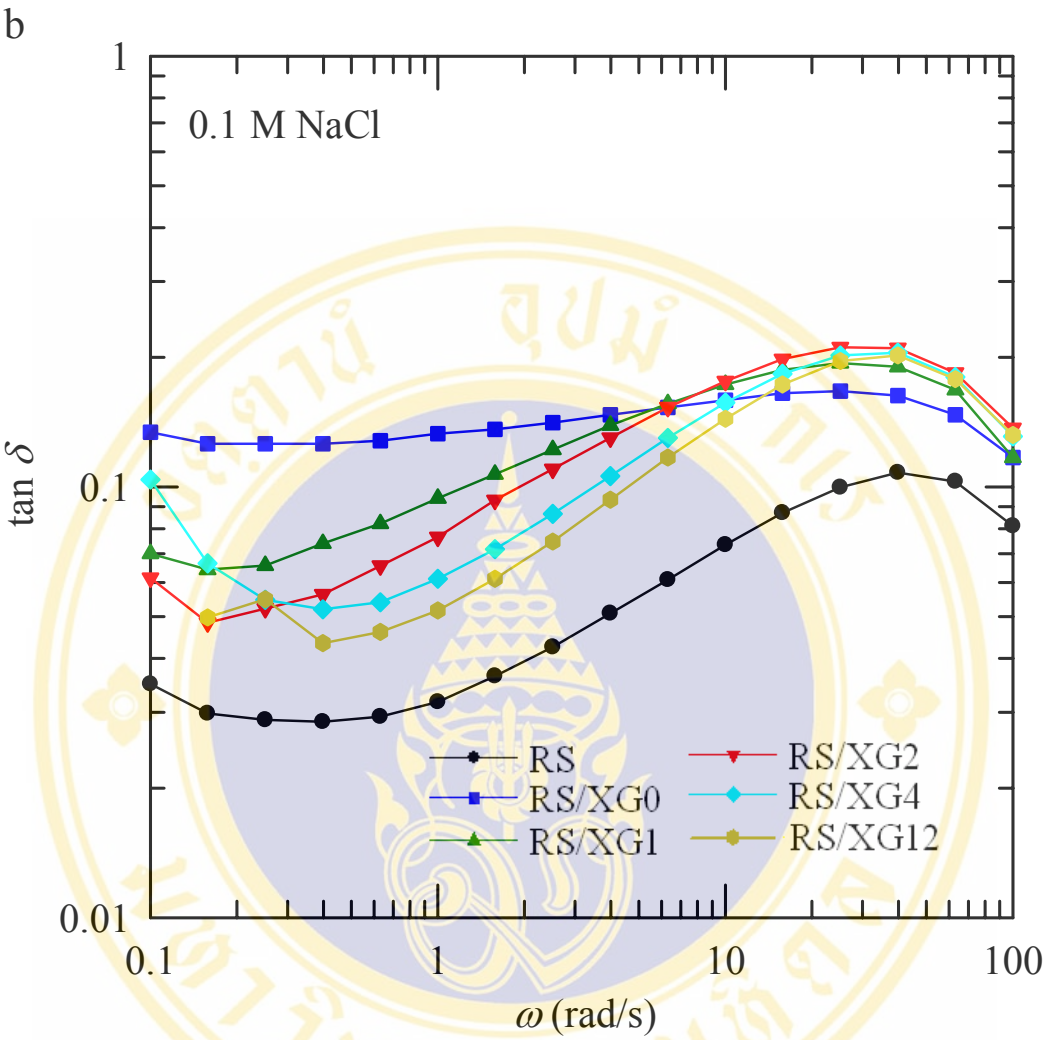


**Figure 4.2** Dynamic mechanical loss tangents ( $\tan\delta$ ) of 6% rice starch (RS) and rice starch (5.7%)/xanthan gum (XG; 0.3%) pastes in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions. Measurements were made at 0.5% strain and 25°C. Refer to Table 4.1 for the sample codes of XG (XG0 – XG12) having various $M_w$ values. (continued)

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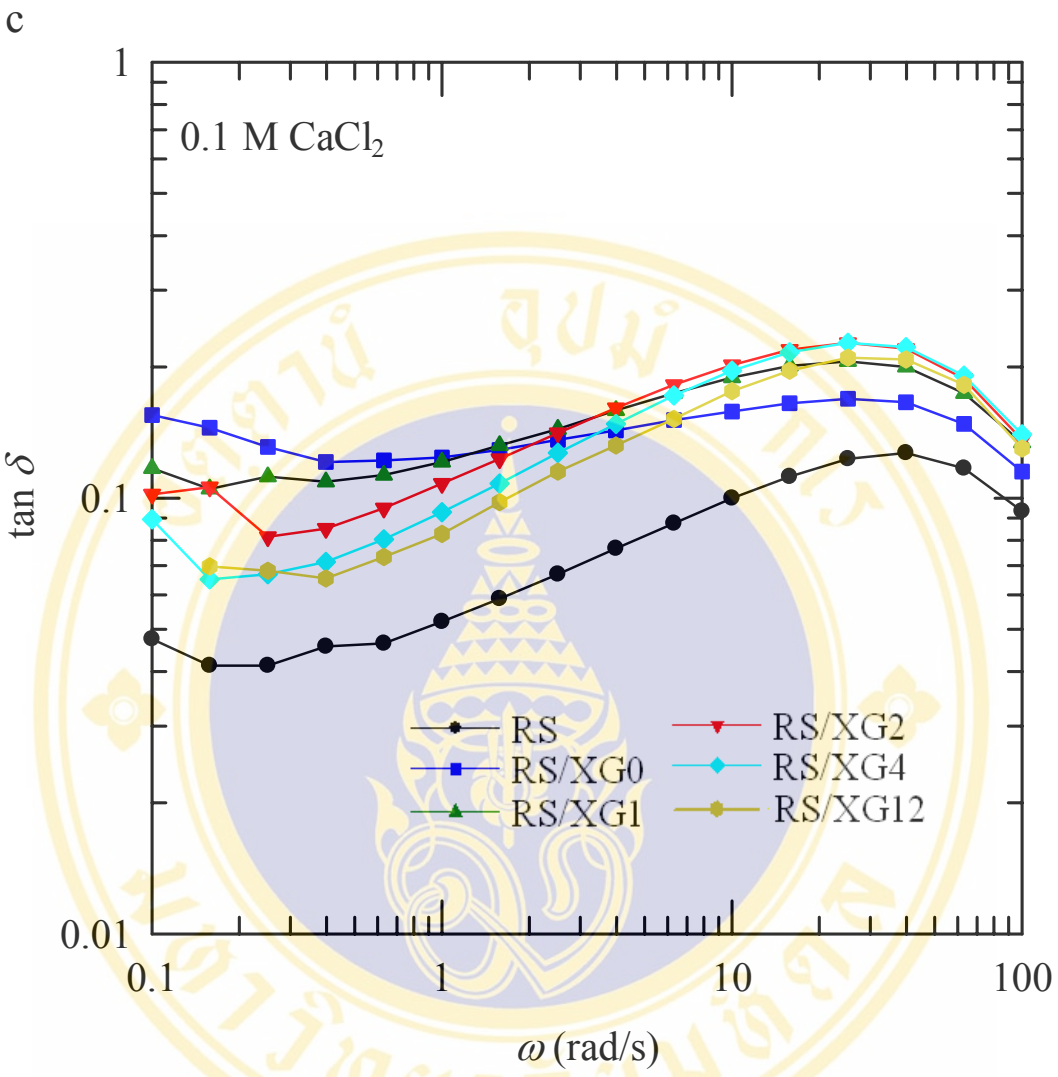


**Figure 4.2** Dynamic mechanical loss tangents ( $\tan\delta$ ) of 6% rice starch (RS) and rice starch (5.7%)/xanthan gum (XG; 0.3%) pastes in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions. Measurements were made at 0.5% strain and 25°C. Refer to Table 4.1 for the sample codes of XG (XG0 – XG12) having various $M_w$ values. (continued)

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**5. Steady shear rheological properties**

The steady flow characteristics of RS alone and RS/XG pastes with various $M_w$ of XG in the presence or absence of salts are presented in Figure 4.6. For the range of shear rates used in this study, all pastes exhibited mainly time-dependent shear-thinning (thixotropic) with yield stress behavior. Both the upward and downward flow curves were adequately described by the Herschel-Bulkley model. Though many models describe the flow behavior of fluids (e.g. the power law, Bingham, Herschel-Bulkley, and Casson model) (Rao, 1999), we used the Herschel-Bulkley model because of the best fit to the flow curves ($R^2 \approx 0.989$) of all pastes (Table 4.4). Consistency coefficients ($K$), flow behavior indices ($n$), and yield stresses ($\sigma_0$) along with 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.

As shown in Table 4.4, a hysteresis loop area was observed in all pastes studied. However, the hysteresis loop of RS alone pastes was significantly ($P \leq 0.05$) reduced when XG was added to the systems. This effect was more pronounced for XG with high $M_w$ and in the presence of salts.

Yield stress $(\sigma_0)$ was observed in all pastes studied. The results demonstrated that a significantly ($P \leq 0.05$) higher magnitude of $\sigma_0$ was observed in the upward flow than in the downward flow curve. The $\sigma_0$ of RS/XG gels decreased with decreasing $M_w$ of XG and slightly increased with the addition of salts.

The consistency coefficients ($K$) and the flow behavior indices ($n$) of RS/XG pastes without salt showed that the $K$ values significantly ($P \leq 0.05$) decreased and the $n$ values increased with decreasing $M_w$ of XG for both the upward and downward curves. In the presence of salts, the opposite trends were apparently observed for both salts.

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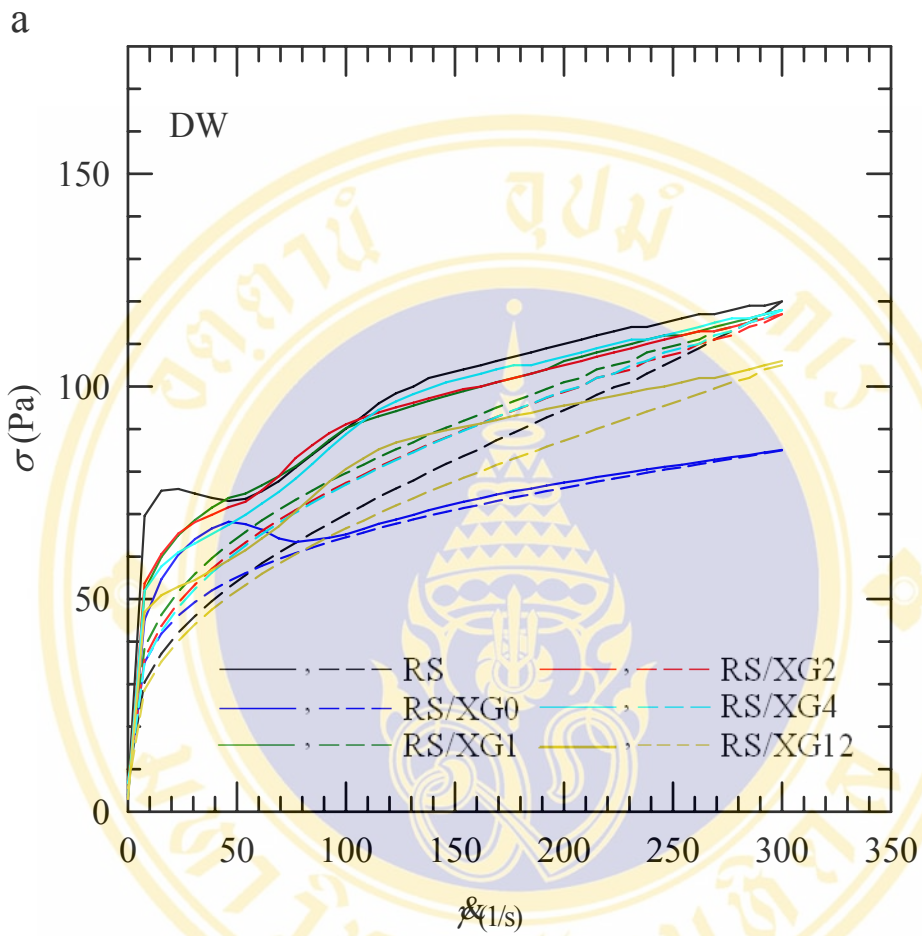


**Figure 4.3** Flow curves of 6% rice starch (RS) and rice starch (5.7%)/xanthan gum (XG; 0.3%) pastes in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions measured at 25°C. Solid lines represent upward curves and dashed lines represent downward curves. Refer to Table 4.1 for the sample codes of XG (XG0 – XG12) having various $M_w$ values.

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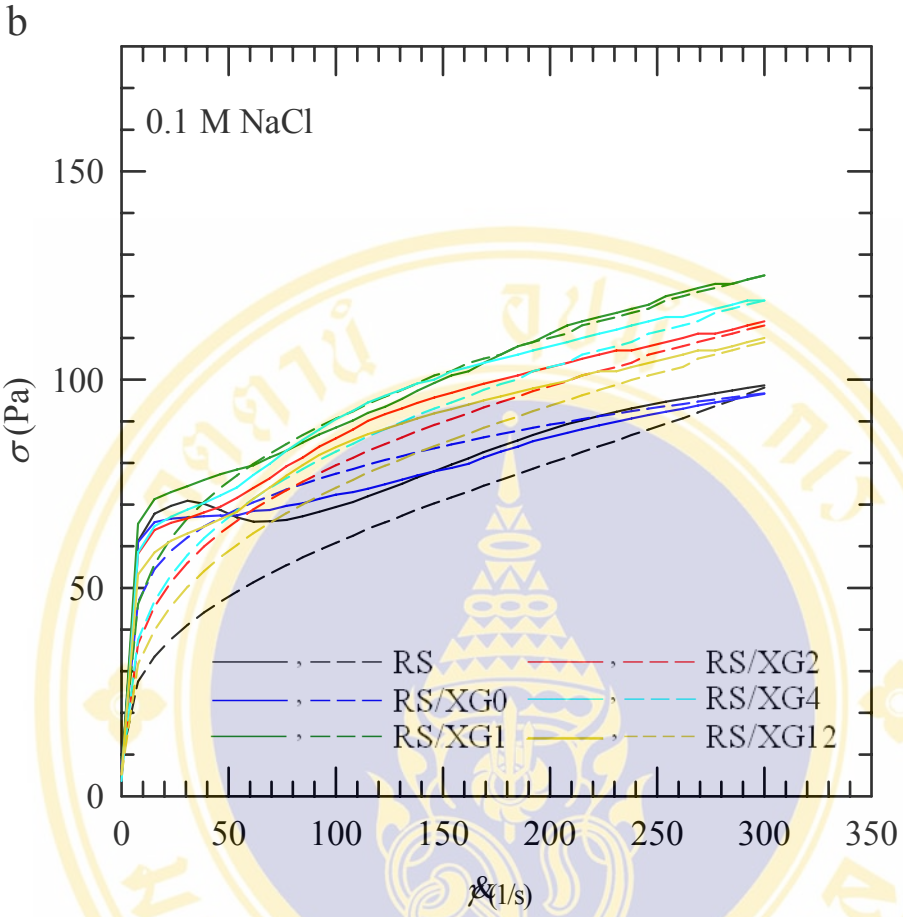


**Figure 4.3** Flow curves of 6% rice starch (RS) and rice starch (5.7%)/xanthan gum (XG; 0.3%) pastes in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions measured at 25°C. Solid lines represent upward curves and dashed lines represent downward curves. Refer to Table 4.1 for the sample codes of XG (XG0 – XG12) having various $M_w$ values. (continued)

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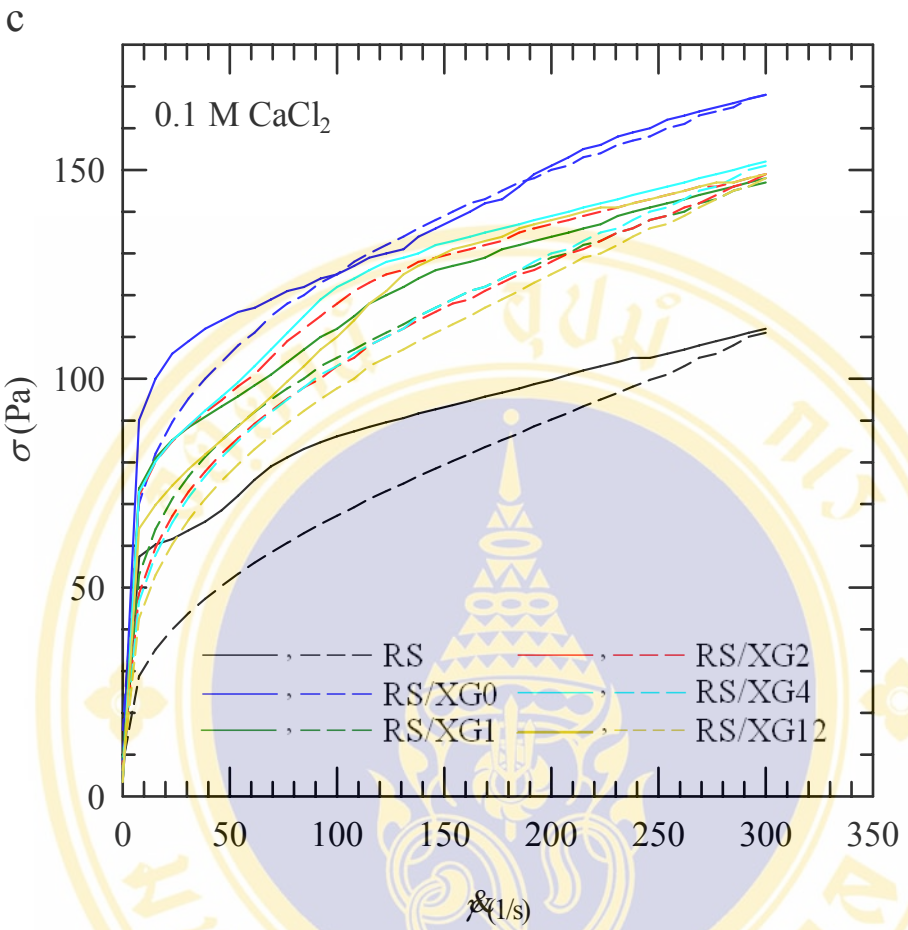


**Figure 4.3** Flow curves of 6% rice starch (RS) and rice starch (5.7%)/xanthan gum (XG; 0.3%) pastes in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions measured at 25°C. Solid lines represent upward curves and dashed lines represent downward curves. Refer to Table 4.1 for the sample codes of XG (XG0 – XG12) having various $M_w$ values. (continued)

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**Table 4.4** The Herschel-Bulkley parameters for 6% rice starch (RS) pastes and RS (5.7%)/xanthan gum (XG; 0.3%) mixed pastes in the presence or absence of salts at 0.1 M concentration[1,2]

| Salt | Hydrocolloid[3] | Hysteresis loop area (Pa $s^{-1}$) | Upward curve | | | | Downward curve | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $\sigma_0$ (Pa) | $K$ (Pa $s^n$) | $n$ (-) | $R^2$ | $\sigma_0$ (Pa) | $K$ (Pa $s^n$) | $n$ (-) | $R^2$ |
| None | None | 5126 ± 131$^{a}$ | 60.3 ± 4.3$^{a}$ | 0.8 ± 0.3$^{d}$ | 0.78 ± 0.06$^{a}$ | 0.972 | 23.6 ±1.8$^{a}$ | 4.7 ± 0.5$^{d}$ | 0.52 ± 0.02$^{a}$ | 0.862 |
| | XG0 | 1058 ± 43$^{e}$ | 30.6 ± 5.3$^{b}$ | 12.6 ± 4.7$^{b}$ | 0.27 ± 0.04$^{c}$ | 0.952 | 8.8 ± 2.7$^{d}$ | 21.3 ± 3.2$^{a}$ | 0.23 ± 0.01$^{d}$ | 0.941 |
| | XG1 | 2086 ± 80$^{d}$ | 25.9 ± 4.9$^{b}$ | 11.7 ± 2.3$^{bc}$ | 0.36 ± 0.03$^{bc}$ | 0.990 | 19.9 ± 0.7$^{ab}$ | 11.4 ± 0.9$^{b}$ | 0.38 ± 0.01$^{c}$ | 0.968 |
| | XG2 | 2538 ±113$^{c}$ | 29.9 ± 2.2$^{b}$ | 10.4 ± 1.5$^{bc}$ | 0.38 ± 0.03$^{b}$ | 0.995 | 18.1 ± 1.0$^{bc}$ | 10.8 ± 0.7$^{bc}$ | 0.39 ± 0.01$^{bc}$ | 0.960 |
| | XG4 | 2657 ±108$^{bc}$ | 32.4 ± 1.7$^{b}$ | 7.8 ± 1.8$^{bc}$ | 0.43 ± 0.04$^{b}$ | 0.993 | 18.4 ± 1.1$^{bc}$ | 9.6 ± 1.0$^{bc}$ | 0.41 ± 0.02$^{bc}$ | 0.963 |
| | XG12 | 2826 ± 88$^{b}$ | 29.8 ± 0.4$^{b}$ | 6.0 ± 0.7$^{cd}$ | 0.46 ± 0.02$^{b}$ | 0.987 | 15.2 ± 0.4$^{c}$ | 7.9 ± 0.5$^{c}$ | 0.43 ±0.01$^{b}$ | 0.952 |
| NaCl | None | 3422 ± 137$^{a}$ | 65.4 ± 0.9$^{a}$ | 0.01 ±0.01$^{c}$ | 1.51 ± 0.13$^{a}$ | 0.970 | 23.4 ± 1.2$^{c}$ | 4.2 ± 0.2$^{c}$ | 0.50 ± 0.02$^{a}$ | 0.818 |
| | XG0 | -453 ± 78$^{d}$ | 62.9 ± 0.5$^{a}$ | 0.1 ± 0.1$^{c}$ | 1.08 ± 0.10$^{b}$ | 0.995 | 46.3 ± 0.1$^{a}$ | 3.3 ± 0.6$^{c}$ | 0.48 ± 0.02$^{ab}$ | 0.971 |
| | XG1 | 977 ±516$^{c}$ | 63.8 ± 1.2$^{a}$ | 1.1 ± 0.5$^{bc}$ | 0.74 ± 0.10$^{c}$ | 0.996 | 34.9 ± 3.8$^{b}$ | 8.4 ± 2.2$^{b}$ | 0.42 ± 0.04$^{b}$ | 0.976 |
| | XG2 | 1904 ± 187$^{b}$ | 49.2 ± 1.6$^{b}$ | 3.7 ± 0.8$^{ab}$ | 0.52 ± 0.03$^{cd}$ | 0.995 | 16.3 ± 0.9$^{d}$ | 14.4 ± 1.6$^{a}$ | 0.34 ± 0.01$^{c}$ | 0.962 |
| | XG4 | 1942 ± 36$^{b}$ | 45.7 ± 1.4$^{b}$ | 4.4 ± 0.5$^{a}$ | 0.50 ± 0.02$^{d}$ | 0.996 | 16.2 ± 1.0$^{d}$ | 13.9 ± 0.4$^{a}$ | 0.36 ± 0.01$^{c}$ | 0.967 |
| | XG12 | 2290 ± 190$^{b}$ | 38.7 ± 2.6$^{c}$ | 5.9 ± 2.1$^{a}$ | 0.45 ± 0.06$^{d}$ | 0.997 | 7.7 ± 2.8$^{e}$ | 15.4 ± 2.0$^{a}$ | 0.33 ± 0.02$^{c}$ | 0.950 |
| $CaCl_2$ | None | 4181 ± 260$^{a}$ | 43.0 ± 0.6$^{c}$ | 4.7 ± 0.3$^{ab}$ | 0.48 ± 0.01$^{b}$ | 0.986 | 17.3 ± 1.0$^{c}$ | 7.3 ± 0.1$^{c}$ | 0.46 ± 0.01$^{a}$ | 0.948 |
| | XG0 | 822 ± 95$^{e}$ | 89.4 ± 3.1$^{a}$ | 1.5 ± 0.3$^{c}$ | 0.69 ± 0.04$^{a}$ | 0.992 | 45.7 ± 14.9$^{a}$ | 14.3 ± 6.4$^{ab}$ | 0.38 ± 0.05$^{bc}$ | 0.984 |
| | XG1 | 1862 ± 104$^{d}$ | 58.8 ± 2.9$^{b}$ | 5.1 ± 1.3$^{ab}$ | 0.51 ± 0.04$^{b}$ | 0.997 | 28.7 ± 9.8$^{bc}$ | 14.0 ± 3.5$^{ab}$ | 0.38 ± 0.03$^{bc}$ | 0.987 |
| | XG2 | 3095 ± 67$^{c}$ | 40.4 ± 3.1$^{c}$ | 14.4 ± 1.9$^{a}$ | 0.36 ± 0.02$^{b}$ | 0.996 | 17.4 ± 2.8$^{c}$ | 20.1 ± 1.5$^{a}$ | 0.33 ± 0.01$^{c}$ | 0.963 |
| | XG4 | 3589 ± 104$^{b}$ | 36.3 ± 1.0$^{c}$ | 14.1 ± 9.7$^{a}$ | 0.39 ± 0.12$^{b}$ | 0.995 | 11.9 ± 4.8$^{c}$ | 19.5 ± 5.6$^{a}$ | 0.33 ± 0.03$^{c}$ | 0.958 |
| | XG12 | 3399 ± 96$^{bc}$ | 41.2 ± 5.1$^{c}$ | 8.1 ± 1.8$^{ab}$ | 0.47 ± 0.04$^{b}$ | 0.993 | 21.2 ± 3.5$^{c}$ | 12.7 ± 1.2$^{ab}$ | 0.41 ± 0.02$^{ab}$ | 0.970 |

[1] Assays were performed in triplicate. Mean ± standard deviation values in the same column for each solution followed by different superscripts are significantly different ($P \leq 0.05$).

[2] Herschel-Bulkley parameters: $\sigma_0$, yield stress; $K$, consistency coefficient; $n$, flow behavior index.

[3] Refer to Table 4.1 for the sample codes of XG (XG0 –XG12) having various $M_w$ values.





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**6. Syneresis determination**

Syneresis was determined by measuring separation of water from the composite gel (RS alone or RS/XG mixed gel in the presence or absence of salts) by centrifugation method at 1180 g after storage at 4°C for 1, 3, 7, 14, 21, 35, 49, and 63 days (Figure 4.4 a-c).

The syneresis of RS alone gels in the presence or absence of salts showed the highest syneresis production. At 7 and 14 days, syneresis more rapidly increased and around 21-63 days their values were slightly increase. In the case of RS/XG mixed gel, syneresis production are three times lower than in RS alone and this effect became greater in order XG0 > XG1 > XG2> XG4 > XG12, which was in accordance with the order of the $M_w$ and [$\eta$] of XG. The presence of $CaCl_2$ at the concentration used in this study seemed to be exhibit more pronounced effect on lower production of syneresis of the system than did NaCl.

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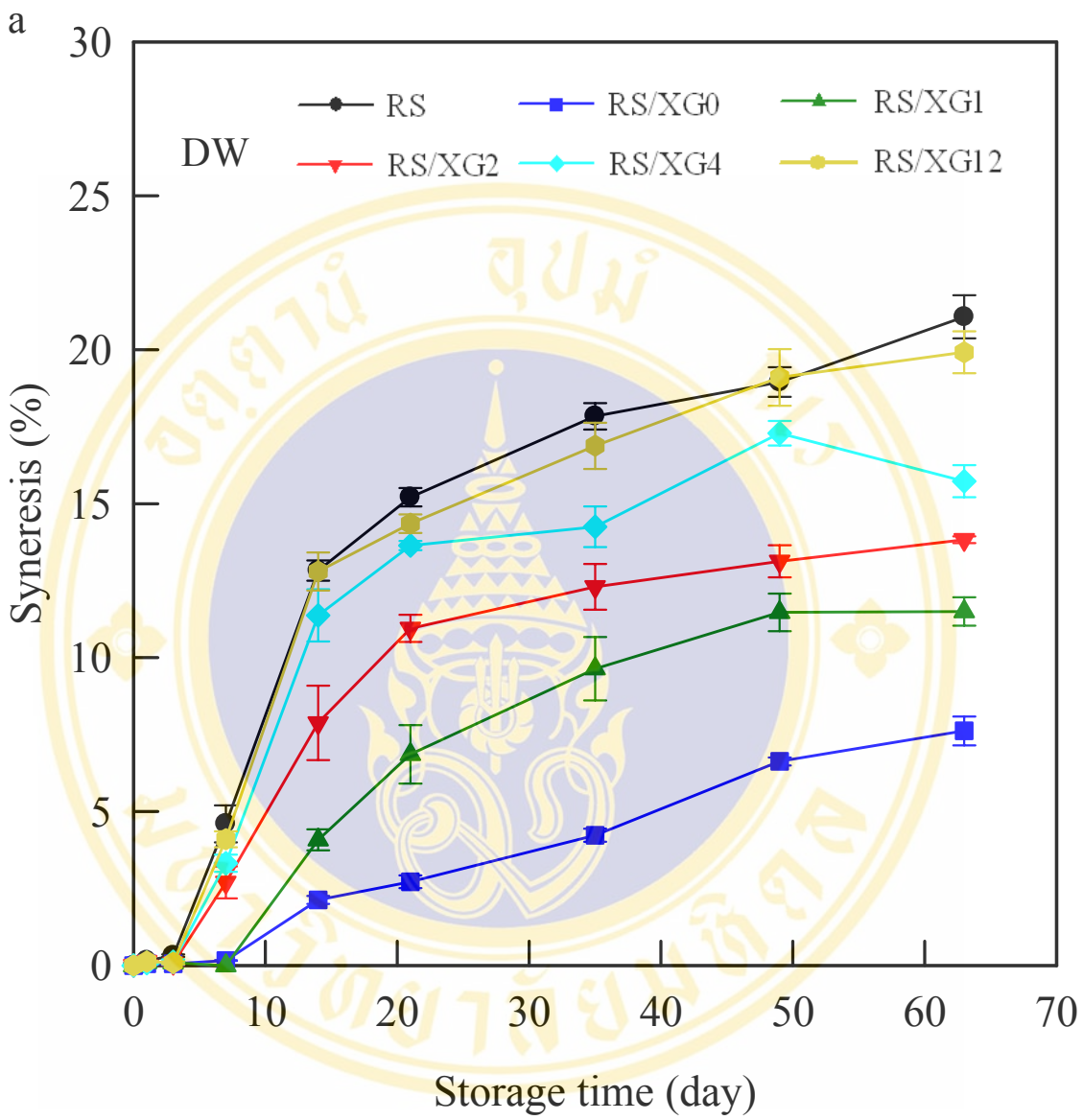


**Figure 4.4** Syneresis production of 6% rice starch (RS) and rice starch (5.7%)/xanthan gum (XG)(0.3%) pastes in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions as a function of storage time at 4°C. Error bars represent standard deviations. Refer to Table 4.1 for the sample codes of XG (XG0 – XG12) having various $M_w$ values.

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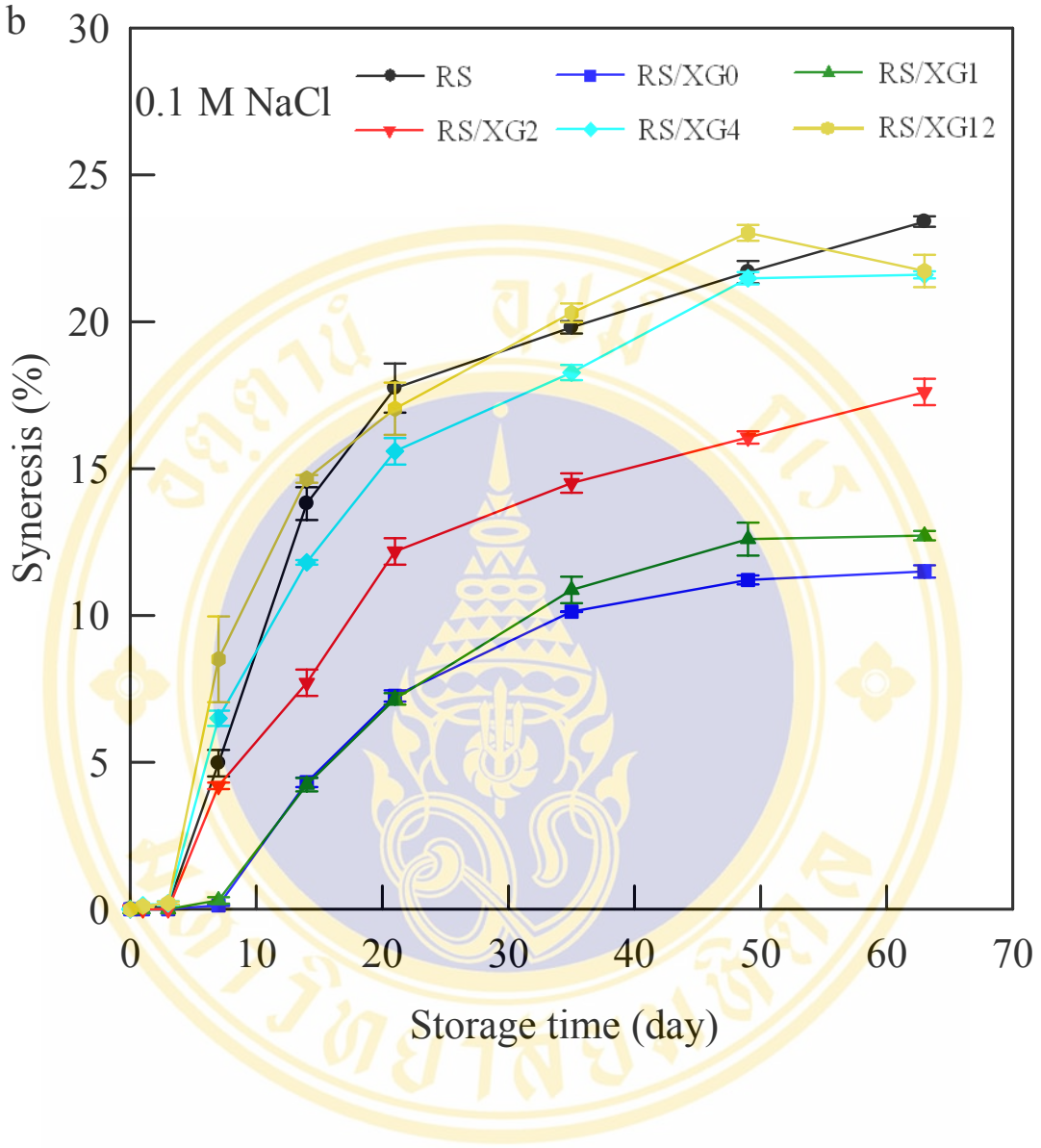


**Figure 4.4** Syneresis production of 6% rice starch (RS) and rice starch (5.7%)/xanthan gum (XG)(0.3%) pastes in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions as a function of storage time at 4°C. Error bars represent standard deviations. Refer to Table 4.1 for the sample codes of XG (XG0 – XG12) having various $M_w$ values. (Continued)

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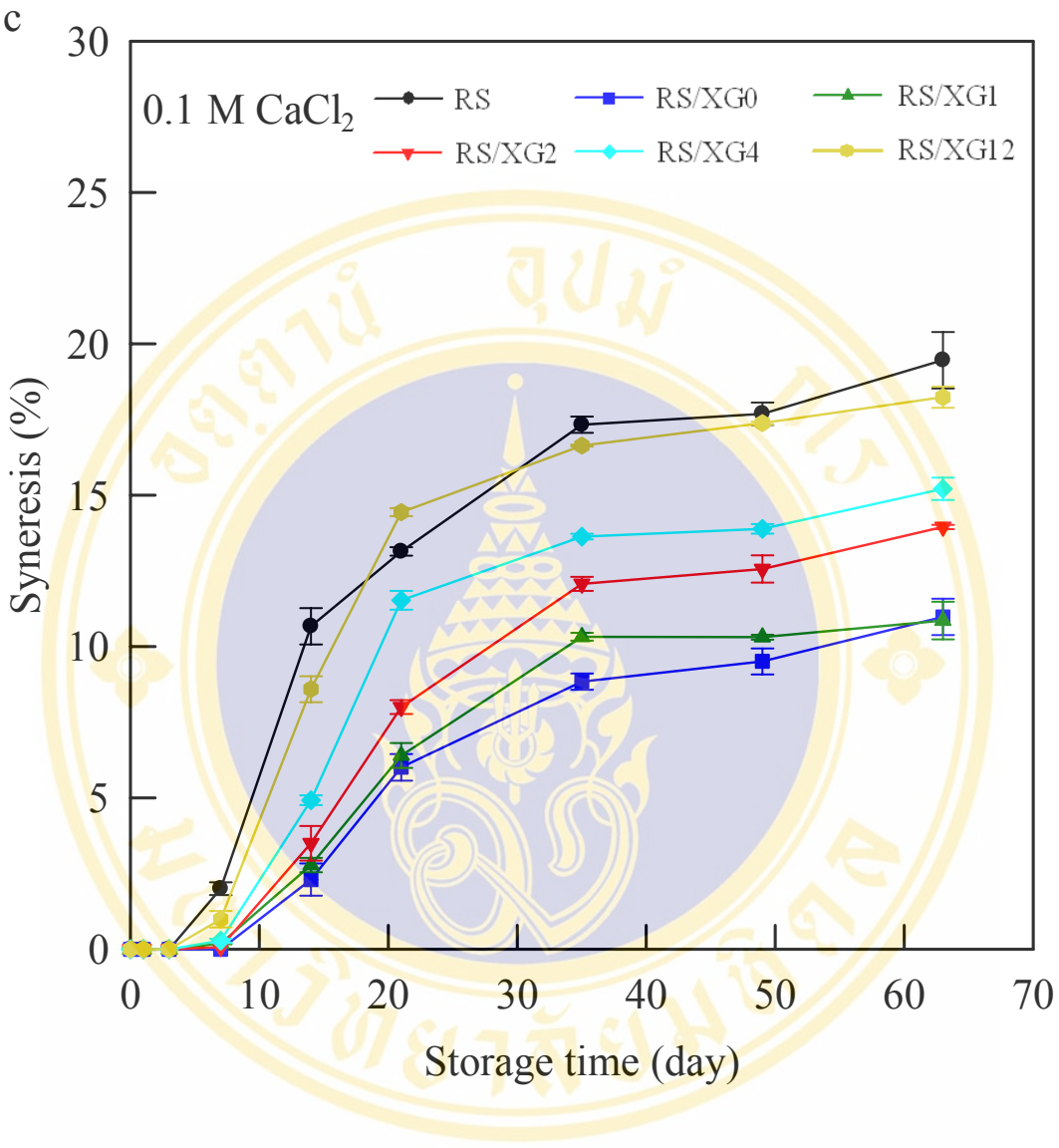


**Figure 4.4** Syneresis production of 6% rice starch (RS) and rice starch (5.7%)/xanthan gum (XG)(0.3%) pastes in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions as a function of storage time at 4°C. Error bars represent standard deviations. Refer to Table 4.1 for the sample codes of XG (XG0 – XG12) having various $M_w$ values. (Continued)

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**7. Thermal properties of retrograded RS/XG mixed gel**

The thermal properties of RS alone and RS/XG mixtures, in the presence or absence of salts, and their corresponding retrograded gels determined by DSC, are summarized in Table 4.5. For the first run, addition of 0.1 M NaCl or $CaCl_2$ to RS alone suspensions resulted in a significant ($P \leq 0.05$) increase in $T_o$, $T_p$, and $T_c$ and a decrease in $T_c$-$T_o$ and $\Delta H_1$ values as compared with the control. Addition of XG to RS alone or RS/NaCl systems resulted in a significant increase in $T_o$ and $T_p$ and a decrease in $\Delta H_1$, whereas $T_c$ and $T_c$-$T_o$ seemed to be unaffected. However, addition of XG to RS/$CaCl_2$ suspensions did not affect the gelatinization parameters except $\Delta H_1$ which was significantly decreased by XG addition.

For the second run data of the retrograded gels, the endothermic transition temperatures ($T_o$, $T_p$, and $T_c$) and the melting enthalpies ($\Delta H_2$) were much lower than those observed during gelatinization of native starch (the first run). The retrogradation temperature range ($T_c$-$T_o$) were more than twofold broader than the gelatinization temperature ranges for a given sample. Addition of XG as well as salts did not affect the thermal properties of the retrograded RS gels under the conditions tested. However, the retrogradation ratios ($\Delta H_2/\Delta H_1$) were significantly enhanced by XG addition either in the presence or absence of salts.

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**Table 4.5** Gelatinization temperature, enthalpy, and retrogradation ration for 12% rice starch (RS) and RS/xanthan gum (XG; 5.7/0.3, w/w ratio) mixtures dispersed in aqueous solutions with or without salts at 0.1 M concentration.[1,2]

| | | First run | | | | | Second run (30 days at 4°C) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Salt | Hydrocolloid | $T_o$ (°C) | $T_p$ (°C) | $T_c$ (°C) | $T_c$-$T_o$ (°C) | $\Delta H_1$ (J/g) | $T_o$ (°C) | $T_p$ (°C) | $T_c$ (°C) | $T_c$-$T_o$ (°C) | $\Delta H_2$ (J/g) | $\Delta H_2/\Delta H_1$ |
| None | None | 71.0±0.5$^{b}$ | 76.0±0.4$^{b}$ | 80.4±0.6$^{a}$ | 9.4±0.6$^{a}$ | 21.2±4.7$^{a}$ | 43.4±3.8$^{a}$ | 51.8±0.1$^{a}$ | 63.7±2.5$^{a}$ | 20.4±5.8$^{a}$ | 10.3±4.5$^{a}$ | 0.47±0.10$^{b}$ |
| | XG0 | 71.8±0.3$^{a}$ | 76.7±0.1$^{a}$ | 81.1±0.2$^{a}$ | 9.3±0.4$^{a}$ | 11.8±1.6$^{b}$ | 41.2±4.1$^{a}$ | 53.5±2.2$^{a}$ | 61.1±4.6$^{a}$ | 19.9±8.6$^{a}$ | 8.5±1.6$^{a}$ | 0.76±0.12$^{a}$ |
| | XG1 | 72.0±0.4$^{a}$ | 76.9±0.2$^{a}$ | 81.3±0.4$^{a}$ | 9.3±0.3$^{a}$ | 11.9±0.8$^{b}$ | 43.7±2.1$^{a}$ | 53.4±1.8$^{a}$ | 62.9±1.1$^{a}$ | 19.3±3.0$^{a}$ | 7.2±1.1$^{a}$ | 0.61±0.13$^{ab}$ |
| | XG2 | 71.7±0.4$^{a}$ | 76.7±0.1$^{a}$ | 81.1±0.3$^{a}$ | 9.4±0.4$^{a}$ | 11.3±1.2$^{b}$ | 44.3±1.4$^{a}$ | 53.6±2.5$^{a}$ | 62.6±1.5$^{a}$ | 18.3±2.6$^{a}$ | 7.4±1.1$^{a}$ | 0.66±0.15$^{ab}$ |
| | XG4 | 71.8±0.4$^{a}$ | 76.7±0.1$^{a}$ | 81.0±0.1$^{a}$ | 9.2±0.3$^{a}$ | 10.8±1.2$^{b}$ | 43.5±2.4$^{a}$ | 53.6±2.0$^{a}$ | 63.4±1.0$^{a}$ | 19.9±3.2$^{a}$ | 8.1±1.5$^{a}$ | 0.76±0.19$^{ab}$ |
| | XG12 | 71.6±0.1$^{ab}$ | 76.9±0.1$^{a}$ | 81.2±0.3$^{a}$ | 9.7±0.4$^{a}$ | 10.4±0.7$^{b}$ | 46.0±3.1$^{a}$ | 55.8±2.7$^{a}$ | 63.3±2.0$^{a}$ | 17.3±3.9$^{a}$ | 6.0±1.4$^{a}$ | 0.58±0.13$^{ab}$ |
| NaCl | None | 74.1±0.2$^{b}$ | 79.0±0.2$^{b}$ | 83.1±0.3$^{b}$ | 8.9±0.2$^{a}$ | 19.8±1.6$^{a}$ | 43.3±1.7$^{a}$ | 53.4±2.1$^{a}$ | 62.6±0.6$^{a}$ | 19.3±2.3$^{a}$ | 9.1±1.8$^{a}$ | 0.46±0.09$^{b}$ |
| | XG0 | 74.6±0.2$^{a}$ | 79.4±0.1$^{a}$ | 83.6±0.3$^{ab}$ | 9.0±0.1$^{a}$ | 13.1±1.1$^{b}$ | 44.0±2.6$^{a}$ | 54.6±2.4$^{a}$ | 64.2±0.8$^{a}$ | 20.2±3.0$^{a}$ | 8.9±0.6$^{a}$ | 0.68±0.07$^{a}$ |
| | XG1 | 74.7±0.2$^{a}$ | 79.4±0.1$^{a}$ | 83.6±0.2$^{ab}$ | 8.9±0.2$^{a}$ | 12.7±0.9$^{b}$ | 44.4±2.0$^{a}$ | 53.9±1.6$^{a}$ | 63.1±0.9$^{a}$ | 18.8±2.6$^{a}$ | 8.1±1.3$^{a}$ | 0.64±0.07$^{ab}$ |
| | XG2 | 74.7±0.2$^{a}$ | 79.4±0.1$^{a}$ | 83.5±0.2$^{ab}$ | 8.7±0.1$^{a}$ | 12.0±0.6$^{b}$ | 44.4±2.9$^{a}$ | 54.6±1.8$^{a}$ | 64.1±1.9$^{a}$ | 19.8±4.1$^{a}$ | 7.8±0.8$^{a}$ | 0.65±0.07$^{a}$ |
| | XG4 | 74.8±0.2$^{a}$ | 79.4±0.1$^{a}$ | 83.6±0.2$^{ab}$ | 8.8±0.1$^{a}$ | 12.7±1.0$^{b}$ | 45.6±2.4$^{a}$ | 54.4±1.9$^{a}$ | 63.7±1.9$^{a}$ | 18.2±4.2$^{a}$ | 7.7±1.5$^{a}$ | 0.60±0.10$^{ab}$ |
| | XG12 | 74.7±0.2$^{a}$ | 79.5±0.0$^{a}$ | 83.9±0.3$^{a}$ | 9.1±0.5$^{a}$ | 12.9±1.6$^{b}$ | 45.3±1.4$^{a}$ | 54.9±1.8$^{a}$ | 63.7±0.9$^{a}$ | 18.4±2.0$^{a}$ | 7.1±0.9$^{a}$ | 0.56±0.09$^{ab}$ |
| $CaCl_2$ | None | 75.8±0.2$^{a}$ | 80.5±0.1$^{a}$ | 84.3±0.1$^{a}$ | 8.6±0.2$^{a}$ | 16.0±1.4$^{a}$ | 46.2±1.2$^{a}$ | 53.8±1.9$^{a}$ | 63.2±2.3$^{a}$ | 17.0±2.1$^{a}$ | 7.1±1.2$^{a}$ | 0.44±0.08$^{b}$ |
| | XG0 | 76.1±0.2$^{a}$ | 80.6±0.2$^{a}$ | 84.6±0.3$^{a}$ | 8.5±0.4$^{a}$ | 11.5±2.1$^{b}$ | 44.9±3.6$^{a}$ | 55.1±2.7$^{a}$ | 63.6±0.8$^{a}$ | 18.6±3.4$^{a}$ | 7.4±1.4$^{a}$ | 0.65±0.11$^{ab}$ |
| | XG1 | 76.0±0.2$^{a}$ | 80.6±0.1$^{a}$ | 84.8±0.0$^{a}$ | 8.8±0.2$^{a}$ | 12.5±0.7$^{b}$ | 44.4±2.8$^{a}$ | 54.9±2.3$^{a}$ | 64.3±1.1$^{a}$ | 19.9±3.5$^{a}$ | 8.7±1.7$^{a}$ | 0.69±0.12$^{ab}$ |
| | XG2 | 75.7±0.2$^{a}$ | 80.3±0.1$^{a}$ | 84.4±0.2$^{a}$ | 8.7±0.2$^{a}$ | 12.3±0.8$^{b}$ | 42.1±4.6$^{a}$ | 54.5±2.3$^{a}$ | 63.3±1.5$^{a}$ | 21.2±6.1$^{a}$ | 8.2±1.9$^{a}$ | 0.67±0.17$^{ab}$ |
| | XG4 | 75.9±0.2$^{a}$ | 80.4±0.1$^{a}$ | 84.5±0.1$^{a}$ | 8.6±0.2$^{a}$ | 12.7±0.9$^{b}$ | 45.3±3.0$^{a}$ | 55.7±2.1$^{a}$ | 63.6±1.4$^{a}$ | 18.3±3.7$^{a}$ | 9.2±1.4$^{a}$ | 0.73±0.12$^{a}$ |
| | XG12 | 76.1±0.3$^{a}$ | 80.4±0.2$^{a}$ | 84.4±0.3$^{a}$ | 8.3±0.6$^{a}$ | 11.4±2.8$^{b}$ | 45.4±3.4$^{a}$ | 54.8±2.3$^{a}$ | 64.2±1.4$^{a}$ | 18.8±3.2$^{a}$ | 7.9±2.2$^{a}$ | 0.69±0.08$^{ab}$ |

[1]Assays were performed in triplicate. Mean ± standard deviation values in the same column for each solution with different superscripts are significantly different ($P \leq 0.05$).

[2]$T_o$, onset temperature; $T_p$, peak temperature; $T_c$, conclusion temperature; $\Delta H_1$, gelatinization enthalpy; $\Delta H_2$, retrogradation enthalpy; $\Delta H_2/\Delta H_1$, retrogradation ratio.

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**8. Dynamic viscoelastic measurements**

Changes in viscoelastic properties; storage modulus ( $G'$ ), loss modulus ( $G''$ ), loss tangent ( $\tan\delta$ ), of RS alone and RS/XG gels in the presence or absence of salts, after storage at 4°C for 1, 3, 7, 14, 21, 35, 49, and 63 days are shown in Figures 4.5 a-c, 4.6 a-c, 4.7 a-c, respectively.

In general, the $G'$ and $G''$ values of all gels steadily increased, whereas the $\tan\delta$ values tended to decrease with storage time. The most pronounced effect was observed for the controls (RS alone), either in the presence or absence salts, in which their $G'$ and $G''$ values were the highest and $\tan\delta$ values were the lowest among those of the gels tested. This effect was enhanced by addition of salts in the order $CaCl_2$ > NaCl > DW.

Addition of XG, having various $M_w$ values, to RS retarded an increase in $G'$ and $G''$ values and raised $\tan\delta$ values of RS gels during storage in the order XG0 > XG1 > XG2 > XG4 > XG12.

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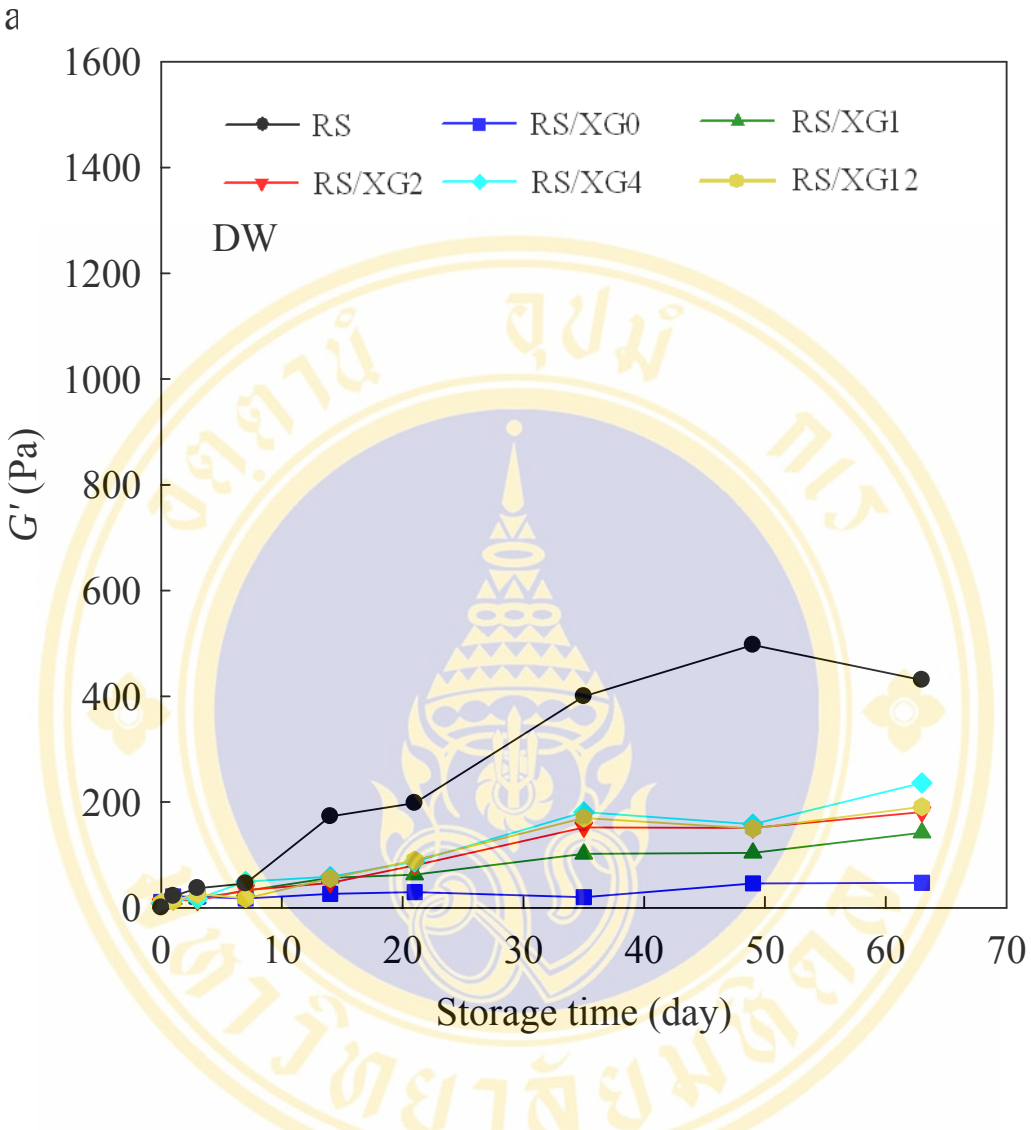


**Figure 4.5** Changes in storage modulus ($G'$) of 3.5% rice starch (RS) and RS/xanthan gum (XG) (5.7/0.3, w/w ratio) mixtures in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions as a function of storage time at 4°C. Measurements were made at 1 rad/s, 0.5% strain and 25°C.

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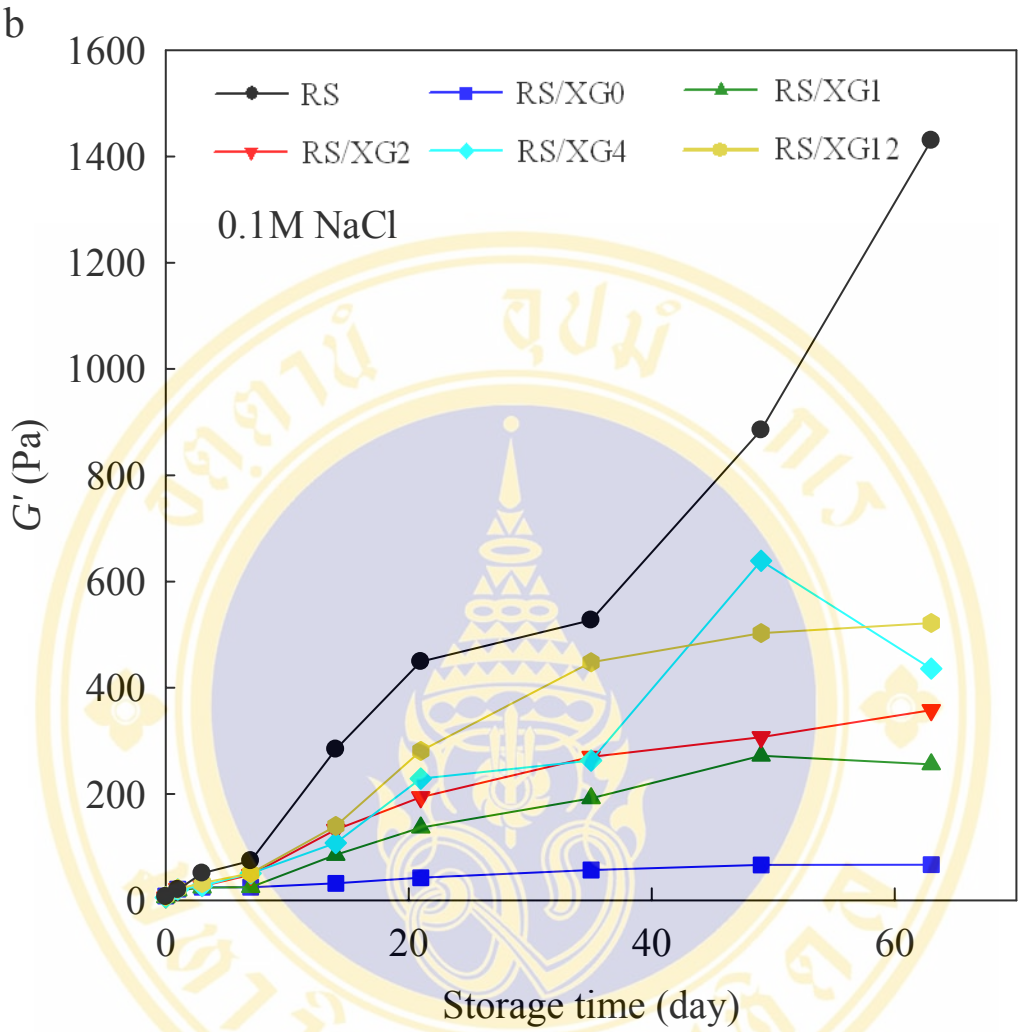


**Figure 4.5** Changes in storage modulus ($G'$) of 3.5% rice starch (RS) and RS/xanthan gum (XG) (5.7/0.3, w/w ratio) mixtures in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions as a function of storage time at 4°C. Measurements were made at 1 rad/s, 0.5% strain and 25°C. (Continued)

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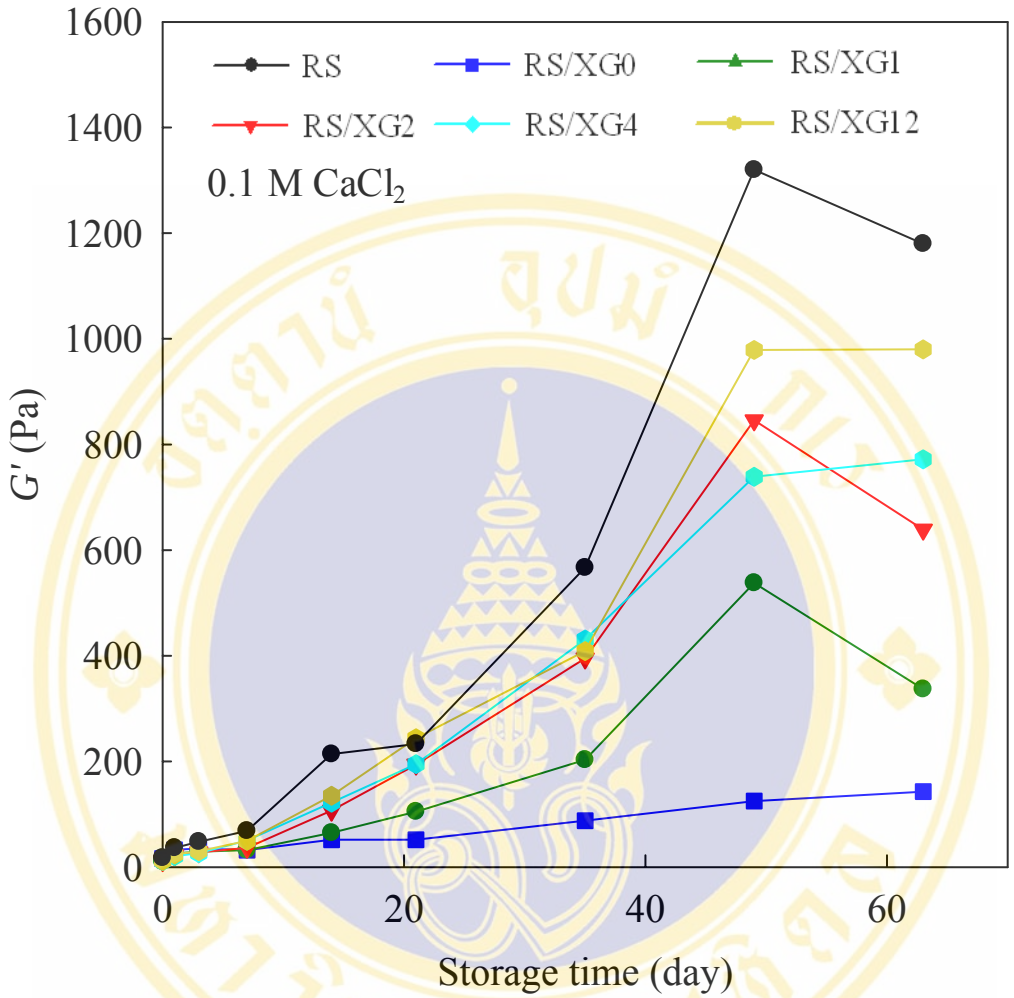


**Figure 4.5** Changes in storage modulus ($G'$) of 3.5% rice starch (RS) and RS/xanthan gum (XG) (5.7/0.3, w/w ratio) mixtures in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions as a function of storage time at 4°C. Measurements were made at 1 rad/s, 0.5% strain and 25°C. (Continued)

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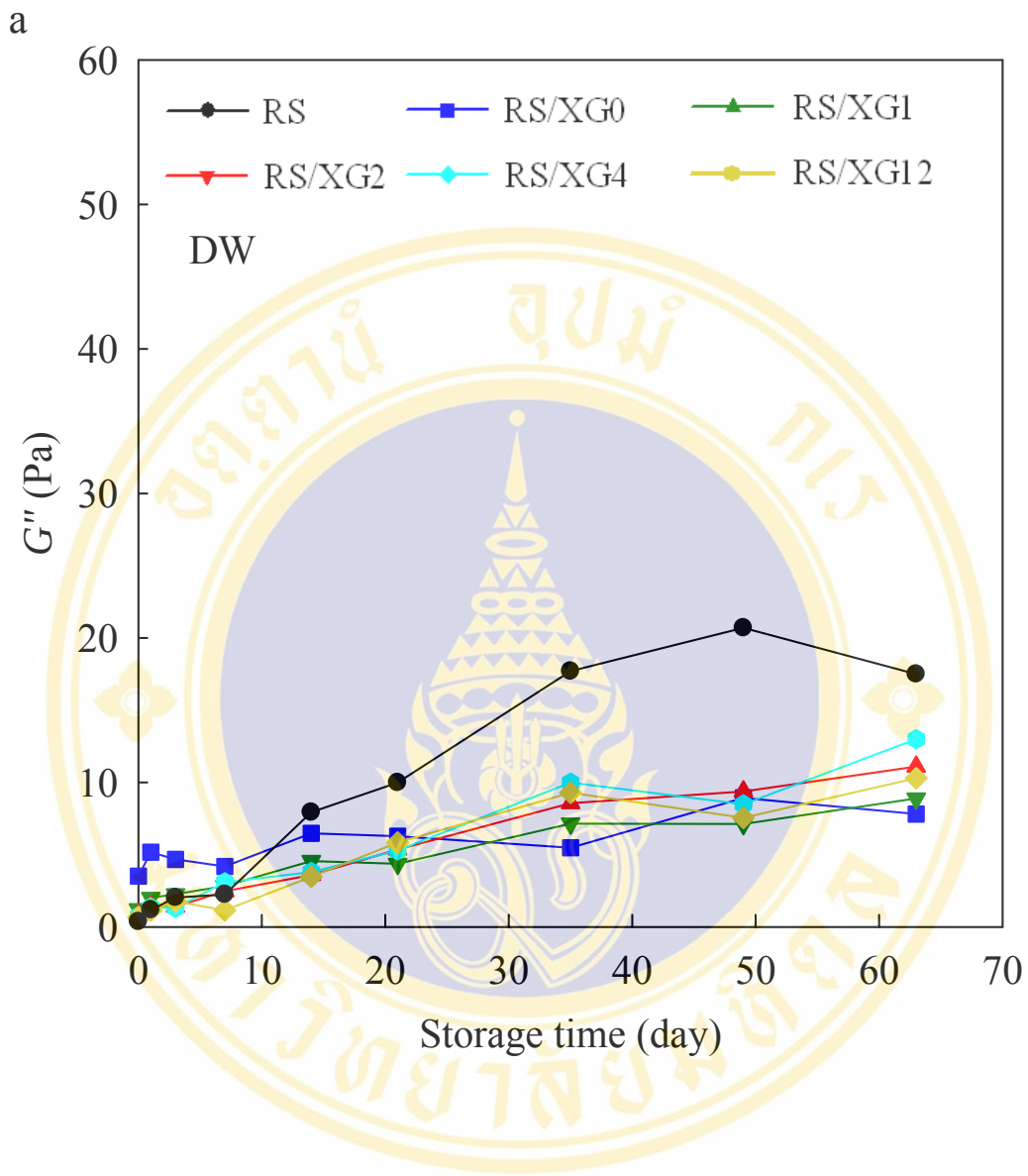


**Figure 4.6** Changes in loss modulus ($G''$) of 3.5% rice starch (RS) and RS/xanthan gum (XG) (5.7/0.3, w/w ratio) mixtures in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions as a function of storage time at 4°C. Measurements were made at 1 rad/s, 0.5% strain and 25°C.

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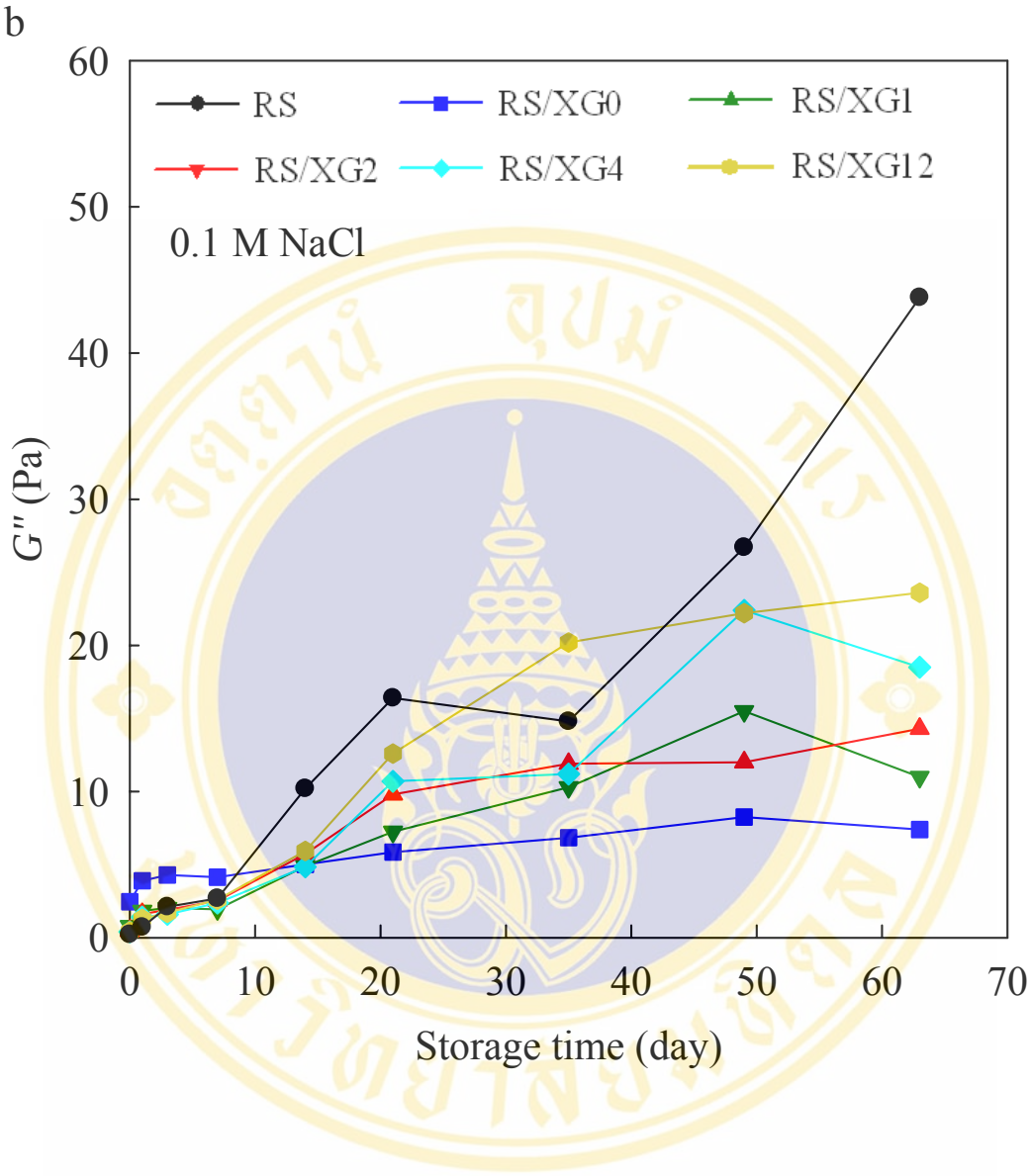


**Figure 4.6** Changes in loss modulus ($G''$) of 3.5% rice starch (RS) and RS/xanthan gum (XG) (5.7/0.3, w/w ratio) mixtures in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions as a function of storage time at 4°C. Measurements were made at 1 rad/s, 0.5% strain and 25°C. (Continued)

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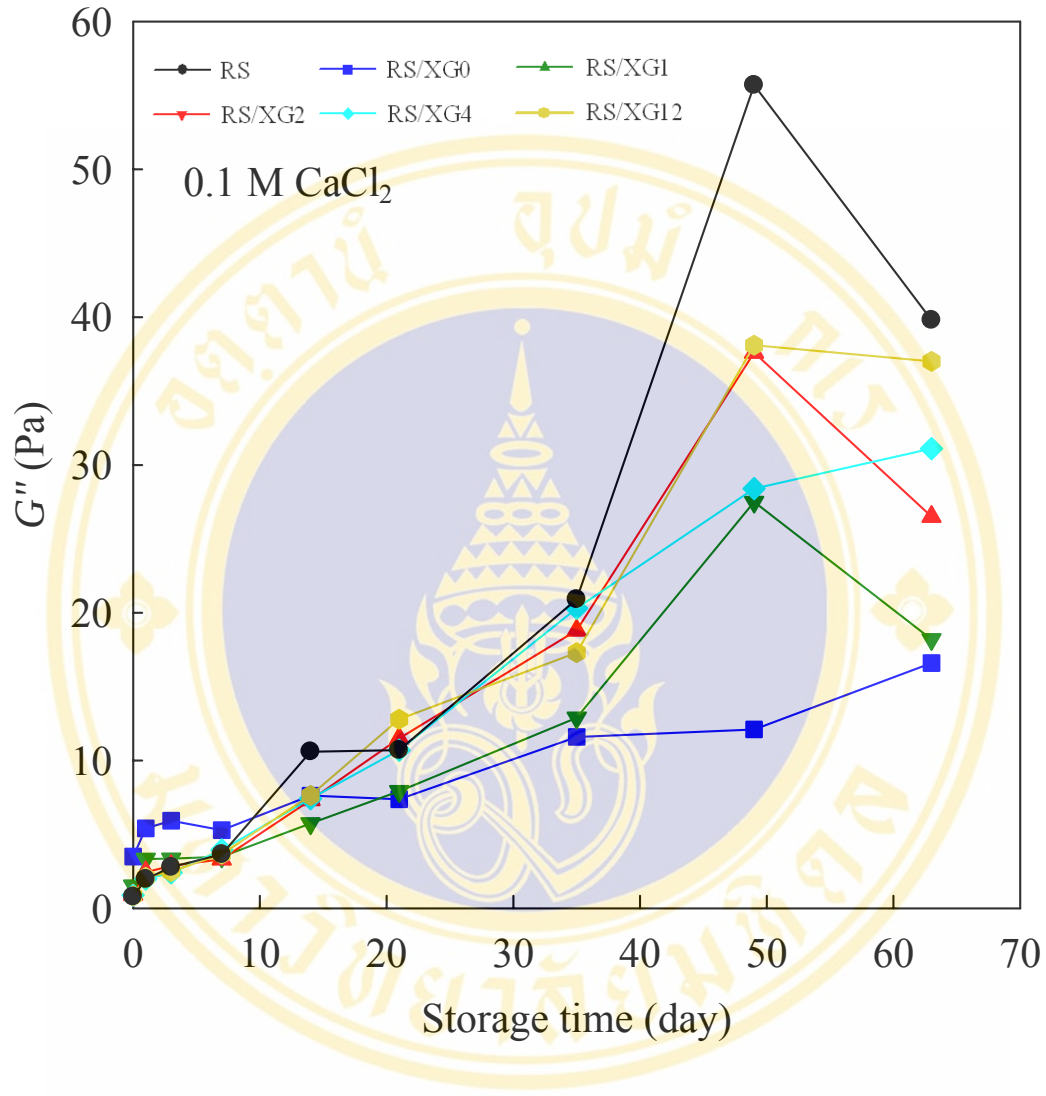


**Figure 4.6** Changes in loss modulus ($G''$) of 3.5% rice starch (RS) and RS/xanthan gum (XG) (5.7/0.3, w/w ratio) mixtures in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions as a function of storage time at 4°C. Measurements were made at 1 rad/s, 0.5% strain and 25°C. (Continued)

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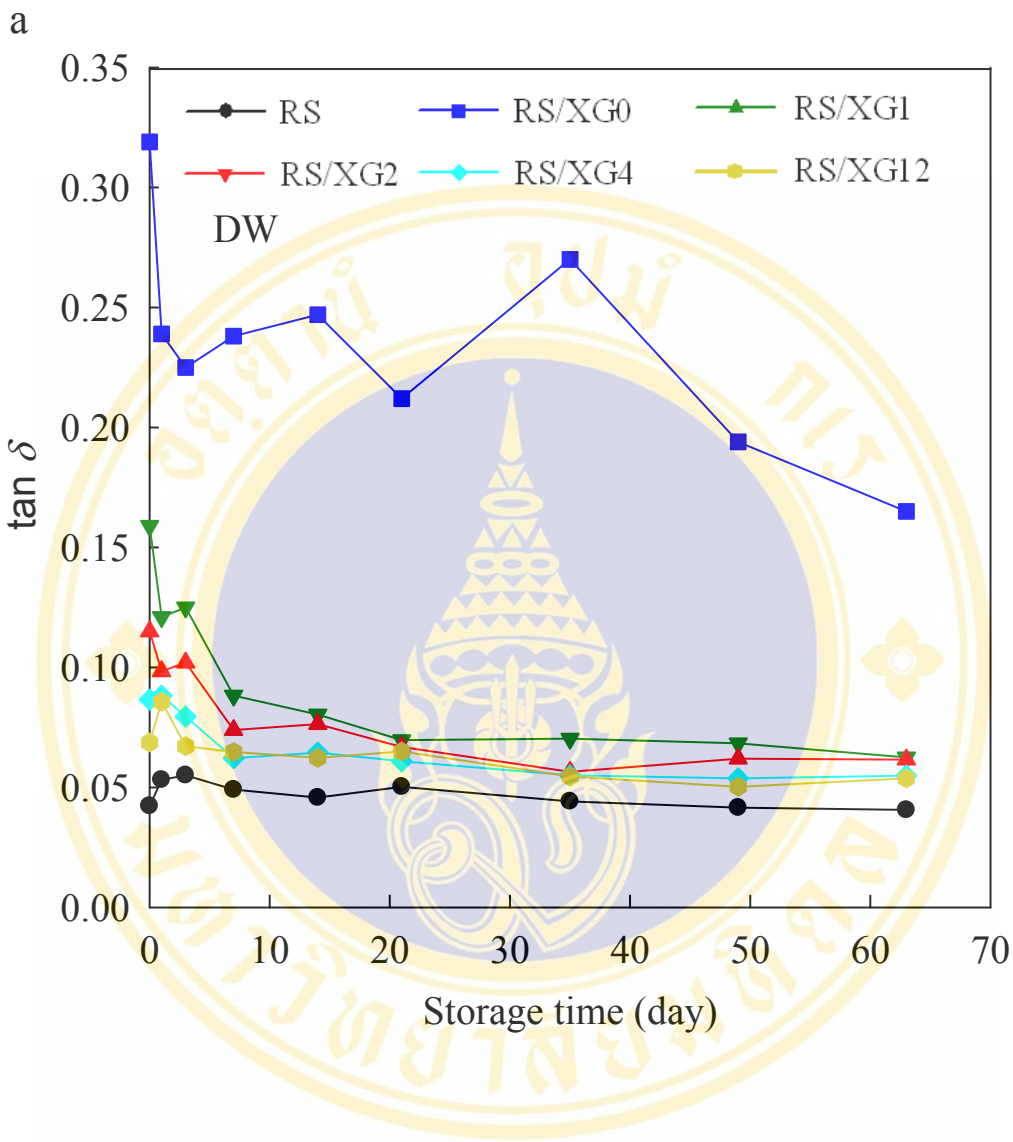


**Figure 4.7** Changes in loss tangent ( $\tan\delta$ ) of 3.5% rice starch (RS) and RS/xanthan gum (XG) (5.7/0.3, w/w ratio) mixtures in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions as a function of storage time at 4°C. Measurements were made at 1 rad/s, 0.5% strain and 25°C.

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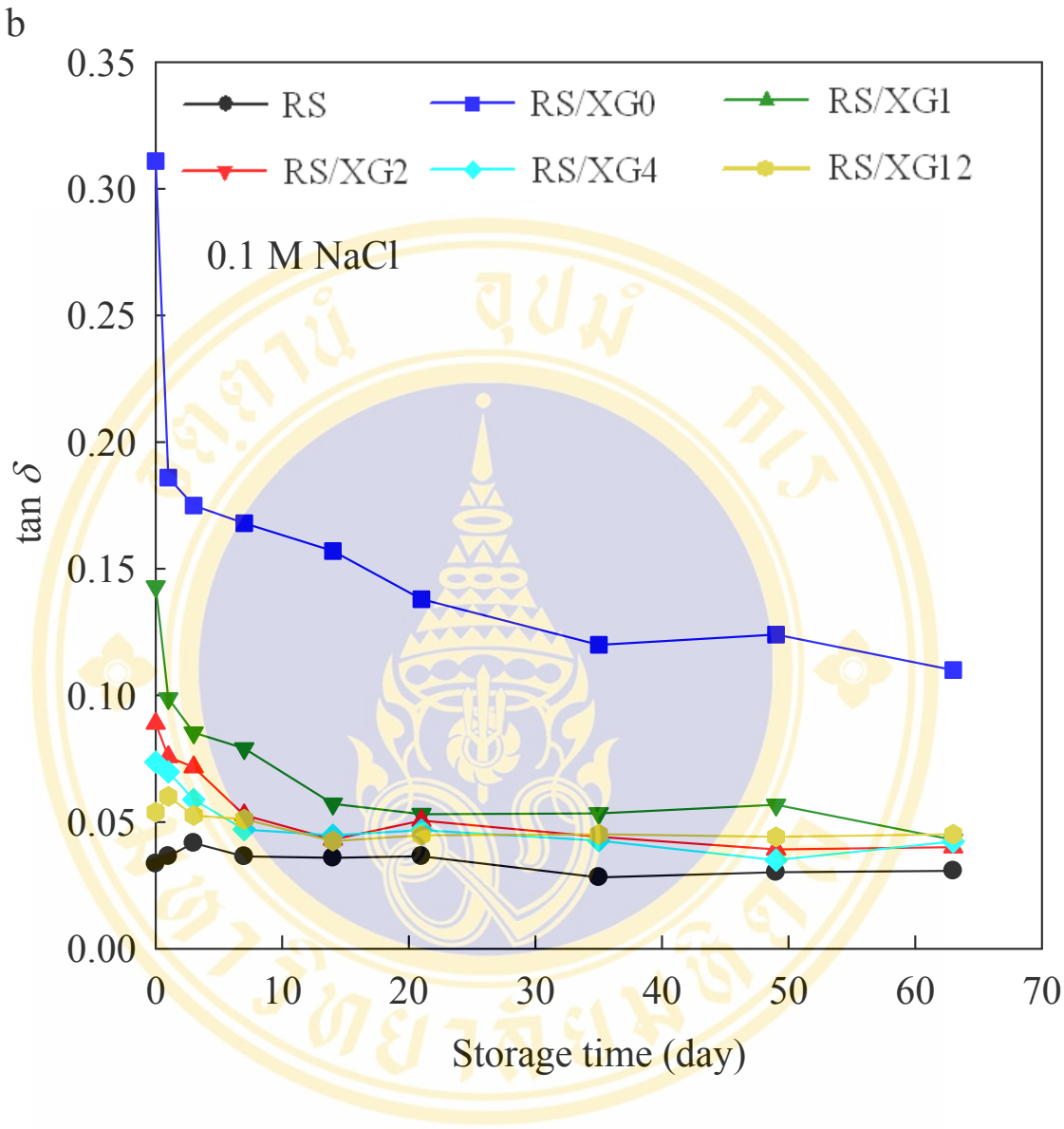


**Figure 4.7** Changes in loss tangent ( $\tan\delta$ ) of 3.5% rice starch (RS) and RS/xanthan gum (XG) (5.7/0.3, w/w ratio) mixtures in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions as a function of storage time at 4°C. Measurements were made at 1 rad/s, 0.5% strain and 25°C. (Continued)

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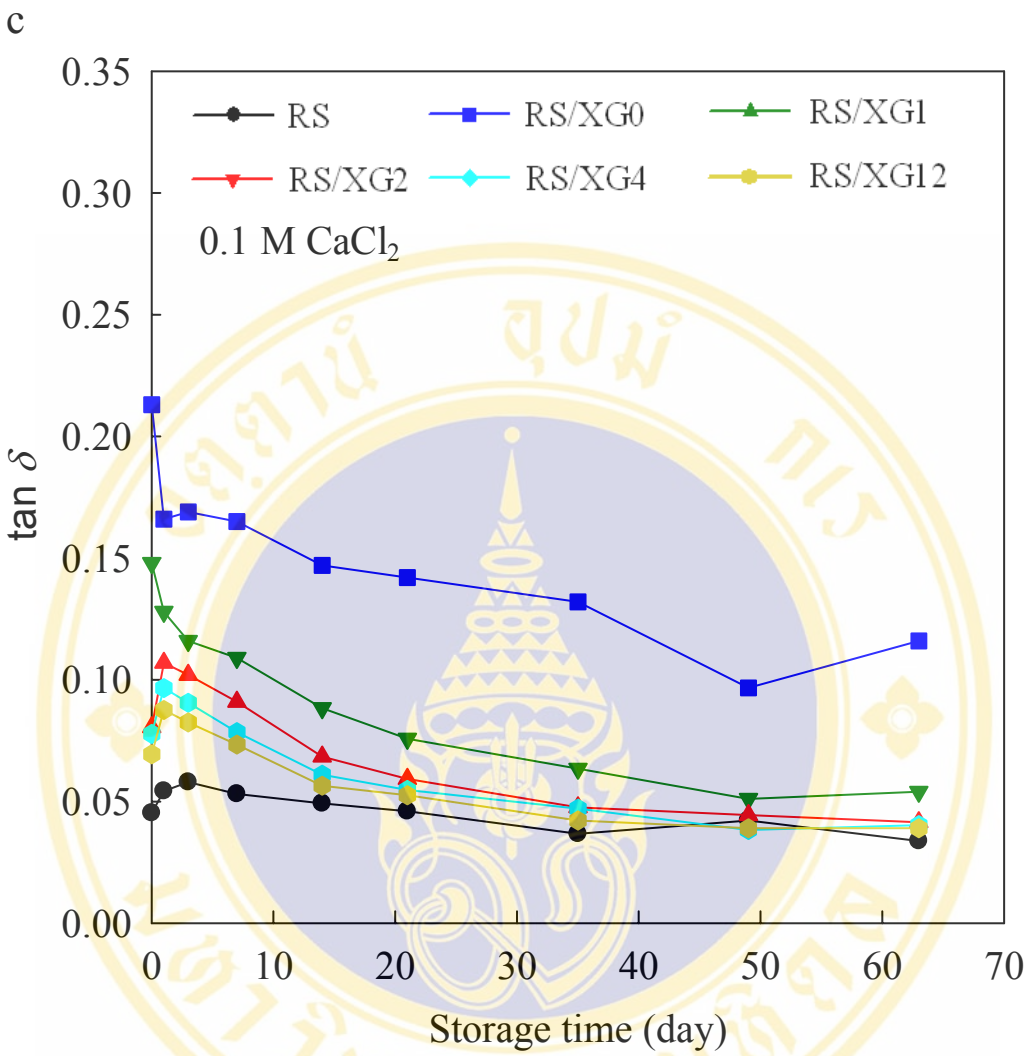


**Figure 4.7** Changes in loss tangent ( $\tan\delta$ ) of 3.5% rice starch (RS) and RS/xanthan gum (XG) (5.7/0.3, w/w ratio) mixtures in (a) distilled water (DW), (b) 0.1 M NaCl, and (c) 0.1 M $CaCl_2$ solutions as a function of storage time at 4°C. Measurements were made at 1 rad/s, 0.5% strain and 25°C. (Continued)

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# CHAPTER V
# DISCUSSION

## 1. Molecular weight and intrinsic viscosity of xanthan

It is known that strong mechanical effects, such as sonication (Holzwarth, 1978; Sato et al., 1984) or homogenization (Koczo, Wasan, Borwankar, & Gonsalves, 1998), split xanthan (XG) molecules into smaller fragments. Table 4.1 shows that homogenization of 1% XG solutions in the presence or absence of salts resulted in a decrease in weight-average molecular weight ($M_w$) with increasing number of passes during homogenization. The presence of salts caused about a double increase in $M_w$ of XG in the non-homogenized solutions. This can be attributed to a salt induced self-aggregation of XG molecules, as pointed out by Meyer et al. (1993). When salt is added to the solution, XG, like other polyelectrolytes, undergoes a disorder (random coil) to order (helix) conformational transition in which the backbone takes on a helical conformation and the charged trisaccharide side chains collapse down onto the backbone (due to charge screening effects) and stabilize the order conformation. In this rod-like configuration, with the electrostatic repulsions screened, the molecules are easily aligned and strongly associate (due to hydrogen bonding and ionic interactions) to form (nonpermanent) intermolecular associations (Rochefort & Middleman, 1987). By using light scattering measurement, Sato et al. (1984) found that XG in 0.1 M aqueous NaCl consisted of paired chains having Mw about two times higher than that of single chain molecules. In fact, differences in $M_w$ have been attributed to the degree of aggregation of XG by Meyer et al. (1993). However, these XG aggregates were easily ruptured by only a few passes through the homogenizer. When homogenization was performed for more than four passes, XG molecules in both distilled water and salt solutions were disrupted, by the same extents, to about $2.0\times10^6$ Da at 12 passes.

The intrinsic viscosity, $[\eta]$, of XG solutions, with and without added salts significantly decreased with decreasing $M_w$ of XG. This is expected since the $[\eta]$ is a measure of the hydrodynamic volume occupied by a macromolecule, which is

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closely related to the size and conformation of the macromolecular chains in a particular solvent (Higiro, Herald, Alavi, & Bean, 2007). Furthermore, the addition of salts was found to greatly reduce (5-10 times) the [$\eta$] of XG, providing further proof that XG molecules have a highly expanded conformation at low ionic strength (in distilled water) due to electrostatic repulsions of the negatively charged side chains (Carrington et al., 1996). When salt is added, charge screening causes the side chains to collapse down to the backbone, hence giving the XG molecule a more compact structure and reducing the hydrodynamic volume (Rochefort & Middleman, 1987).

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**2. Pasting properties**

The result showed the addition of 0.1 M NaCl or $CaCl_2$ to RS alone suspensions increase in peak viscosities as compared with the control. This could be attributed to the starch/salt interactions which reduced mobility of the starch granules, leading to higher viscosity (Bircan & Barringer, 1998). Oosten (1983) proposed that starch, being a weak acidic ion-exchanger, adsorbs the more voluminous cations, namely sodium or calcium ion, from the solution in exchange with the smaller hydrogen ion. A consequence is that the starch matrix is stretched to some extent, resulting in an increase of granule volume. This also seems to be responsible for the increase in viscosity of a starch suspension by adding salts. Jyothi et al. (2005) reported that a higher peak viscosity of a starch suspension during pasting in the presence of salts generally indicates a higher swelling power of the starch granules due to the weakening of the granules are more affected by prolonged stirring at high temperature, and this leads to the large breakdown value and lower final and setback viscosities. This also explains a significant increase in breakdown viscosities and decrease in final and setback viscosities of RS alone in the presence of salts, with the exception of $CaCl_2$, which increased final viscosity and did not affect setback viscosity of RS alone. There was no significant change in pasting temperatures of RS alone in the presence of either NaCl or $CaCl_2$ as compared with the control.

In all cases, addition of XG increase in peak, breakdown, final, and setback viscosities, whereas pasting temperatures were not significantly affected. This result can be interpreted by assuming that the system is biphasic, with the hydrocolloid located entirely in the continuous phase. Its concentration would then increase as the volume of the phase accessible to the hydrocolloid was reduced due to swelling of the starch granules during pasting. This resulted in a pronounced increase in the viscosity of the continuous phase and in turn the overall viscosity of the suspension itself, owing to the thickening properties of these hydrocolloids (Alloncle et al., 1989) added to the thickening produced by swollen starch granules. Therefore, the thickening capacity of the starch/hydrocolloid pastes. This hypothesis was confirmed by the fact that the effect of XG on viscosity of RS/XG mixtures significantly decreased with a decrease in their $M_w$ (Table 4.2) due to their lower viscosities (Table 4.1).

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Addition of salts significantly increased peak, breakdown, final, and setback viscosities of RS/XG mixtures with various $M_w$ of XG. As discussed earlier, salts caused a disorder to order conformational transition of XG molecules which affected the solution viscosity (Carrington et al., 1996). However, this effect depends on the concentration of XG in solution. At low gum concentrations (<0.25%), salt cause a decrease in viscosity whereas, at high gum concentrations, as used in this study (0.3%), viscosity increases with added salt (Sworn, 2000). Within each RS/XG blend, divalent cations from $CaCl_2$ showed a more pronounced effect on the peak and final viscosities than did monovalent cations from NaCl. This was possibly due to molecular cross-linking of XG by calcium ions, which resulted in a greater extent of molecular contraction (Higiro et al., 2007). The opposite trend to this result was observed for addition of NaCl or $CaCl_2$ to corn starch/xanthan mixtures, which may be attributed to the lower concentration of XG used (≤0.25%) in those experiments (Sudhakar et al., 1995). For the pasting temperature, it is generally known that the pasting temperature of starch is lowed by gum addition, due to the interactions between gum and leached starch molecules, primarily amylose (Christianson et al., 1981; Shi & BeMiller, 2002). These molecular interactions were found to become stronger with increasing $M_w$ of gum (Funami et al., 2005).

However, there were no significant differences in the pasting temperatures of any systems studied in the present work, indicating negligible interactions between leached amylose and XG with various $M_w$ in either the presence or absence of salts. These findings were similar to those previously reported for the pasting temperatures of 5% corn starch alone (86.6°C) and 5% corn starch/0.25% XG/0.5% (~0.1 M) NaCl or 1.0% (~0.1 M) $CaCl_2$ combination (86.0°C; Sudhakar et al., 1995).

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**3. Thermal properties**

The result showed the addition of 0.1 M NaCl did not significantly affect the magnitudes of $T_o$, $T_p$, $T_c$ and $\Delta T$, whereas 0.1 M $CaCl_2$ additions significantly increased $T_o$ and $T_p$ values without any effect on $T_c$ and $\Delta T$ as compared with those of the control. The $\Delta H$ was found to be significantly decreased by the addition of both salts used in this study. Salts have been reported to cause an elevation or depression of gelatinization temperature and gelatinization enthalpy, depending on the types of salt and their concentrations used (Ahmad & Williams, 1999, 2002; Chungcharoen & Lund, 1987; Jane, 1993; Jyothi et al., 2005; Maaurf et al., 2001; Oosten, 1982, 1983, 1990). It has been reported that NaCl and $CaCl_2$ at low concentrations, slightly increased the $T_p$ of sago starch (Ahmad & Williams, 1999, 2002; Maauf et al., 2001) and $T_o$ of corn starch (Jane, 1993) but, when the concentrations increased further (up to ~2 M for NaCl and ~1 M for $CaCl_2$), these temperature decreased. The $\Delta H$ showed a behavior similar to the gelatinization temperature. In the case of various rice flours and their isolated starches, Chungcharoen and Lund (1987) found that gelatinization temperatures of rice flour or starch suspension shifted to higher temperatures and enthalpy decreased in the presence of NaCl. The presence of $CaCl_2$ at the concentration used in this study seemed to exhibit a more pronounced effect on gelatinization of RS than did NaCl. This could be attributed to the fact that the effects of salts on starch gelatinization generally follow the order of the Hofmeister series (Ahmad & Williams, 1999, 2002; Jane, 1993). It is known that cations, coupled with the same anion counterpart, $Cl^-$ in this case, affect the gelatinization process in the following order $Mg^{2+} > Ca^{2+} > Li^+ > Na^+ > K^+$.

Various explanations have been proposed to elucidate the mechanism of starch gelatinization affected by various salts. It seem that the influence of salts on the gelatinization properties of starch can be attributed to various factors, especially the influence on polymer-solvent interaction, the effect on water structure and the electrostatic interaction between starch and the ions. According to Oosten (1982, 1983, 1990), when NaCl or $CaCl_2$ was added to a starch suspension, some alcoholic groups in the starch granules were converted to sodium or calcium alcoholate groups. These compounds were better dissociated, thus causing a rise in the Donnan potential, which

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more effectively excluded the gelatinizing $Cl^-$ from the granules. However, the absorption of $Na^+$ or $Ca^{2+}$ was rather limited since, in our systems, there was no agent to bind the released $H^+$; hence, the increase in gelatinization temperature was also limited. Consequently, once gelatinization began (at a higher temperature), $Cl^-$ assisted gelatinization by breaking hydrogen bonds between starch chains, as evidenced by a reduction between starch and ions. These two effects conflict with one another and result in complex effect patterns, depending on the type and concentration of salts.

Surprisingly, addition of XG with various $M_w$, at a concentration used in this study, did not significantly affect the $T_o$, $T_p$, $T_c$, and $\Delta T$ values of RS/XG blends as compared with those of the corresponding control samples without XG. It is clear that, in combinations of RS, XG and salt, the effect of salt, particularly $CaCl_2$ in increasing the $T_o$ and $T_p$ values of RS, and in turn the Donnan potential, is unchanged by an increase in the viscosity of RS/XG systems. The magnitude of $\Delta H$ values, in contrast, was significantly decreased by XG addition, except for the systems with added $CaCl_2$, and this effect seemed to be independent of $M_w$ of XG. The decrease in $\Delta H$ values could be attributed, at least in part, to a reduction in water availability and/or mobility, causing partial gelatinization of crystalline regions in the starch granules, and the effect of starch/gum interactions.

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**4. Dynamic viscoelastic properties**

The magnitudes of $G'$ of RS/XG0 gels were comparable to those of RS alone gels, whereas those of $G''$ were about 2.4-4.6 times higher at a $\omega$ of 1 rad/s, indicating that RS/XG0 gels were more viscous than RS alone gels. This result is consistent with a substantial increase in $\tan\delta$ observed for all RS/XG gels as compared with RS alone gels. However, the effects appeared to be less pronounced when $M_w$ of XG was decreased but the gel strengths of the pure RS pastes having the lowest $\tan\delta$ values are never attained. From this it can be deduced that the hydrocolloids employed are not able to provide an effective contribution to the further structural build-up of the starch network. This finding supports the hypothesis that these systems involve mixtures in which intermolecular interactions between like molecules are favored, which locally at least results in a macroscopic phase separation (Alloncle & Doublier, 1991; Annable, Fitton, Harris, Phillips, & Williams, 1994; Eidam, Kulicke, Kuhn, & Stute, 1995).

Interestingly, there were almost no differences in viscoelastic properties of RS alone and RS/XG pastes in distilled water when compared to those with the addition of sodium or calcium ions. This may be attributed, at least in part, to the relatively low level (0.1 M) of salts used in this study. All previous research on the effects of various salts, including NaCl and $CaCl_2$, on the viscoelastic properties of starch gels occurred at much higher concentrations (0.5 M; Ahmad & Williams, 1999) than those that were effective for xanthan gels (0.01 M; Ma & Barbosa–Canovas, 1997). Our results demonstrated that, in combination of RS, XG, and salt, XG dominates the viscoelastic behavior of the mixed gels and the addition of salts only slightly affects these properties. This is in accordance with an earlier report for wheat starch/XG/NaCl mixed systems (Mandala et al., 2004).

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**5. Steady shear rheological properties**

As show in Table 2.4, a hysteresis loop areas was observed in all pastes studied, which can be interpreted as structural breakdown by the shear field to alter a structure or form a new structure, which then maintained a shear-thinning characteristic on following shear sweeps (Achayuthakan & Suphantharika, 2008). However, the hysteresis loop of RS alone pastes was significantly reduced when XG was added to the systems, indicating that XG apparently promoted re-association after destruction of structure due to high shear. In general, it is know that XG has high shear stability and extremely short times for recovery of its original structure after alteration (Achayuthakan & Suphantharika, 2008; Rochefort & Middleman, 1987). This effect was more pronounced for XG with high $M_w$ and in the presence of salts, possibly due to their ordered configuration, removing any hindrance to reassociation between molecules. Similar findings were previously reported for other starch/hydrocolloid mixed pastes, in which it was also shown that hydrocolloids enhanced the in-shear structural recovery of the starch pastes (Achayuthakan & Suphantharika, 2008; Tye, 1988). This result is also consistent with the dynamic viscoelastic data in which the more structured gels having low $\tan\delta$ values (Figure 4.2 (a, b, and c)) exhibited higher hysteresis loop areas than did the less structured ones.

Another important flow characteristic observed for all pastes studied was the presence of yield stress ($\sigma_0$), which represents a finite stress required to achieve flow. Its existence indicates that there is a cross-linked or other interactive structure in a material which must be broken down before flow can occur at an appreciable rate (Achayuthakan, Suphantharika, & Rao, 2006; Cheng 1986). Our results demonstrated that a significantly higher magnitude of $\sigma_0$ was observed in the upward flow than in the downward flow curve. Many researchers termed the former one as static and the latter one as dynamic yield stress (Achayuthakan et al., 2006; Cheng, 1986). These observation on $\sigma_0$ implied that there could be more than one type of structure of these thixotropic gels some structure become insensitive to shear and serve to define the equilibrium flow curve while others are broken-down by the least shear but the weak structure can be formed again over a certain period of time during rest (Cheng, 1986). The $\sigma_0$ of RS/XG gels decreased with decreasing $M_w$ of XG and slightly increased with the addition of salts.

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The consistency coefficients ($K$) and the flow behaviour indices ($n$) of RS/XG pastes without salt showed that the K values significantly decreased and the n values increased with decreasing $M_w$ of XG for both the upward and downward curved, indicating a decrease in pseudoplasticity of these gels. This could be explained by the hypothesis that XG, especially those with low $M_w$ values, realized a smooth flow and prevented the abrasion of the starch granules by existing in the continuous phase without interacting with amylose or amylopectin leached (Funami et al., 2005). Similar findings were previously reported for the other starch/XG gels and their flow curves fitted well to the Herschel-Bulkley model (Sikora, Kowalski, & Tomasik, 2007). However, the cited authors found that their $K$ and $n$ values changed non-linearly with the starch/gum composition. In the presence of salts, the opposite trends were apparently observed for both salts, possibly due to the more rigid molecular structure of the small XG molecules as compared with the semiflexible structure of the larger molecules (Sato et al., 1984). However, it has been reported that a salt has a considerable effect on both starch and XG, as well as their interaction in complicated patterns, depending on the type and concentration of the salt (Sudhakar et al., 1995).

From these dynamic and steady shear results, it was concluded that all the paste samples had a weak gel-like structure that can readily be broken to give a free flowing solution, and that their steady shear properties were apparently influenced by $M_w$ of XG and salt addition.

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**6. Syneresis determination**

Syneresis is a consequence of continuous reassociation of gelatinized starch polymer, amylose and amylopectin, during storage at low temperature, which results in expulsion of water from the gel structure (Lapasin & Pricl, 1995). The rate and extent of starch recrystallization are determined primarily by the mobility of the system (Slade & Levine, 1987). In our results, the syneresis of RS alone gels was increased by NaCl addition, but slightly decreased by $CaCl_2$ addition during storage at 4°C. It is known that starch chain re-ordering in composite gels was related to the compatibility of a solute with water's structure (Biliaderis & Zawistowski, 1990), in our case was salt-water. In terms of salt-water interactions, ions of high charge density, such as $Ca^{2+}$, have strong electrostatic interactions with water molecules. These ions (structure makers) reduce the fraction of free water and increase the viscosity of the solution. Ions with low charge densities (e.g. $Na^{+}$), known as structure breakers, decrease the viscosity of the aqueous solution by breaking or weakening hydrogen bonds between water molecules and, thus, increase the fraction of free water (Jane, 1993). Zeleznak and Hoseney (1986) proposed that the extent of retrogradation in starch gels was controlled by the amount of water present during storage at low temperatures.

In the case of RS/XG mixed gels, syneresis production was lower than in the RS alone gels, either in the presence or absence of salts, the extent of which increased with increasing $M_w$ of XG. Similar results were also reported for corn starch/guar gum systems in which the syneresis was reduced by the addition of guar samples with high $M_w$ values (Funami et al., 2005b). It is generally known that a hydrocolloid acts as a water binder in aqueous starch systems, and effectively depriving the amylose or amylopectin of available water for crystallization, thus leading to prevention of starch retrogradation. In this study, therefore, the effect of XG might include not only the hydration or the water-holding of XG itself but also the retardation of starch retrogradation, especially the inhibition of amylose-amylose association as pointed out by Lee, Baek, Cha, Park, and Lim (2002). Addition of salts apparently reduced the effect of XG on retrogradation of the RS gels possibly due to the disorder to order conformational change of XG molecules, resulting a reduction of the water-holding capacity of the gel systems.

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**7. Thermal properties**

In the presence or absence of salts, the retrograded gels after 30 days of storage showed lower melting transition temperatures ($T_o$, $T_p$, and $T_c$) and enthalpies ($\Delta H_2$) but about twofold wider peak melting ranges ($T_c$-$T_o$) than the original gelatinization data (Table 4.5). These results suggest that retrogradation results in reassociation of the gelatinized starch molecules, but in less ordered and hence less perfect or stable forms and more heterogeneous in stability than those existing in the native starch granules (Karim, Norziah, & Seow, 2000). This agreed with the observations of Chang and Liu (1991) for waxy and non-waxy rice starches. An increased $T_c$-$T_o$ in starch gels stored at refrigerated temperatures was also reported by white et al. (1989).

In the case of retrograded RS alone gels, the amylopectin retrogradation enthalpies ($\Delta H_2$) were slightly decreased by addition of salts at 0.1 M (0.59% NaCl or 1.47% $CaCl_2$) – concentration. However, at higher NaCl concentrations (2 – 5 %), a marked decrease in the $\Delta H_2$ values reported for rice starch (Chang & Liu, 1991), and amaranth starch (Baker & Rayas–Duarte, 1998a,b) gels during aging. It is known that retrogradation in starch gels is controlled by the amount of water present during aging, regardless of the amount present during gelatinization (Zeleznak & Hoseney, 1987). Water is well established as a plasticizer. When salts are present, they restrict the mobility of plasticizer, resulting in a retardation in the retrogradation of amylopectin fraction of starch gels during aging. Fenema (1985) postulated that salts may be competing with the starch for the water to such a degree that the water remains bound in the gel, resulting in less retrogradation. The $Na^+$ and $Cl^-$ ions may also form partial ionic bonds with the starch molecules and the water and keep the starch molecules from realigning.

Addition of XG to RS gels did not affect the $\Delta H_2$ values compared to those of their control counterparts, i.e. RS in the presence or absence of salts. This effect was independent of $M_w$ of XG. This result demonstrated that XG failed to prevent amylopectin retrogradation which could be attributed to the fact that gums act outside the starch granule, while amylopectin retrogradation takes place within the

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granule (Ferreo et al., 1994). The retrogradation ratios ($\Delta H_2/\Delta H_1$) of the retrograded RS/XG gels with and without added salts were slightly higher than those of the RS alone gels. This could be explained by the fact that the $\Delta H_1$ values of the RS alone, either in the present or absence of salts, were significantly higher than those of the RS/XG gels as discussed earlier.

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**8. Dynamic viscoelastic properties**

Development of viscoelastic properties; storage modulus ( G′ ), loss modulus ( G″ ), and loss tangent ( tan δ ), of RS alone and RS/XG mixed gels in the presence or absence of salts, after storage at 4°C for 1, 3, 7, 14, 21, 35, 49, and 63 days, are shown in Figures 4.5 a-c, 4.6 a-c, and 4.7 a-c, respectively. The storage modulus $G'$ is a measure of the energy that is stored in the material or recoverable per cycle of deformation, whereas $G''$ is a measure of the energy that is lost as viscous dissipation per cycle of deformation. One characteristic value for evaluation of the viscoelastic behavior can be described as $\tan\delta$ stating directly the ratio of $G''/G'$ value. A $\tan\delta$ < 1 indicates predominantly elastic behavior while a $\tan\delta$ > 1 indicates predominantly viscous behavior.

The control RS alone gels, either in the presence or absence of salts, exhibited the highest G′ and G″ values and the lowest tan δ values compared to those of the RS/XG gels throughout the storage period. Addition of salts markedly increased the magnitude of both moduli, but slightly affected the tan δ values. Russell and Oliver (1989) reported that the elastic modulus of the wheat starch gels changed during storage at 21°C in a more complicated way with increasing NaCl concentration. It increased at low levels (0.44 and 0.88%) and decreased at higher levels (2.21 and 4.43%). These authors postulated that the re-ordering process in the amylopectin is responsible for the long-term changes in rheological characteristics of the gels. Ciacco and Fernandes (1979) found that the anions increased the retrogradation rate in the order $I^-$ < $Br^-$ < $Cl^-$ < and $F^-$, and the cations decreased in the order of $K^+$ < $Li^+$ < $Na^+$.

Addition of XG, having various $M_w$ values, to RS retarded an increase in $G'$ and $G''$ values and raised the $\tan\delta$ values of RS gels during storage in the order XG0 > XG1 > XG2 > XG4 > XG12. These results suggest that these XG samples should have great impacts on the gelatinized starch molecules to prevent their gelation. The synergistic effect of XG and salts on the rheological properties of RS gels during storage could be explained by the fact that salts at the concentration tested not only enhanced the re-ordering process in the amylopectin (Russell & Oliver, 1989) but also augmented the entanglement networks by specific association between the ordered XG chains (Ross-Murphy, 1995). The presence of 0.1 M $CaCl_2$ seemed to exhibit more

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pronounced effect on the rheological properties of RS/XG mixed gels than did NaCl. It is generally known that the extent of XG association is enhanced by salt, with the order of effectiveness of common counterions being $Na^+ < K^+ < Ca^{2+}$ (Ross-Murphy et al., 1995). Entanglement networks decreased by decreasing $M_w$ of XG possibly due to a decrease in their hydrodynamic volume and thickening properties.

RS gelatinized in XG 'weak gel' networks. Amylose released into XG matrix in which XG-amylose interaction probably competed against amylose-amylose interactions thus avoiding retrogradation (Ferrero et al., 1994). In this study, it was concluded that RS/XG gels behave as a composite material composed of a dispersed phase (the swollen gelatinized starch granules) in a continuous polymer solution (the amylose/XG matrix). The rheological properties of such a system depend on properties of the components themselves as well as their ratio and interactions between them (Eliasson & Gudmundsson, 2006).

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# CHAPTER VI
# CONCLUSION

This work clarified that the gelatinization and retrogradation characteristics of RS were modified by XG and salts additions and the extent of these effects depended upon the $M_w$ of XG and the types of salt added. The pasting viscosities of RS were enhanced by XG addition either in the presence or absence of salts. The thermal properties of RS seemed to be unaffected by XG and salts addition except for the gelatinization enthalpies ($\Delta H_1$), which were significantly decreased when compared with the controls. Immobilization of water molecules by XG and reduction of free water molecules by salts limited the amount of water available for starch gelatinization and the lower of the heat transfer rate were possible reasons for these results. The measurement of gel viscoelasticity revealed weak gel-like behavior in all the gels tested in which their rigidity was decreased by XG addition. The flow behavior of all the gels exhibited time-dependent shear thinning (thixotropic) behavior and appeared to have a yield value, i.e. the system is behaving like an elastic solid, while beyond the yield point, the system behaves like a viscous liquid. The thixotropic hysteresis loop areas of RS gels decreased with XG addition either in the presence or absence of salts.

Storage of all the gelatinized gels at 4°C resulted in a reduction of the transition temperatures and enthalpies of the retrograded gels compared to those obtained during gelatinization of the native starch. The syneresis and viscoelastic characteristics of all the gels tested increased with storage time. The addition of XG retarded the syneresis production and improved stability of RS gels over the storage period. In this case, there was synergistic effect when XG was incorporated into RS gels. RS gave gel structure while XG gave some body (viscosity) and controlled syneresis. In general, all the aforementioned effects of XG on the gelatinization and retrogradation of RS were more pronounced with increasing $M_w$ of XG and, to a much less extent, dependent on the addition of salts.

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# APPENDIX

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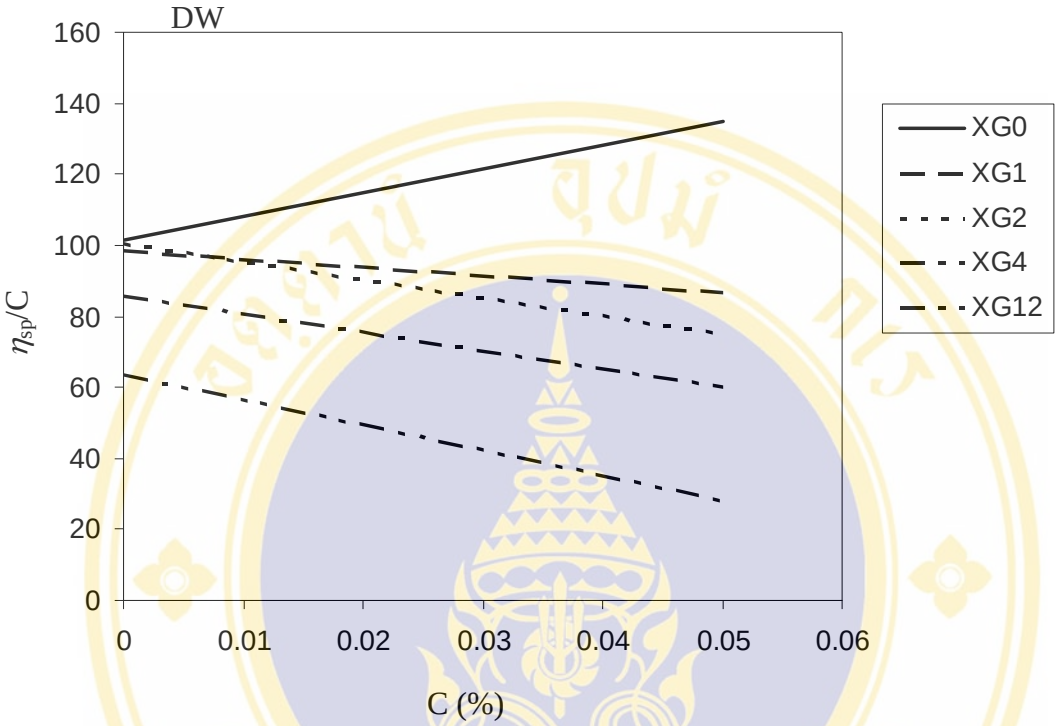


**Figure A 1** The plot of Huggins equation of xanthan with various $M_w$ in distilled water at 25°C

**Table A 1** Huggins equation of xanthan with various $M_w$ in distilled water at 25°C

| Homogenization (Number of passes) | Huggins equation |
|---|---|
| 0 (XG0) | y = 664.5x + 101.44 |
| 1 (XG1) | y = -236x + 98.34 |
| 2 (XG2) | y = -508x + 85.43 |
| 4 (XG4) | y = -515.5x + 100.5 |
| 12 (XG12) | y = -707x + 63.46 |

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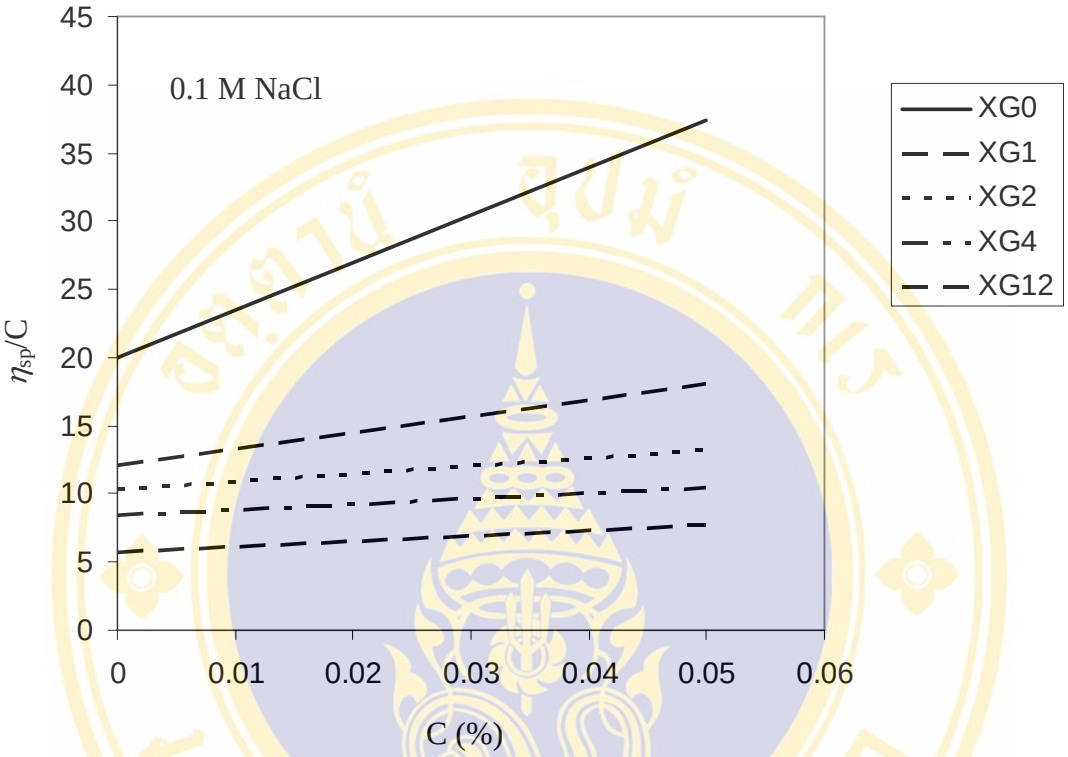


**Figure A 2** The plot of Huggins equation of xanthan with various $M_w$ in 0.1 M NaCl at 25°C

**Table A 2** Huggins equation of xanthan with various $M_w$ in 0.1 M NaCl at 25°C

| Homogenization (Number of passes) | Huggins equation |
|---|---|
| 0 (XG0) | y = 346.5x + 20.03 |
| 1 (XG1) | y = 117.5x + 12.16 |
| 2 (XG2) | y = 58x + 10.27 |
| 4 (XG4) | y = 42x + 8.39 |
| 12 (XG12) | y = 42x + 5.66 |

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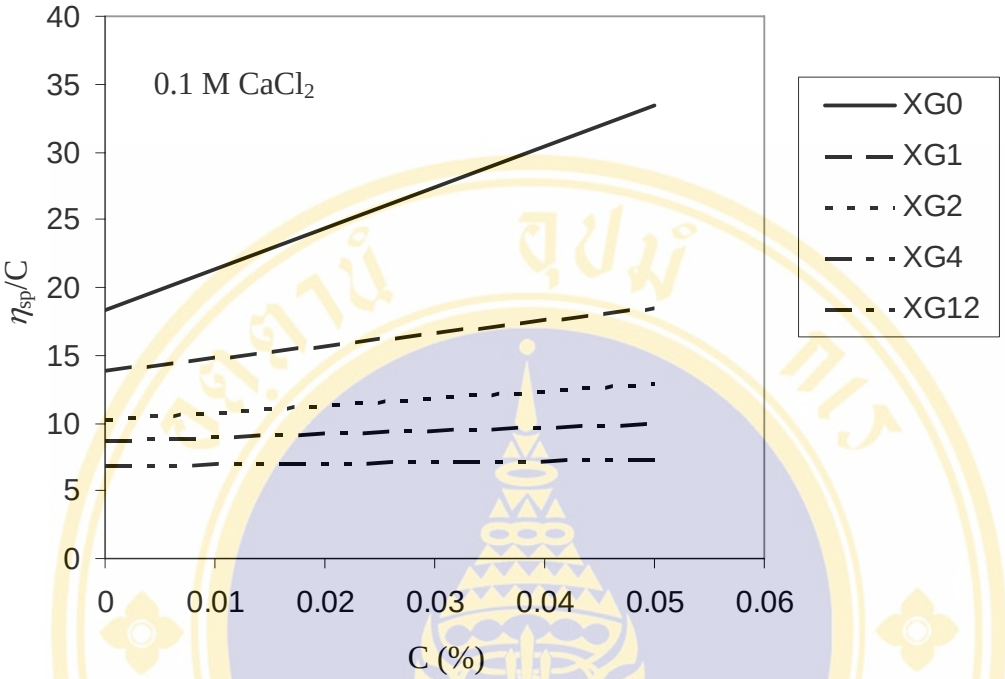


**Figure A 3** The plot of Huggins equation of xanthan with various $M_w$ in 0.1 M $CaCl_2$ at 25°C

**Table A 3** Huggins equation of xanthan with various $M_w$ in 0.1 M $CaCl_2$ at 25°C

| Homogenization (Number of passes) | Huggins equation |
|---|---|
| 0 (XG0) | y = 302.5x + 18.31 |
| 1 (XG1) | y = 92x + 13.83 |
| 2 (XG2) | y = 52.5x + 10.28 |
| 4 (XG4) | y = 25x + 8.62 |
| 12 (XG12) | y = 10.5x + 6.82 |

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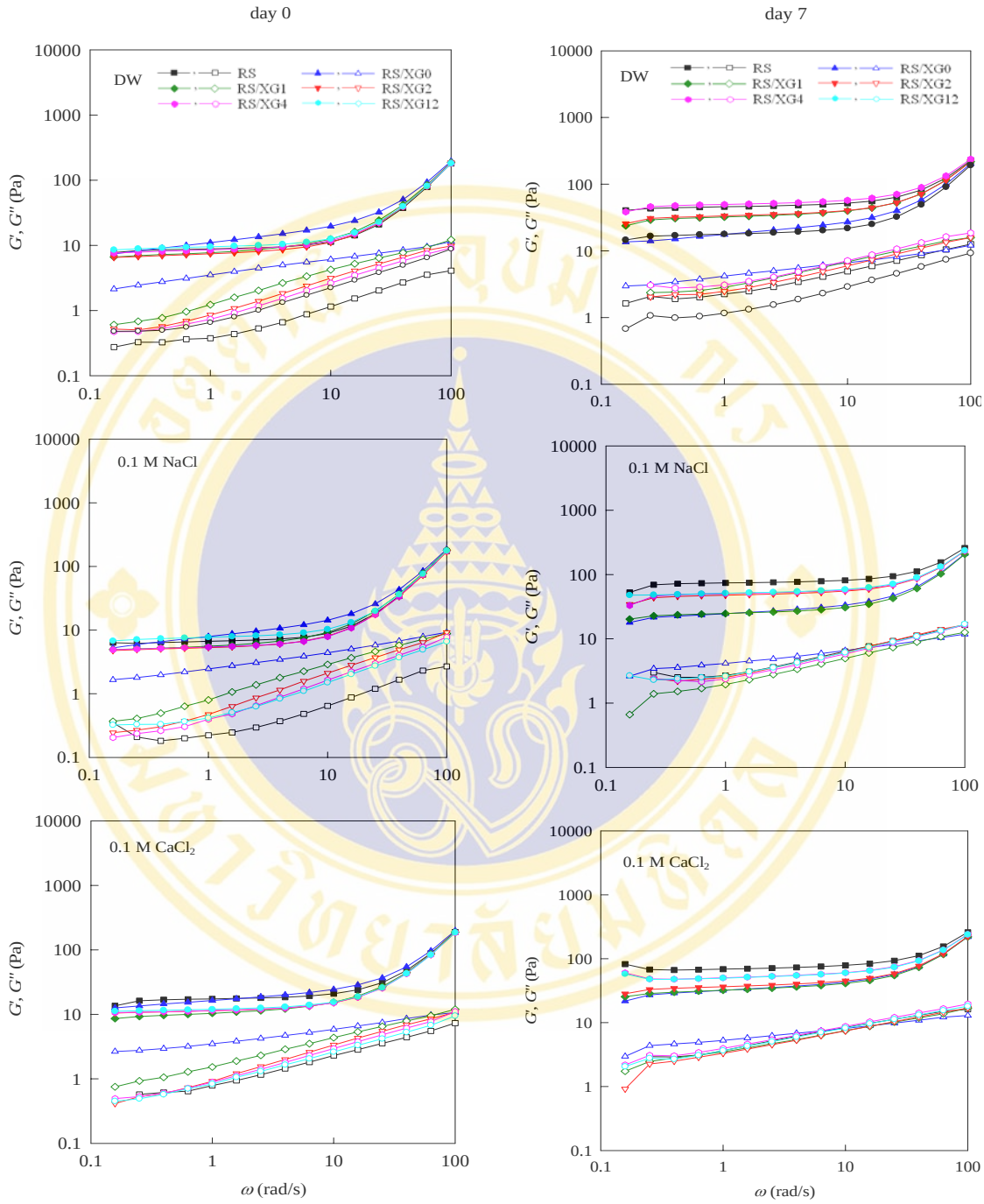


**Figure A 4** Frequency dependence of storage modulus, $G'$ (closed symbol) and loss modulus, $G''$ (open symbol) of 3.5% RS alone and RS/XG (5.7/0.3, w/w ratio) gels, in the presence or absence of salts, (a) immediately after gelatinization (b) 7, (c) 21, and (d) 63 days after storage at 4°C. Measurements were made at 0.5% strain and 25°C.

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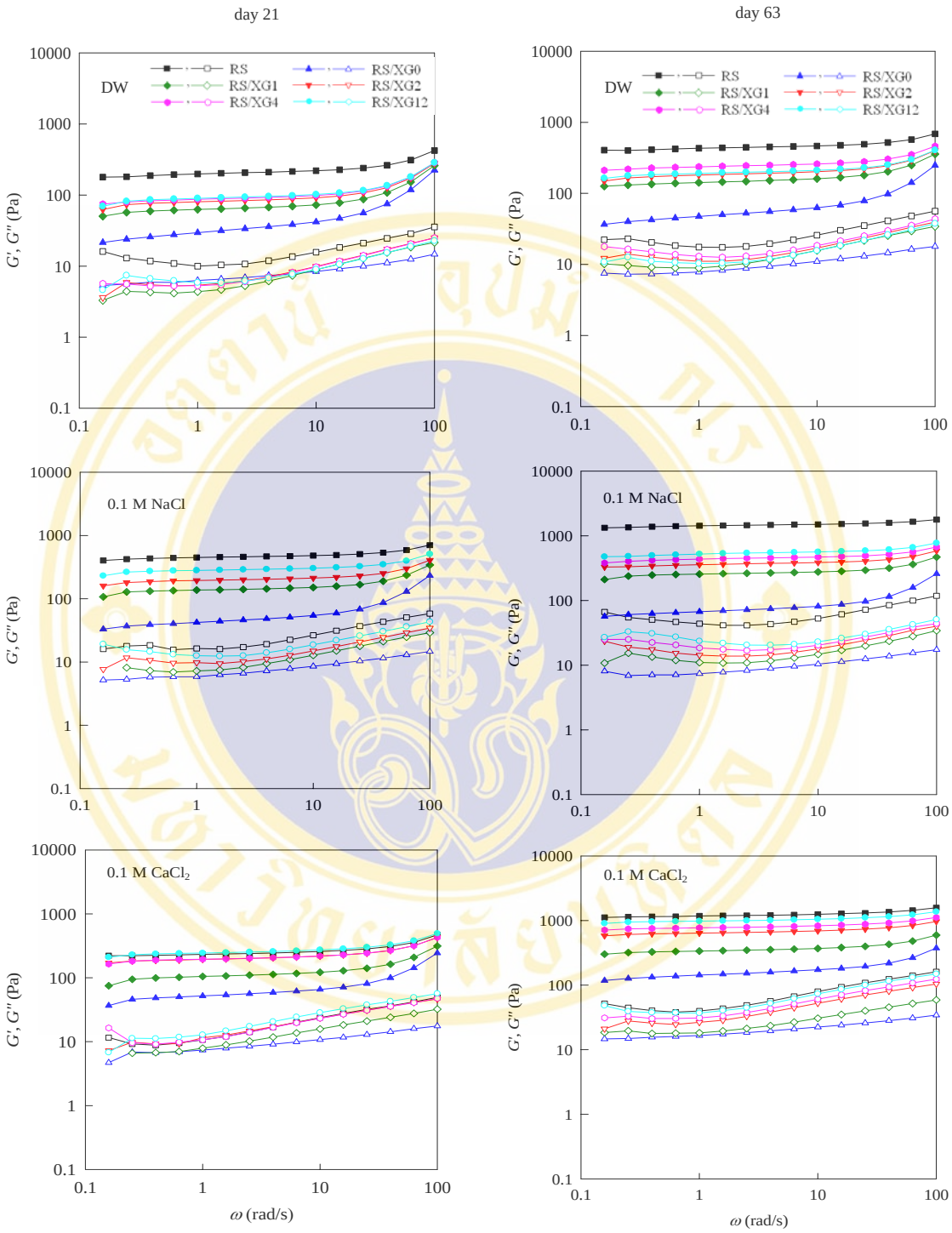


**Figure A 4** Frequency dependence of storage modulus, $G'$ (closed symbol) and loss modulus, $G''$ (open symbol) of 3.5% RS alone and RS/XG (5.7/0.3, w/w ratio) gels, in the presence or absence of salts, (a) immediately after gelatinization (b) 7, (c) 21, and (d) 63 days after storage at 4°C. Measurements were made at 0.5% strain and 25°C. (Continued)

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# BIOGRAPHY

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**PRESENTATION**

1. Viturawong, Y., & Suphatharika, M. (2007, November 6-7). Pasting and rheological properties of rice starch-xanthan mixtures: Effects of molecular weight of xanthan and salt. *Poster presentation at the 4th International Conference on Starch Technology, National Center for Genetic Engineering and Biotechnology (BIOTEC)*, National Convention Center, Bangkok, Thailand.
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