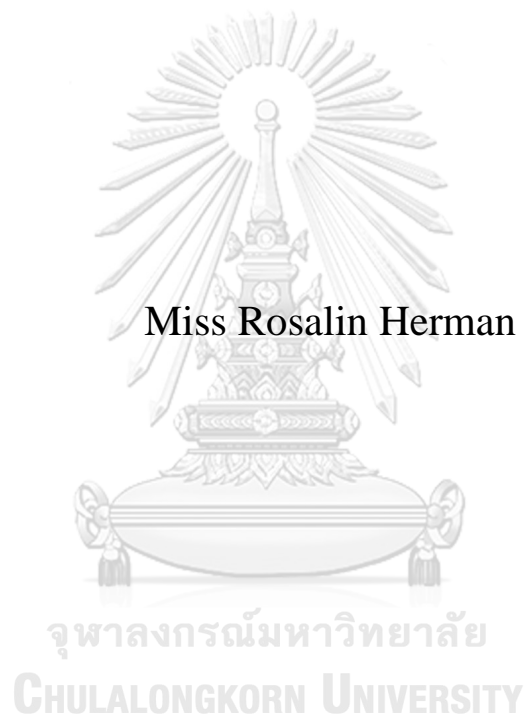


**EFFECTS OF HYDROCOLLOIDS ON PASTING  
BEHAVIOR AND GELLING PROPERTIES OF BANANA  
FLOUR AND ITS APPLICATION IN BREAD**



A Thesis Submitted in Partial Fulfillment of the Requirements  
for the Degree of Master of Science in Food Science and Technology  
Department of Food Technology  
FACULTY OF SCIENCE  
Chulalongkorn University  
Academic Year 2020  
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ผลของไฮโดรคอลลอยด์ต่อพฤติกรรมการเกิดเพสต์และสมบัติการเกิดเจลของฟลาวาร์กัวยและการ  
ประยุกต์ในขนมปัง



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต  
สาขาวิชาวิทยาศาสตร์และเทคโนโลยีทางอาหาร ภาควิชาเทคโนโลยีทางอาหาร  
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รศลิน เฮอร์แมน : ผลของไฮโดรคอลลอยด์ต่อพฤติกรรมการเกิดเพสต์และสมบัติการเกิดเจลของฟลาวาร์กกล้วยและ  
การประยุกต์ในขนมปัง. ( EFFECTS OF HYDROCOLLOIDS ON PASTING  
BEHAVIOR AND GELLING PROPERTIES OF BANANA FLOUR  
AND ITS APPLICATION IN BREAD) อ.ที่ปรึกษาหลัก : ธนจันทร์ มหาวณิช

กล้วยเป็นผลไม้ที่มีศักยภาพในการเป็นพืชอาหารหลักสำหรับอนาคต เนื่องจากมีอัตราในการเจริญเติบโตที่เร็ว ระยะเวลาการเก็บเกี่ยวที่สั้น และปริมาณสารที่สูง แต่อย่างไรก็ตามฟลาวาร์กกล้วยมีการเกิดริโทรเกรเดชันที่สูงซึ่งเป็นข้อจำกัดหลักของการนำไปใช้ งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของไฮโดรคอลลอยด์ต่อสมบัติการเกิดเพสต์และเจลที่สัมพันธ์กับการเกิดเจลในเซชันและริโทรเกรเดชันของฟลาวาร์กกล้วย รวมถึงการประยุกต์ฟลาวาร์กกล้วยเพื่อทดแทนแป้งสาลีในขนมปัง ไฮโดรคอลลอยด์ที่ใช้ในงานวิจัยนี้ ได้แก่ แซนแทนกัม (XG) กัมอะราบิก (GA) ไฮดรอกซีโพรพิลเมทิลเซลลูโลส (HPMC) และกัวร์กัม (GG) โดยเพิ่มความเข้มข้น 0.5, 1.0 และ 1.5% ในด้านพฤติกรรมการเกิดเพสต์ XG และ GA มีผลทำให้ peak viscosity ลดลงเมื่อความเข้มข้นเพิ่มขึ้น ในขณะที่ HPMC และ GG มีผลทำให้ peak viscosity เพิ่มขึ้นเมื่อความเข้มข้นเพิ่มขึ้น ไฮโดรคอลลอยด์ทุกชนิดมีผลลด setback viscosity ยกเว้น GG ที่ทำให้ setback viscosity มีค่าเพิ่มขึ้น ไฮโดรคอลลอยด์ทุกชนิดทำให้อุณหภูมิการเกิดเพสต์เพิ่มขึ้นเล็กน้อย ยกเว้น XG ที่ความเข้มข้น 1.5% และ GG ที่ความเข้มข้น 1.0% ในด้านเจลในเซชัน ไฮโดรคอลลอยด์ทุกชนิดไม่ส่งผลต่ออุณหภูมิเจลในเซชัน แต่มีผลทำให้เอนทาลปีของการเกิดเจลในเซชันมีค่าเพิ่มขึ้น ในส่วนของริโทรเกรเดชัน การเติมไฮโดรคอลลอยด์ไม่มีผลต่อเอนทาลปีของการเกิดริโทรเกรเดชัน การเติมไฮโดรคอลลอยด์ไม่มีผลที่เห็นได้ชัดต่อค่าความแน่นเนื้อของเจลที่เตรียมใหม่ แต่เมื่อเก็บตัวอย่างเจลไว้ที่อุณหภูมิ 4 องศาเซลเซียส เป็นเวลา 7, 10 และ 14 วัน พบว่า XG เข้มข้น 0.5% GA เข้มข้น 1.5% HPMC เข้มข้น 0.5% และ GG เข้มข้น 1.0% สามารถลดค่าความแน่นเนื้อของเจลได้อย่างมีนัยสำคัญ ในขณะที่ไฮโดรคอลลอยด์ที่ความเข้มข้นอื่นอาจทำให้ความแน่นเนื้อของเจลเพิ่มขึ้นหรือไม่เปลี่ยนแปลง จึงสรุปได้ว่าไฮโดรคอลลอยด์แต่ละชนิดที่ความเข้มข้นต่างกันมีผลต่อสมบัติการเกิดเพสต์และเจลของฟลาวาร์กกล้วยที่แตกต่างกันไป จากผลที่ได้จากการวิจัยนี้จึงคัดเลือกความเข้มข้นของไฮโดรคอลลอยด์ที่เหมาะสมเพื่อนำมาประยุกต์ในขนมปังที่ทดแทนแป้งสาลีด้วยฟลาวาร์กกล้วย 30% โดยวิเคราะห์คุณภาพของขนมปังที่ผลิตเสร็จใหม่ๆ และขนมปังที่เก็บที่อุณหภูมิห้องเป็นเวลา 7, 10 และ 14 วัน พบว่าปริมาณความชื้นของขนมปังมีค่าลดลงเมื่อเวลาการเก็บรักษาเพิ่มขึ้น แต่ตัวอย่างที่เติม HPMC มีปริมาณความชื้นที่สูงกว่าตัวอย่างควบคุมในระยะแรกของการเก็บรักษา การเติมไฮโดรคอลลอยด์ไม่มีผลต่อค่าเอนทาลปีของขนมปัง ในด้านของปริมาณการพองตัวของก้อนขนมปัง พบว่าขนมปังที่เติม HPMC มีปริมาณการพองตัวที่ต่ำที่สุด ระยะเวลาการเก็บรักษาไม่มีผลต่อโครงสร้างของเนื้อในขนมปัง ในด้านเนื้อสัมผัส พบว่าการเติม GA มีผลทำให้ขนมปังมีความแข็งต่ำกว่าตัวอย่างควบคุม โดยความแข็งมีค่าเพิ่มขึ้นเมื่อระยะเวลาการเก็บรักษาเพิ่มขึ้นสำหรับขนมปังทุกตัวอย่าง ส่วนในด้านสี พบว่าตัวอย่างขนมปังไม่มีความแตกต่างที่เห็นได้ชัด



สาขาวิชา วิทยาศาสตร์และเทคโนโลยีทางอาหาร  
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ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

## 6172616223 : MAJOR FOOD SCIENCE AND TECHNOLOGY

KEYWORD: Banana flour, Pasting behavior, Gelling properties, Retrogradation

Rosalin Herman : EFFECTS OF HYDROCOLLOIDS ON PASTING BEHAVIOR AND GELLING PROPERTIES OF BANANA FLOUR AND ITS APPLICATION IN BREAD.

Advisor: Asst. Prof. THANACHAN MAHAWANICH, Ph.D.

Bananas have a high potential in becoming a future staple crop due to its rapid growth rate, short harvest time, and high starch content. However, banana flour is highly retrograded which is its major limitation. The objective of this study was to study the effects of selected hydrocolloids on the pasting and gelling properties as related to the gelatinization and retrogradation of banana flour as well as its application in bread as a wheat flour substitute. Xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG) were added to banana flour at different concentrations (0.5, 1.0, and 1.5%). Regarding the pasting behavior, XG and GA decreased the peak viscosity with increasing concentration while HPMC and GG increased the peak viscosity with increasing concentration. All of the hydrocolloids reduced the setback viscosity except for GG which increased it. There were slight increases in pasting temperature but this was not significantly different except for XG at 1.5% and GG at 1.0%. In terms of gelatinization, although all of the hydrocolloids showed no effect on the gelatinization temperature, it did increase the gelatinization enthalpy (DH<sub>G</sub>). Concerning retrogradation, the addition of hydrocolloids did not significantly affect the retrogradation enthalpy (DH<sub>R</sub>). As for the gel firmness, hydrocolloids did not show a significant effect on firmness of the freshly prepared gels. However, after being stored at 4°C for 7, 10, and 14 days, XG at 0.5%, GA at 1.5%, HPMC at 0.5%, and GG at 1.0% resulted in a significant reduction in gel firmness. Other concentrations may cause an increase in firmness or have no effect at all. In summary, different types of hydrocolloids at different concentrations affected the pasting and gelling properties of banana flour in a different way. From the results mentioned above, the optimum concentration of each hydrocolloid (XG at 0.5%, GA at 1.5%, HPMC at 0.5% and GG at 1.0%) was selected to add to white pan breads with 30% banana flour substitution to investigate the quality of freshly prepared breads (Day 0) and breads stored at room temperature for 7, 10 and 14 days. Moisture content of the bread was found to decrease as the storage time increased. HPMC-added breads showed significantly higher moisture content than the control during the beginning of the storage time. As for the water activity, addition of hydrocolloids did not affect water activity of the bread samples. In terms of specific loaf volume, HPMC-added breads resulted in the lowest specific loaf volume for all storage days. However, there were no significant differences observed in the crumb structure. For the crumb texture, freshly prepared GA-added breads resulted in lower hardness than the control. An increase in crumb hardness was observed in all samples during storage. All the breads showed no obvious difference in terms of color.

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Field of Study: Food Science and Technology

Student's Signature .....

Academic Year: 2020

Advisor's Signature .....

## ACKNOWLEDGEMENTS

Firstly, I would have to thank my thesis advisor Assistant Professor Dr. Thanachan Mahawanich of the Faculty of Science, Chulalongkorn University. Dr. Thanachan was always ready to advise whenever I come across a question or concern about my research. She helped me overcome any problems and at the same time inspired me to experiment and learn something new every step of the way.

I would also like to thank the Chair and all members of the committee which include Associate Professor Dr. Jirarat Anuntagool, Assistant Professor Dr. Thanachan Mahawanich, Associate Professor Dr. Chaleeda Borompichaichartkul, Assistant Professor Dr. Sirima Puangpraphant, and Assistant Professor Dr. Withida Chantrapornchai, for advice and suggestions that guide this thesis in the right direction. Thank you for your insights and knowledge that made this thesis more complete.

Thank you to Eunice Muute Muema and Arati Bhatta for the support and company throughout this research.

Finally, I would like to express my sincerest gratitude to my parents, my family, and to my friends for the continuous support and motivation during my years of study and research as well as the process of writing this thesis. This accomplishment would not have been possible without each and every one of the people mentioned above. Thank you.

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## CHAPTER 1

### INTRODUCTION

There have been speculations about how global warming will eventually affect the production of current staple crops such as corn, rice and wheat (McGrath, 2012). In addition, this decrease in agricultural production will counteract the increase in demand for food due to increasing human population, making the problem even worse. Food and Agriculture Organization of the United Nations (FAO) estimated that the world must increase its food production by 70% in order to satisfy the food demand of the growing population, which is estimated to reach 9 billion in 2050 (FAO, 2009). Scientists from all over the world as well as various global organizations like the World Food Program (WFP) and the FAO have been aware of and warned about this threatening situation. They are currently trying to find solutions as well as looking for the 'next' staple crops by focusing on plants that can endure heat and drought conditions and plants that have short harvest time with high starch content.

Bananas are among the plants that have potential in being developed into the future staple crop due to the fact that it has rapid growth rate and short harvest time, with the unripe fruit containing as high as 60% starch on a dry weight basis. Banana flour and starch are also reported to be able to withstand severe processing condition. However, the major limitation of banana flour and starch is that they are highly retrograded, resulting in a gel with high hardness when compared to flour and starch from other plant sources (Zhang & Hamaker, 2012). This is the reason why banana flour and starch are still underutilized, particularly on an industrial scale.

Improvement of pasting behavior and gelling properties of starch-based systems can be done using various techniques such as modification of starch itself and addition of food additives (Fu et al., 2015). It is widely recognized that various food additives can modify the pasting and gelling properties of starch-based systems by affecting the starch gelatinization and retrogradation process. Addition of food additives into a starch-based system can pose an effect on the arrangement of amylose

and amylopectin as well as the interaction between amylose and amylopectin which in turns influence the retrogradation of starch (Schierbaum et al., 1992).

Among many food additives studied, hydrocolloids were demonstrated to hinder starch retrogradation in two main aspects. Some researchers described that incorporation of hydrocolloids could induce a decrease in interaction among starch molecules and an increase in interaction between the hydrocolloids and starch. This interaction may be noncovalent interactions such as hydrogen bonds and Van der Waals forces (Alam et al., 2009; Leite et al., 2012). Another aspect in explaining the effect of additives on gelatinization and retrogradation of starch is based on the assumption that addition of hydrocolloids may alter the availability of water necessary for starch gelatinization or that needed for stabilizing starch crystallites forming upon retrogradation (Baik & Chinachoti, 2001; Baik & Chinachoti, 2000; Ribotta & Le Bail, 2007). In an attempt to increase the usability of banana flour, this research therefore aims to study the effects of various hydrocolloids on the pasting behavior and gelling properties of banana flour. In addition, application of banana flour with selected hydrocolloids as a wheat flour-substitute in bread will also be investigated.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Banana flour

Bananas (*Musa* spp.) are among the plants that have potential in being developed into the future staple crop following rice, wheat, and corn (Arias, 2003). With its rapid growth rate and short harvest time, bananas can also be grown in tropical and subtropical areas which prove its ability to withstand harsh climates. Although the composition of bananas changes from ripening, the primary source of carbohydrate in banana is starch with the unripe fruit containing as high as 60% starch on a dry weight basis (Table 2.1) (Huang et al., 2019; Lii et al., 1982; Zhang et al., 2005).

Table 2. 1 Proximate compositions of the edible portion of banana at different stages as classified by the color of banana peel

Stage	Peel color	Starch (%)	Reducing sugar (%)	Sucrose (%)
1	Green	61.7	0.2	1.2
2	Green	58.6	1.3	6.0
3	Green/a trace of yellow	42.4	10.8	18.4
4	More green than yellow	39.8	11.5	21.4
5	More yellow than green	37.6	12.4	27.9
6	Yellow with a green tip	9.7	15.0	53.1
7	All yellow	6.3	31.2	51.9
8	Yellow/a few brown spots	3.3	33.8	52.0
9	Yellow/many brown spots	2.6	33.6	53.2

Source: Modified from Zhang et al. (2005)

When compared with commercial wheat flour, banana flour has higher carbohydrate content but lower protein content as shown in Table 2.2. Moreover, green banana flour has high resistant starch content which is known for its positive



digestion properties and functional properties (Langkilde et al., 2002; Tribess et al., 2009; Zhang et al., 2005). Banana starch and flour are also reported to be able to withstand severe processing condition. However, the major limitation of banana flour and starch is that they are highly retrograded, resulting in a gel with high hardness when compared to flour and starch from other plant sources (Zhang & Hamaker, 2012). This is the reason why banana flour and starch are still underutilized, particularly on an industrial scale.

Table 2. 2 Proximate composition of banana flour compared with wheat flour

Compositions	Banana <sup>1</sup>	Wheat <sup>2</sup>
<i>Wet basis</i>		
Moisture (g/100 g)	8.18	12.20
Carbohydrates (g/100 g)	83.24	72.45
Starch (g/100 g)	70.83	60.40
Protein (g/100 g)	3.98	12.74
Fat (g/100 g)	1.78	1.51
Ash (g/100 g)	2.82	1.10
Energy (kJ/100 g)	1526.70	1482.60
Calories (kcal/100 g)	364.89	354.35
<i>Dry basis</i>		
Carbohydrates (g/100 g)	90.66	82.52
Starch (g/100 g)	77.14	68.79
Protein (g/100 g)	4.33	14.51
Fat (g/100 g)	1.94	1.72
Ash (g/100 g)	3.07	1.25
Energy (kJ/100 g)	1662.71	1688.61
Calories (kcal/100 g)	397.40	403.59

<sup>1</sup> Banana flour data was obtained from LiveKuna, Kunachia LLC (Davie, FL, USA)

<sup>2</sup> Wheat flour data was obtained from Bulk Barn Foods (Aurora, ON, Canada)

Source: Modified from Huang et al. (2019)

## 2.2 Starch

Starch is the primary storage carbohydrate of plants (Copeland et al., 2009). It is natural, renewable, biodegradable, and the second most abundant biomass material in nature (Le Corre et al., 2010). It is one of the most common energy sources in human diets and has various usages in both food and nonfood applications. Generally, it is commercially isolated from grains, tubers, and seeds with varying diameter from 0.5-175  $\mu\text{m}$  (Whistler & Daniel, 2000; Zobel, 1988). The molecular structure of starch consists of carbon, hydrogen, and oxygen in the ratio of 6:10:5 with the chemical formula  $\text{C}_6\text{H}_{10}\text{O}_5$  (Whistler & Daniel, 2000).

### 2.2.1 Starch molecules

Starch is a polysaccharide of glucose units bonded by glycosidic bonds. It contains two types of D-glucopyranose polymers: amylose and amylopectin. Amylose is a linear (1 $\rightarrow$ 4)-linked  $\alpha$ -D-glucan that are slightly branched and has a molecular weight of about  $10^5$ - $10^6$  g/mol which corresponds to a degree of polymerization (DP) of 1000-10000 glucose units shown in Figure 2.1 (Copeland et al., 2009; Miles et al., 1985; Whistler & Daniel, 2000; Zobel, 1988). Due to its low degree of branching of long glucan chains, the amylose molecules have properties that are almost identical to those of truly linear molecules (Buleon et al., 1998; Takeda et al., 1990). The  $\alpha$ -1,4 links between anhydroglucose units of amylose molecules result in a left-handed helix structure. The glycosidic oxygen atoms are facing the interior part of the helical structure which attributes to the hydrophobic interior of amylose helix. When stained with iodine, amylose forms inclusion complexes which results in a blue color (Copeland et al., 2009; Whistler & Daniel, 2000). Most native starches contain 20-30% amylose but pulse starches may contain higher amounts (Hoover et al., 2010; Wang et al., 2011).

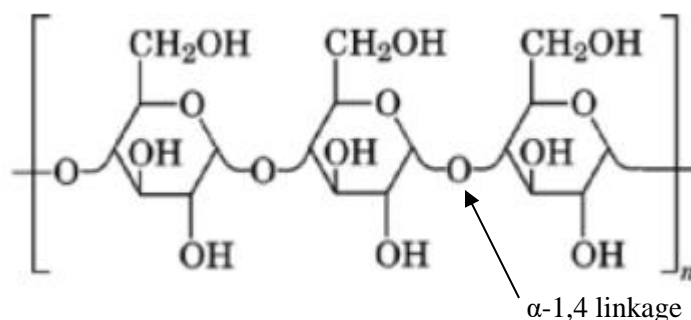


Figure 2. 1 Structure of amylose

Source: Modified from Whistler & Daniel (2000)

On the other hand, amylopectin is a highly branched macromolecule with short chains of (1→4)-linked  $\alpha$ -D-glucose with 5-6% of (1→6)- $\alpha$ -linked bonds at branch points which occur at approximately every 20-30 anhydroglucose units shown in Figure 2.2 (Copeland et al., 2009; Miles et al., 1985; Whistler & Daniel, 2000; Zobel, 1988). Figure 2.3 illustrates the cluster model of amylopectin. The A-chains are joined to other B- or C- chains through  $\alpha$ -D-1,6 linkages by their reducing ends without being branched themselves. B-chains are linked to B- or C-chain but are branched through  $\alpha$ -D-1,6 bonds to one or more A- and/or B-chains on primary hydroxyl groups. B-chains are subcategorized in to B1, B2, and B3. The C-chain is linked with the sole reducing end (BeMiller & Whistler, 2009; Zobel, 1988). When stained with iodine, amylopectin forms a purple to reddish brown complex which depends on the source of amylopectin. Amylopectin has a molecular weight of about  $10^6$ - $10^8$  g/mol and is made up of more than  $10^6$  glucose units (Copeland et al., 2009). Generally, most starches consist of around 60-90% amylopectin but high-amylose starches may contain only 30% amylopectin. Waxy starches from corn, rice, and sorghum may contain almost 100% amylopectin.

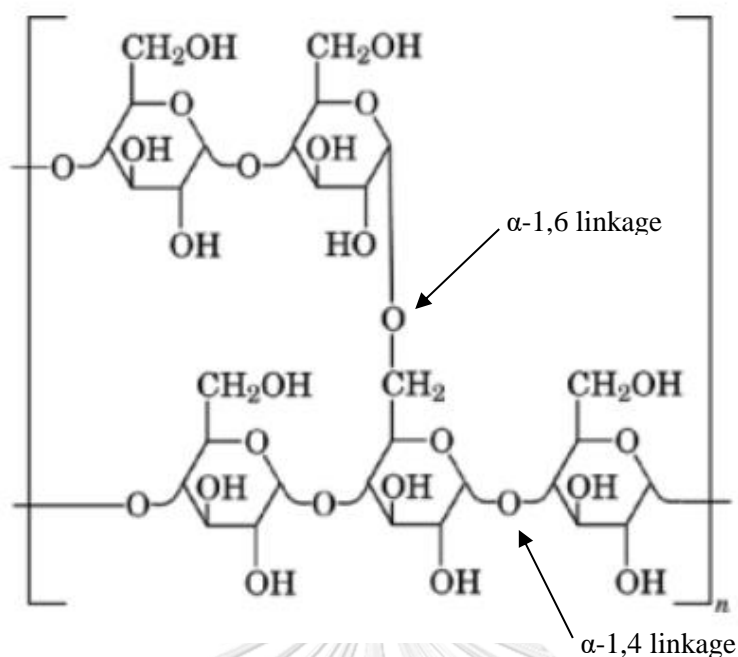


Figure 2. 2 Structure of amylopectin

Source: Modified from Whistler & Daniel (2000)

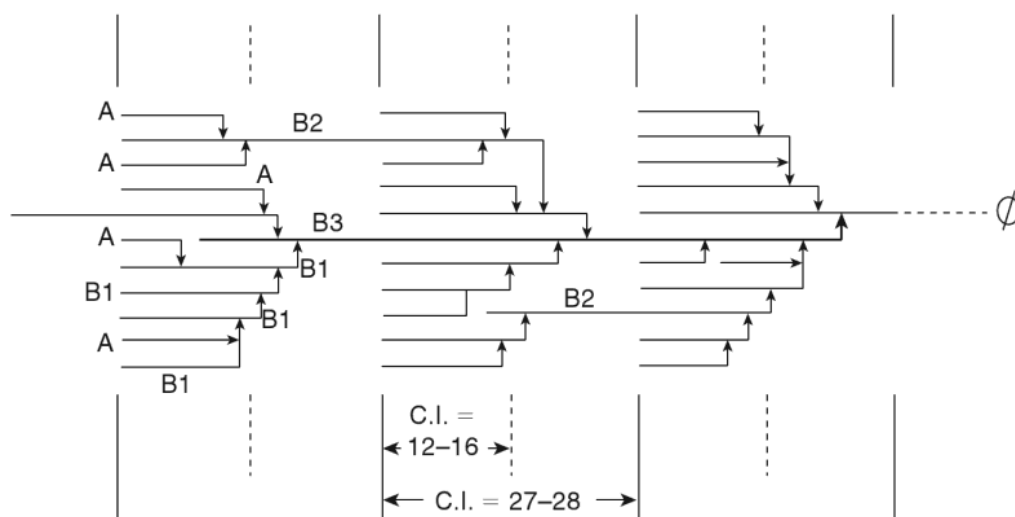


Figure 2. 3 A cluster model of amylopectin with A, and B1-3 chains. The chain carrying the reducing end ( $\emptyset$ ) is the C-chain.

Source: BeMiller & Whistler (2009)

Starch is deposited by plants in organized structures called starch granules. The structure of starch granules consists of an amorphous core surrounded by alternating amorphous and crystalline growth rings shown in Figure 2.4. Typically, the growth rings are 120-400 nm in thickness which is considered to be a representation of diurnal fluctuations of the accumulation of starch in the storage tissue (Donald et al., 2001; Gallant et al., 1997; Sevenou et al., 2002). The crystalline layers are formed from amylopectin chains that are arranged into a crystalline lattice making it higher in density. On the other hand, the amorphous layers consist of the amylopectin branching points, amylose, and amylopectin molecules that are arranged in a disordered conformation due to steric hindrance making it lower in density. The longer chains on amylopectin molecules may pass from the crystalline area into the amorphous area. This granule structure appears to be a universal feature which is independent to the botanical source (Pérez et al., 2009).

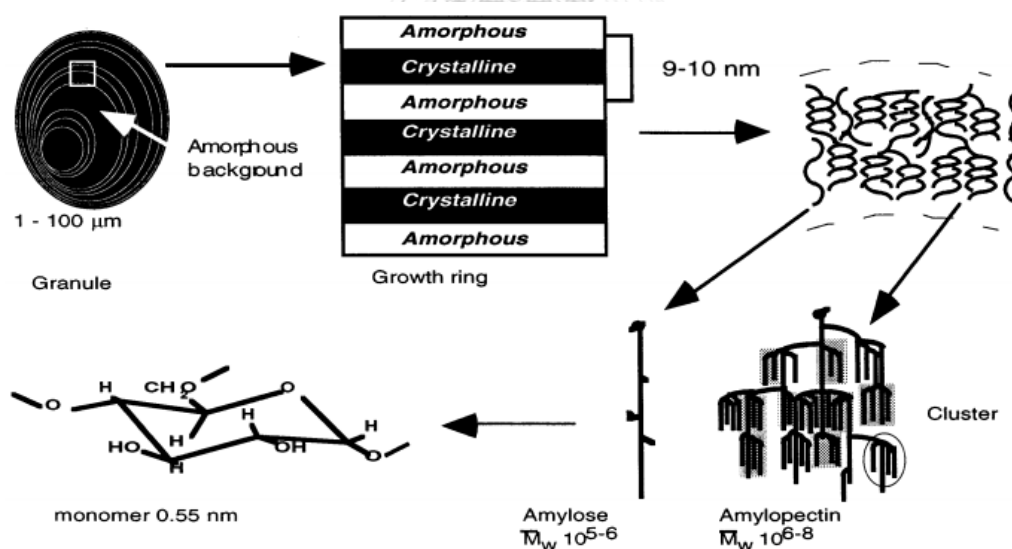


Figure 2. 4 Schematic representation of the different structural levels of the starch granule and the involvement of amylose and amylopectin

Source: Buleon et al. (1998)

### 2.2.2 Starch gelatinization

In the presence of heat and water, starch granules begin to uptake water and swell. With prolonged heating, the fragile granules may disrupt with a release of amylose and amylopectin chains. The starch loses its crystalline structure and transforms into a disordered form resulting in a gelatinized paste. This phase transition associated with the diffusion of water into the starch granule, hydration, swelling, uptake of heat, loss of crystallinity, and amylose leaching is called gelatinization (Fu et al., 2015; Lelievre, 1974; Ratnayake & Jackson, 2008).

Figure 2.5 shows the phase transitions of starch structures during gelatinization. The first phase of swelling begins when water is slowly absorbed by the starch granules which cause the granules to swell to a limited extent. There is no change in viscosity of the suspension at this phase and is still a reversible process by dehydration. During the second phase, the swelling starts within a small temperature range which depends on the type of starch and granules absorb water rapidly and lose birefringence. This phase causes a sudden increase in viscosity of the suspension and a small amount of starch is solubilized. This is an irreversible process because granules will not return to their original when cooled or dehydrated. The third phase of swelling occurs when the granules burst and become formless sacs which allow more soluble fractions of the starch to leach out into the suspension. Gelatinization begins in the amorphous regions of the starch granule because it is more accessible to water and the intermolecular bonding is weak (Ratnayake & Jackson, 2008; Zaslow, 1965).

The swelling behavior of starch was controlled by the degree and type of association within the starch granules. The gelatinization process depends on many factors such as the starch origin, starch characteristics, amylose-to-amylopectin ratio, presence of other food components, agitation, processing temperature and conditions (Leach, 1965).

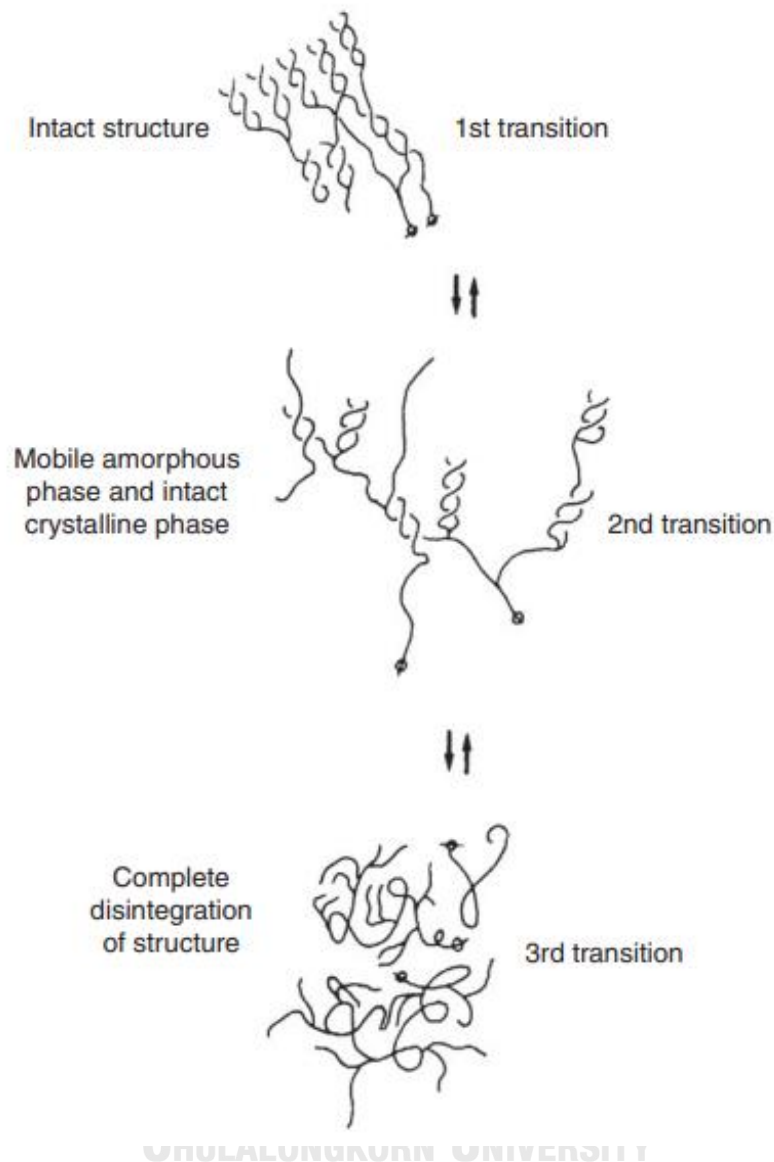


Figure 2. 5 Phase transition of starch structures during gelatinization

Source: Ratnayake & Jackson (2008)

### 2.2.3 Starch retrogradation

When starch is heated in the presence of water, the starch granules begin to swell and eventually burst to release amylose and amylopectin chains. If this viscous solution is cooled long enough, the starch chains will reform into a more crystalline structure but is still different from its native in a process called retrogradation. Starch retrogradation is generally a series of physical changes that

occur when a mixture of starch and water goes through heating, cooling, and storage which may be translated to the industrial operation of processing, distribution, and storage. Some of these changes may include an increase in viscosity, formation of gel, expulsion of water, or even change in crystalline structure (Hoover et al., 2010). Figure 2.6 shows a schematic representation of changes that occur in a starch-water mixture during heating, cooling, and storage. The extent and mechanism of these changes has been the subject of intensive research over many years due to its adverse effects on sensory and storage qualities of starch-rich foods.

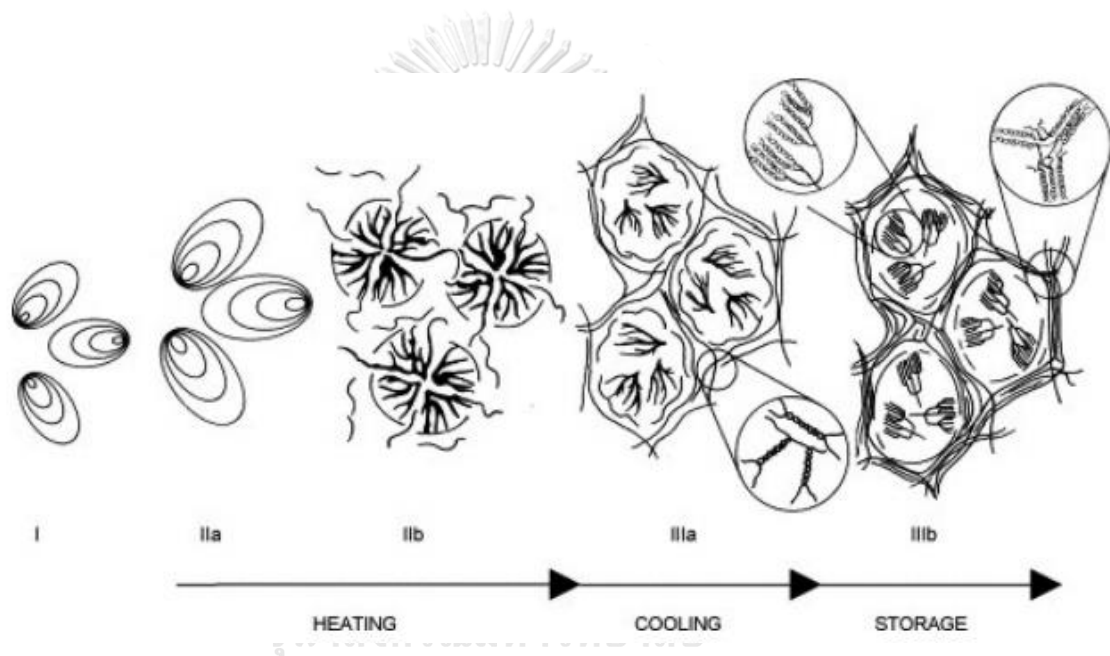


Figure 2. 6 Changes that occur in a starch-water mixture during heating, cooling, and storage

(I) Native starch granules, (II) gelatinization, associated with swelling [a] and amylose leaching and partial granule disruption [b], resulting in the formation of starch paste, (III) retrogradation: formation of an amylose network (gelation/amylose retrogradation) during cooling of the starch paste [a] and formation of ordered or crystalline amylopectin molecules (amylopectin retrogradation) during storage [b]

Source: Wang et al. (2015)



The roles of amylose and amylopectin are different in starch retrogradation. The retrogradation of amylose is quite rapid and begins to realign right after or even before phase separation which is why it is also called short-term retrogradation (Ottenhof & Farhat, 2004). This process is responsible for the initial hardness and stickiness of the starch gel as well as the digestibility of processed foods (Wang et al., 2015). On the other hand, the retrogradation of amylopectin takes a longer time than amylose due to the hindrance caused by its branched structure and is also referred to as long-term retrogradation (Miles et al., 1985). This process is responsible for the gel structure and degree of crystalline development of processed starch. In addition, the interactions between amylose and amylopectin as well as the nature of these molecules have been proposed to affect the rate and extent of retrogradation (Klucinec & Thompson, 1999). For instance, chains that are too short or too long are not preferred for starch retrogradation. The optimum chain length is at about 14 to 24 glucose units (Shi & Seib, 1992). Other factors such as temperature and moisture content also play a role in the kinetics of starch retrogradation (Ottenhof & Farhat, 2004; Slade & Levine, 1991).

Starch retrogradation may have desirable effects in certain products such as improving the sensory properties of breakfast cereals, rice noodles, and other extruded products (Karim et al., 2000). However, starch retrogradation is often recognized for its negative effects on food products such as reducing nutritional value, decreasing the shelf-life, syneresis, and staling of starchy foods (D'appolonia et al., 1981; Karim et al., 2000; Knightly, 1977; Kulp et al., 1981). Since starch has versatile applications in many industries and starch retrogradation can significantly affect the quality of many products, this phenomenon has been widely studied and numerous methods have been developed in order to be able to control it.

### **2.3 Hydrocolloids**

Hydrocolloids are a diverse group of polysaccharides that are readily dispersive, fully or partially soluble, and prone to swell in water. Their affinity for binding water comes from the presence of many hydroxyl groups in their chemical structure. Moreover, hydrocolloids present properties of a colloid and produce a

dispersion which is an intermediate between a true solution and a suspension (Saha & Bhattacharya, 2010). These two properties are the reason they are called ‘hydrophilic colloids’ or ‘hydrocolloids.’ They are widely used in many industries as thickeners, gelling agents, emulsifiers, stabilizers, fat replacers, clarifying agents, flocculating agents, clouding agents and whipping agents to improve quality attributes and shelf-life (BeMiller, 2008; Li & Nie, 2016).

### **2.3.1 Xanthan gum**

Xanthan gum is a natural high molecular weight polysaccharide which can be produced by microbial fermentation of a carbohydrate source. The structure of xanthan gum consists of 1,4-linked  $\beta$ -D-glucose with a trisaccharide side chain that is attached to alternating D-glucosyl residues (Jansson et al., 1975; Sworn, 2021; Urlacher & Noble, 1997). The side chains contain two mannose (half of the terminal mannose units carry a pyruvic acid residue) and one glucuronic acid as shown in Figure 2.7 (Jansson et al., 1975). Its secondary structure has been shown to have a five-fold helical structure (Moorhouse et al., 1977). Xanthan gum is soluble in both cold and hot water. However, it needs intensive agitation when introduced into the aqueous medium to prevent lumping like many other hydrocolloids. Xanthan gum solutions are non-Newtonian and highly pseudoplastic which means its apparent viscosity is significantly influenced with shear stress application—that the higher the shear, the lower the viscosity. At low shear, the associated xanthan gum chains are at rest and are stabilized by hydrogen bonds (Sworn, 2021).

Due to its conformational structure, xanthan gum is widely used in various industries with the most prominent being its use as stabilizer and thickener. Associated xanthan gum chains form a three-dimensional network that prevents separation of insoluble solid particles. Its shear-thinning behavior attributes to mixability, pumpability and pourability (Katzbauer, 1998).

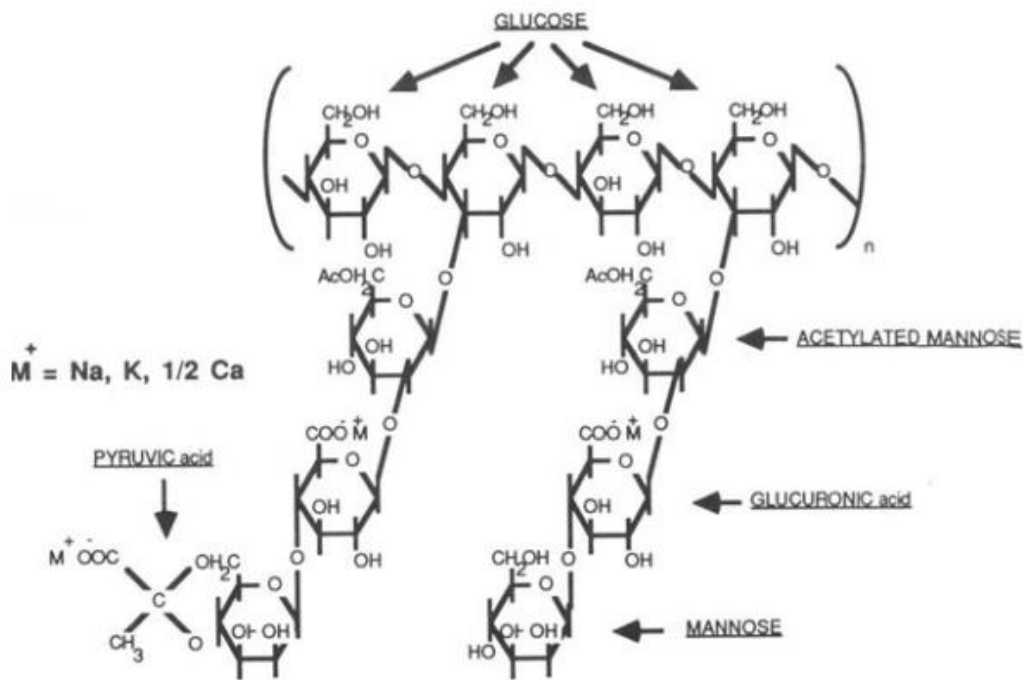


Figure 2. 7 Primary structure of xanthan gum

Source: Jansson et al. (1975)

### 2.3.2 Gum Arabic

Gum arabic, also known as Acacia gum, is a natural polysaccharide that is derived from exudates of *Acacia senegal* and *Acacia seyal* trees. It is widely used in the food industry due to its advantage of being an inexpensive, safe, with less calorific value gum. Moreover, it is a very efficient emulsifier and a long-term stabilizer in food and cosmetic products with oil-water interfaces (Dror et al., 2006). Gum arabic is consisted of three main fractions: a highly branched polysaccharide of  $\beta$ -1,3 galactose backbone with branches of arabinose and rhamnose, arabinogalactan-protein complex, and glycoprotein. The proportions of each sugar differ depending on botanical sources (Williams & Phillips, 2021). Figure 2.8 shows a schematic representation of the structure of the gum Arabic arabinogalactan protein complex. The protein-rich components of the gum contribute to its emulsification properties (Randall et al., 1988). The molecule has a molecular mass of  $(1-2) \times 10^6$  Da and consists of a polypeptide chain containing approximately up to 250 amino acids with

short arabinose side chains and much larger blocks of carbohydrate with the molecular mass of about  $4.0 \times 10^4$  Da. The molecule has a very compact conformation with a radius of gyration of about 36 nm.

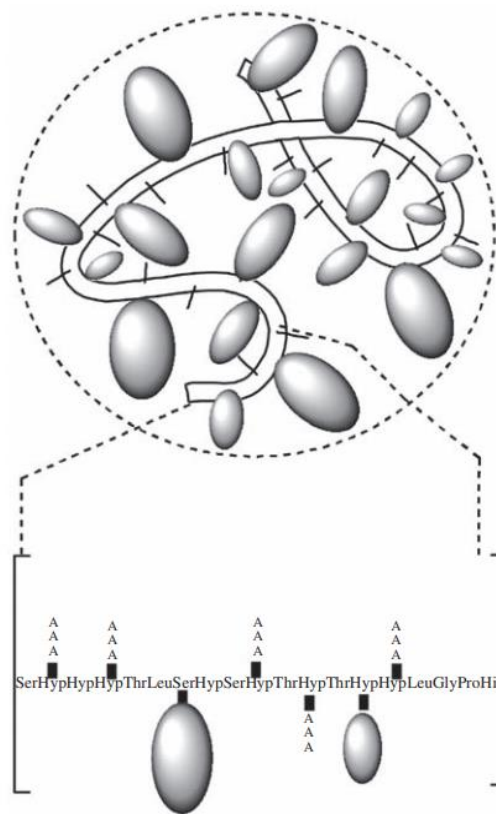


Figure 2. 8 Schematic representation of the structure of gum arabic arabinogalactan-protein complex

Source: Williams & Phillips (2021)

Gum arabic readily dissolves in water. The viscosity of its solution has been found to increase over time when subjected to constant shear and may even form weak gels when allowed to stand to an extent of time which is due to intermolecular aggregation. As for its emulsification properties, it is important to note that *A. senegal* gum is effective in this area while *A. seyal* gum does not produce stable emulsions (Gashua et al. 2016).

### 2.3.3 Hydroxypropyl methylcellulose

Hydroxypropyl methylcellulose (HPMC) belongs to the group of cellulose ethers in which the hydroxyl groups are substituted with one or more of the three hydroxyl groups that are present in the cellulose ring shown in Figure 2.9 (Deshmukh et al., 2017). HPMC is hydrophilic but it has the unusual property of increasing solubility in low temperatures and decreasing solubility in high temperatures (Tundisi et al., 2021) as shown in Figure 2.10. Their solutions exhibit pseudoplastic rheology. Once the solution is heated, it forms a three-dimensional gel structure. This is a reversible process when the solution cools. They also have some interfacial activity and they can form films. Some of the useful properties of HPMC include thickening, surfactancy, film forming ability, adhesiveness, and thermal gelation. The degree of hydrophobicity of HPMC is conferred by its hydroxypropyl and methyl groups, depending on the length of side chains. Therefore, HPMC has the capacity to form gel when they are heated because of this particular characteristic (Grover, 2020). It has a gelation temperature range of 75-90°C.

HPMC as powder or dissolved in water is tasteless and odorless which are important characteristics for the formulation of food, drugs, and cosmetics. It is also biodegradable and biocompatible. It can be used in both aqueous and nonaqueous solvents because it is also soluble in organic solvents (Deshmukh et al., 2017).

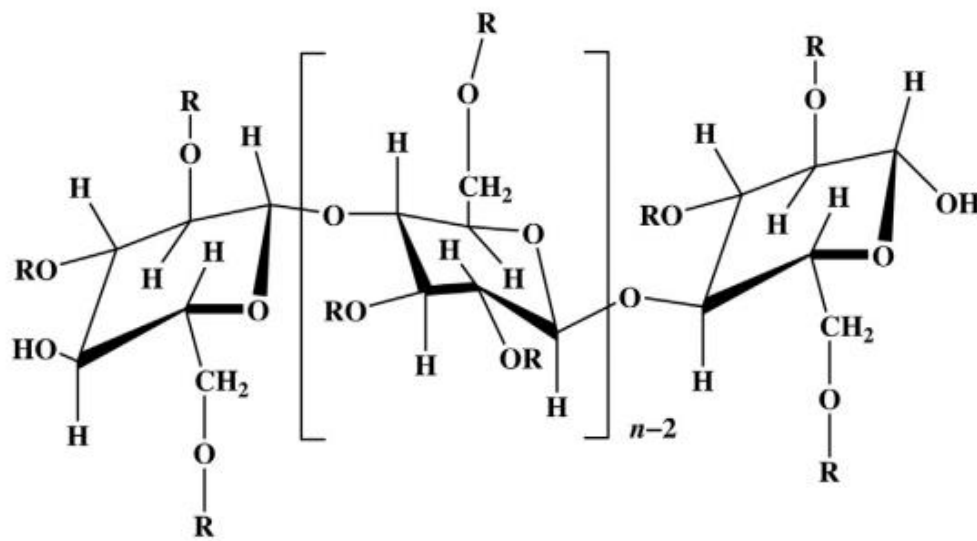


Figure 2. 9 Chemical structure of hydroxypropyl methylcellulose, R = -H, -CH<sub>3</sub> or -(OCH<sub>2</sub>CHCH<sub>3</sub>)<sub>x</sub>OH

Source: Deshmukh et al. (2017)

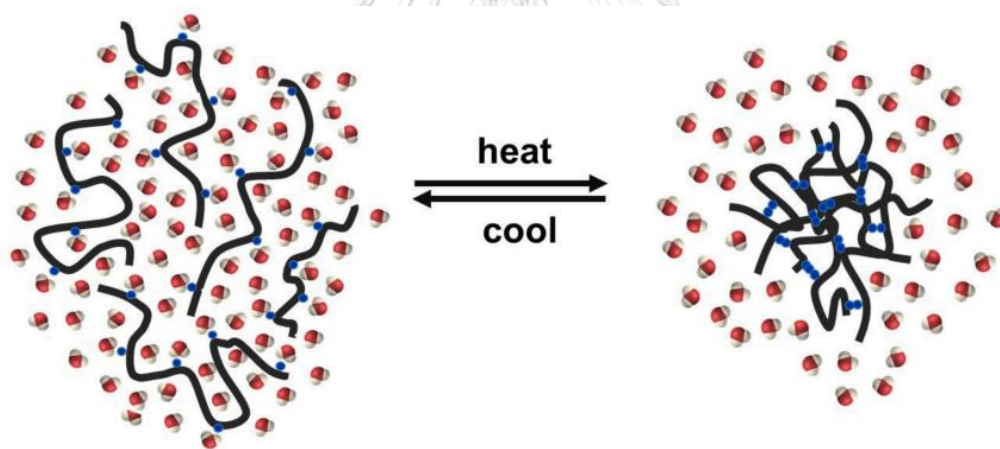


Figure 2. 10 Schematic representation of HPMC in water. At low temperature, the HPMC are highly hydrated, hydrogen bonding between HPMC hydroxyl groups and water favor the hydration. Water molecules close to the HPMC hydrophobic methyl groups form water “cages” (not represented). At high temperature, water “cages” are disrupted, increasing the mobility of water molecules and facilitating the hydrophobic association of methyl groups (blue).

Source: Tundisi et al. (2021)

### 2.3.4 Guar gum

Guar gum is derived from the seeds of an annual pod-bearing, drought-tolerant plant called Guar or cluster bean (*Cyamopsis tetragonolobus* or *C. psoraloides*) of the family *Leguminosae*. It is mainly consisted of the high molecular weight polysaccharides of galactomannans which are linear chain of (1→4)-linked β-D-mannopyranosyl units with (1→6)-linked α-D-galactopyranosyl residues as side chains shown in Figure 2.11 (Mudgil et al., 2014). It is a naturally occurring water soluble polysaccharide with one of the highest molecular weights typically in the range of 0.25-5.0 million (Mudgil et al., 2014; Thombare et al., 2016).

The viscosifying effect of guar gum can vary depending on the molecular weight of the galactomannan. The viscosity of guar gum solution is also enormously dependent on shear rate (Thombare et al., 2016). With increasing shear rate, guar gum solution viscosity decreases which exhibits shear-thinning properties with non-Newtonian, pseudoplastic rheology (Tripathy & Das, 2013). As for temperature, it has an inverse relationship with viscosity which means that an increase in temperature will result in a decrease in viscosity due to the disturbance of the conformation (Parija et al., 2001).

The long chain molecular structure and abundance of hydroxyl groups of the galactomannan molecule make it suitable for a variety of applications which include thickening, emulsifying, gelling, stabilizing, and binding (Thombare et al., 2016). The viscosity and hydration rate of guar gum is affected by factors such as temperature, pH, solute, and concentration which allows it to be further tailored to suit a particular application (Mudgil et al., 2014).

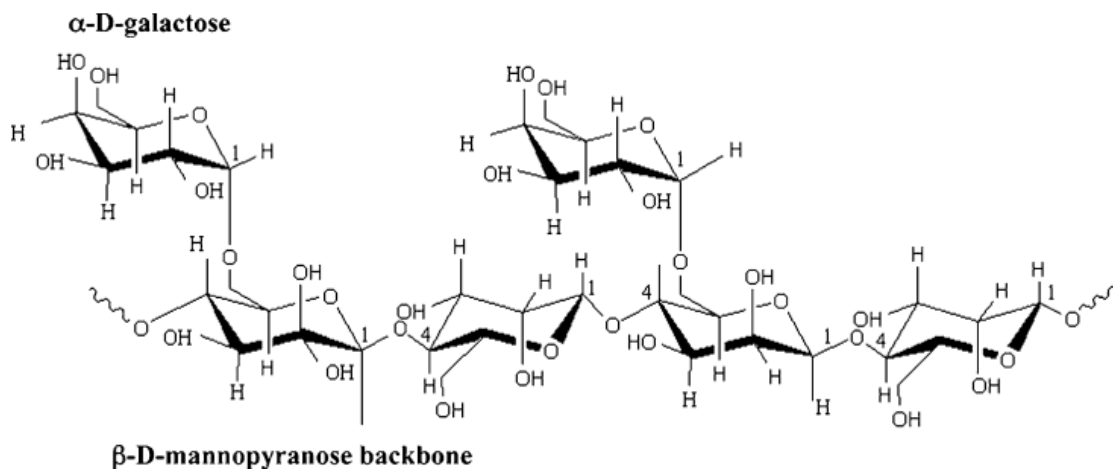


Figure 2. 11 Chemical structure of guar gum

Source: Mudgil et al. (2014)

### 2.3.5 Effects of hydrocolloids on starch-based system

Hydrocolloids are one of the most commonly used food additives due to its ability in network formation, film formation, thickening, and water-holding capacity (BeMiller, 2008). Their functional properties play an important role in the pasting and gelling properties of starch-based systems by affecting the starch gelatinization as well as the short-term and long-term retrogradation of starch. Addition of hydrocolloids into a starch-based system can pose an effect on the arrangement of amylose and branches of amylopectin as well as the interaction between amylose and amylopectin which in turns influence the retrogradation of starch (Schierbaum et al., 1992).

Regarding the effect of hydrocolloids on gelatinization and retrogradation of starch, it is explained in two main aspects. Some researchers described that incorporation of hydrocolloids induce a decrease in interaction among starch molecules and an increase in interaction between the hydrocolloid and starch. This interaction may be noncovalent interactions such as hydrogen bonds and Van der Waals forces (Alam et al., 2009; Leite et al., 2012). Another aspect in explaining the effect of hydrocolloids on gelatinization and retrogradation of starch is based on the assumption that addition of additives may alter the availability of water necessary for



starch gelatinization or that needed for stabilizing starch crystallites forming upon retrogradation (Baik & Chinachoti, 2001; Baik & Chinachoti, 2000; Ribotta & Le Bail, 2007).

The interactions between starch and hydrocolloids play a major role in understanding the mechanism of how hydrocolloids can affect retrogradation. It is suggested that hydrocolloids interact with starch during the gelatinization process. For instance, a study suggests that the hydrocolloid with higher molecular weight can promote the interactions between the hydrocolloid and the highly branched amylopectin. However, when the molecular weight of the hydrocolloid exceeds a certain extent, the effects of molecular weight become insignificant which may be due to the interaction of hydrocolloid with the fixed chain length of amylose (Funami et al., 2005). This study implies the amylose and amylopectin composition of starch should be considered regarding the effects of hydrocolloids on starch retrogradation.

Different types of hydrocolloids were found to influence starch retrogradation differently. For instance, xanthan gum can significantly prevent amylopectin retrogradation while gum arabic and guar gum do not have a strong effect on long-term retrogradation of corn starch (He et al., 2015). Moreover, the properties of each hydrocolloid should be considered before selecting the appropriate one for specific effects. For example, gum arabic can prevent the retrogradation of amylopectin due to its poor water retention properties compared to that of xanthan gum or guar gum. Accordingly, the concentration of hydrocolloid is increased in the system and hinders the cross-linking between molecules of amylopectin.

Therefore, factors such as type and concentration of hydrocolloids as well as of starch should be taken into consideration when discussing the effects of hydrocolloids on starch-based systems.

## CHAPTER 3

### MATERIALS AND METHODS

#### 3.1 Materials and Chemicals

Calcium propionate (Chemipan Corporation Co., Ltd., Bangkok, Thailand)

Guar gum, food grade (Chemipan Corporation Co., Ltd., Bangkok, Thailand)

Gum arabic, food grade (Chemipan Corporation Co., Ltd., Bangkok, Thailand)

Hydroxypropyl methylcellulose, food grade (Chemipan Corporation Co., Ltd., Bangkok, Thailand)

Iodine (KemAus, New South Wales, Australia)

Namwa banana (*Musa acuminata* × *Musa balbisiana*)

Potassium iodide (Ajax Finechem, Sydney, Australia)

Salt (Prung Thip, Bangkok, Thailand)

Shortening (Three Topp Chemicals & Foods Co., Ltd., Bangkok, Thailand)

Sodium metabisulfite (Chemipan Corporation Co., Ltd., Bangkok, Thailand)

Soluble starch, iodometric indicator (Ajax Finechem, Sydney, Australia)

Sugar (Mitr Phol, Bangkok, Thailand)

Unbleached bread flour, Shuttle™ brand (Thai Flour Mill Industry, Samut Prakan, Thailand)

Xanthan gum, food grade (Chemipan Corporation Co., Ltd., Bangkok, Thailand)

### 3.2 Equipments

Chroma meter, model CM-600d (Konica Minolta Sensing, Osaka, Japan)

Desiccators (GL, Wertheim, Germany)

Differential scanning calorimeter (DSC), Diamond DSC® (Perkin Elmer, Waltham, MA, USA)

Dough proofer (Chatcharee Holding Co., Ltd., Bangkok, Thailand)

Infrared food oven, model PL-6 (Kluaynumthai Kitchen, Bangkok, Thailand)

Kenwood® mixer with dough hook attachment (Kenwood Corporation, Nagano, Japan)

Laboratory hot air oven, model 600 (Mettler, Schwabach, Germany)

Rapid Visco Analyzer (RVA), model RVA-4 (Newport Scientific, Warriewood, NSW, Australia)

Shaking water bath, model SW23 (Julabolabortechnik, Seelbach, Germany)

Spectrophotometer, GENESYS 20 (Thermo Scientific, Waltham, MA, USA)

Texture analyzer, TA-XT2i (Stable Micro Systems, Surrey, UK)

Water activity meter, AquaLab™, series 3TE (Decagon Devices, Pullman, WA, USA)

### 3.3 Methodology

#### 3.3.1 Banana flour preparation

The banana used in this research were Namwa (also known as Pisang Awak) banana (*Musa acuminata* × *Musa balbisiana*) at the all-green stage (stage 1) according to the banana color chart (Soltani et al., 2010). To prepare the flour (Figure 3.1), banana fruits were cleaned, peeled, and sliced crosswise into 1 mm-thick pieces. The slices were then soaked in 0.1% sodium metabisulfite solution for 30 min in order to prevent browning. After draining the soaking solution, the banana slices were dried in a hot air oven at 55 °C until reaching the moisture content of around 10% (wet basis). The dried banana slices were then pulverized and sieved through a 100 US mesh to obtain the flour.

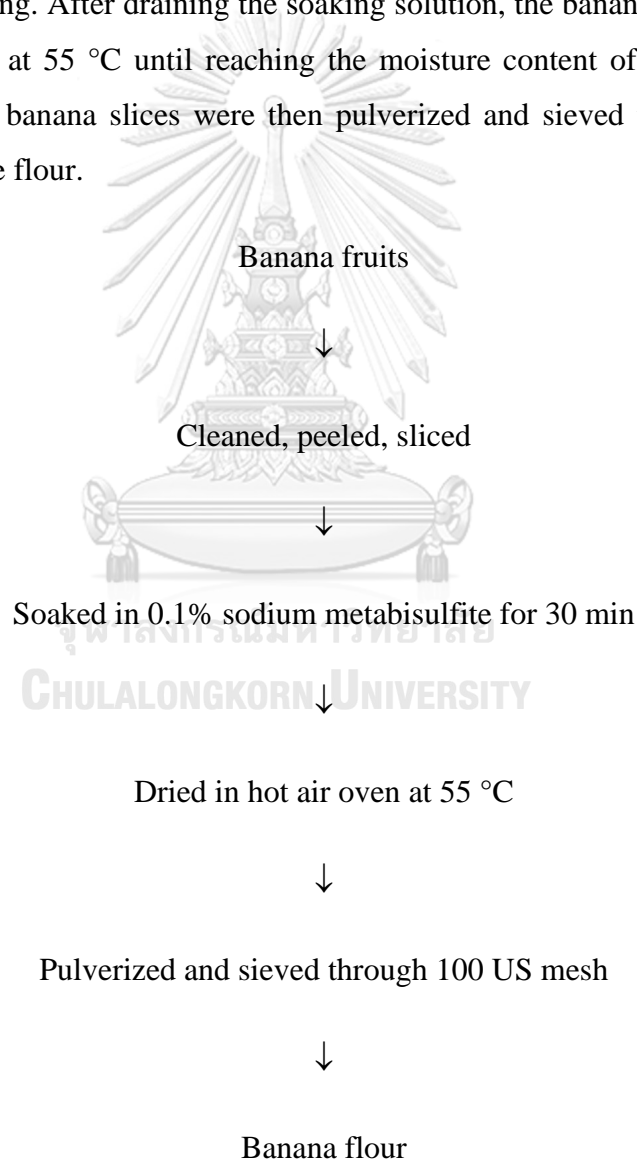


Figure 3. 1 Banana flour preparation steps

### 3.3.2 Composition analysis

The banana flour obtained was analyzed for moisture, protein, fat, ash, and fiber contents according to the AOAC approved methods (AOAC, 1990). Glucoamylase method was used to analyze the starch content (Mason et al., 1982). Additionally, amylose content was analyzed by means of amperometric titration following the previously described method (Takeda et al., 1987).

### 3.3.3 Effects of hydrocolloids on the pasting and gelling properties of banana flour

Hydrocolloids (xanthan gum, gum arabic, HPMC and guar gum) were varied at three levels: 0.5, 1.0, and 1.5% by weight of flour. For the observation of starch gelatinization and retrogradation (3.3.3.2 and 3.3.3.3), the previously mentioned hydrocolloid concentrations were also used accordingly. Banana flour without added hydrocolloid was used as a control. The samples were analyzed for the following properties:

#### 3.3.3.1 Pasting behavior

Pasting behavior of banana flour with and without added hydrocolloids was monitored according to the AACC method (AACC, 2000) using a Rapid Visco Analyzer (RVA) (model RVA-4, Newport Scientific, Warriewood, NSW, Australia). A predetermined amount (2.87 g) of banana flour was weighed into an RVA canister which contained 25.13 mL of hydrocolloid suspension. After mounting the canister to the RVA machine, the experiment was carried out, starting with equilibrating the mixture at 50°C for 1 min. After that the mixture was stirred at 960 rpm for 10 s and reduced to 160 rpm. The temperature was then increased to 95°C and the sample was held at that temperature for 3.5 min. Upon completing the holding step, the temperature was reduced to 50°C and maintain at that temperature for 2 min. The steps are summarized in Table 3.1. Temperature as well as viscosity changes as affected by the heating and cooling of the flour paste were extracted from the RVA curve.

Table 3. 1 Testing profile for RVA

Step	Temperature/Speed	Time
1	50 °C	0 min, 0 sec
2	960 rpm	0 min, 0 sec
3	160 rpm	0 min, 0 sec
4	50 °C	1 min, 0 sec
5	95 °C	4 min, 42 sec
6	95 °C	7 min, 12 sec
7	50 °C	11 min, 0 sec
End of test		13 min, 0 sec
Time between readings		4 sec

### 3.3.3.2 Gelatinization study

Thermal properties related to starch gelatinization were measured using a Differential Scanning Calorimeter (DSC) (Diamond DSC<sup>®</sup>, Perkin Elmer, Waltham, MA, USA) according to previous report (Karim et al., 2008). Firstly, hydrocolloid suspensions were prepared at 0.5, 1.0, and 1.5% concentration by slowly adding the hydrocolloids into distilled water and stirred at 1200 rpm for 5 min, 800 rpm for 20 min, and 1200 rpm for 5 min with a magnetic stirrer. Banana flour (3 mg) and hydrocolloid suspension (12 mg) at 1:4 ratio was sealed in a stainless steel DSC pan (Perkin Elmer, Waltham, MA, USA) and equilibrated at room temperature (25°C) for 24 h before analysis. During analysis, the sample pan was heated from 30 to 120°C at a rate of 10 °C/min. An empty pan was used as a

reference. Onset ( $T_o$ ), peak ( $T_p$ ), and conclusion temperatures ( $T_c$ ), together with enthalpy of gelatinization ( $\Delta H_G$ ) were obtained from the DSC curve.

### 3.3.3.3 Retrogradation study

Long-term retrogradation was investigated using a Differential Scanning Calorimeter (DSC) (Diamond DSC<sup>®</sup>, Perkin Elmer, Waltham, MA, USA) by measuring the temperature and enthalpy required for melting of amylopectin crystallites according to earlier study (Karim et al., 2008). The sample pans obtained from the gelatinization study (3.3.3.2) were stored at 4°C for 7, 10, and 14 days. Measurements were done from 10 to 80°C at a scanning rate of 10 °C/min. An empty pan was used as a reference.  $T_o$ ,  $T_p$ , and  $T_c$ , together with melting enthalpy of amylopectin crystallites ( $\Delta H_R$ ) were extracted from the DSC curve.

### 3.3.3.4 Gel firmness

Gel samples were prepared using the method of Muadklay & Charoenrein (2008) with some modifications. Banana flour slurry containing hydrocolloid (0.5, 1.0, and 1.5% of total solids) was prepared by slowly adding the banana flour into the hydrocolloid suspension to be stirred at 1200 rpm for 5 min, 800 rpm for 20 min, and 1200 rpm for another 5 min. The slurry was poured into a silicone mold. The samples were gelatinized in a water bath at 95 °C for 60 min. The gel samples were cooled at room temperature for 60 minutes. The freshly prepared gel samples (Day 0) and those stored in a tightly sealed container at 4°C for 7, 10, and 14 days were analyzed for gelling properties. Stored samples were allowed to equilibrate at room temperature for 60 min before analysis. Gel firmness was measured using a Texture Analyzer (TA-XT2i, Stable Micro Systems, Surrey, UK) fitted with a stainless steel spherical probe (P/0.25S). The test was conducted using 1 mm/s pre-test speed, 10 mm/s test speed, and 50% deformation.

### 3.3.4 Application of banana flour with selected hydrocolloids as a wheat-flour substitute in bread production

White pan bread samples were prepared using a straight dough method with some modification to the recipe described by Choondee (2003). Wheat flour in the recipe was replaced with the plain banana flour at 30% of wheat flour substitution level with and without selected hydrocolloid as shown in Table 3.2. The selected treatments were XG at 0.5%, GA at 1.5%, HPMC at 0.5%, and GG at 1.0% by weight of banana flour. Bread without added hydrocolloid was used as a control.

Table 3. 2 Recipe for white pan bread with 30% banana flour substitution

	Control	0.5%	1.0%	1.5%
Wheat flour (g)	350	350	350	350
Banana flour (g)	150	150	150	150
Hydrocolloid (g) <sup>1</sup>	-	0.75	1.5	2.25
Yeast (g)	5	5	5	5
Shortening (g)	25	25	25	25
Sugar (g)	25	25	25	25
Salt (g)	7.5	7.5	7.5	7.5
Water (mL)	300	300	300	300
Calcium propionate (g)	1.5	1.5	1.5	1.5

<sup>1</sup> Selected hydrocolloids were XG at 0.5%, GA at 1.5%, HPMC at 0.5%, and GG at 1.0% by weight of banana flour



Bread preparation:

1. Mix the dry ingredients which include the wheat flour, banana flour, hydrocolloid, yeast, and calcium propionate together and sieve it. Pour the mixture into the Kenwood mixer mixing bowl and stir it a spatula.
2. Dissolve the salt and sugar into 300 mL of water and pour it into the dry mixture.
3. Mix with a dough hook attachment at level 3 speed for 1-2 min or until the mixture does not stick to the side of the mixing bowl.
4. Add shortening and mix at level 6 speed for 5-10 min.
5. Knead the dough into a round shape into a basin and let it rest in the proofer at 32 °C for 1 h or until the dough doubles in size.
6. Cut and weigh the dough into 250 g pieces. Knead the divided dough to remove air with a rolling pin and knead it into a round shape to rest for another 30 min at 32 °C in the proofer.
7. Knead to remove air and roll the dough into a bread pan and allow it to rest for 1 h at 32 °C in the proofer.
8. Bake at 200 °C in the infrared food oven (model PL-6, Kluaynumthai Kitchen, Bangkok, Thailand) for 30 min and leave it to cool at room temperature.

The freshly baked bread samples (Day 0) and those stored at room temperature for 7, 10 and 14 days in sealed polyethylene (PE) bags were determined for the following properties:

#### **3.3.4.1 Moisture content and water activity**

Moisture content of bread samples were determined as loss in weight of a sample when heated at 105 °C according to the AACC approved method

(AACC, 2000). The water activity values were measured at 25 °C with the AquaLab™ water activity meter (series 3TE, Decagon Devices, Pullman, WA, USA). Only bread crumbs were used in this assay.

#### 3.3.4.2 Specific volume of the loaf

Specific volume of the loaf of bread samples were obtained by using the rapeseed displacement method (Bárceñas & Rosell, 2006). The bread loaves were first weighed and placed into a container of known volume. The container is then filled with rapeseed. The bread loaf is removed and the rapeseed left is measured. The volume of the bread was determined by the difference and specific loaf volume was calculated according to Equation (3.1).

$$\text{Specific volume (v)} = V/m \quad \dots(3.1)$$

where V is volume

m is weight of the sample (g)

#### 3.3.4.3 Texture profile analysis

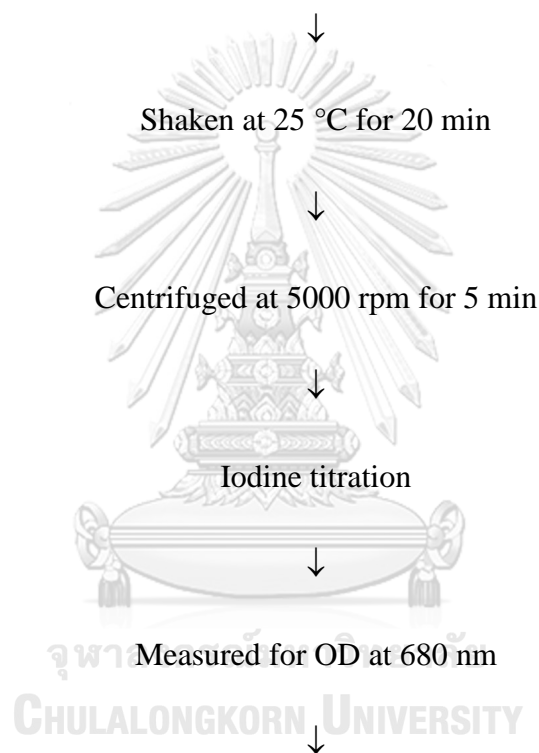
The bread samples were cut in to 3×3×3 cm to be analyzed. The crust was not included. Texture profile analysis (TPA) was carried out using a Texture Analyzer (TA-XT2i, Stable Micro Systems, Surrey, UK) with the P/100 aluminium platen at 1 mm/s test speed and 70% deformation. Hardness, adhesiveness, cohesiveness, springiness, gumminess and chewiness were extracted from the TPA curve.

#### 3.3.4.4 Water-soluble starch content

Water-soluble starch content was reported to associate with degree of starch retrogradation. The content was determined according to Shaikh et al. (2007) shown in Figure 3.2. In brief, 200 mg of bread crumb was added to 15 mL of distilled water. The mixture was shaken at 25°C for 20 min. The slurry obtained was centrifuged at 5000 rpm for 5 min and the supernatant were subjected to starch content determination using iodine titration technique. The standard iodine solution

consisted of 2 mg of iodine and 20 mg potassium iodide in 100 mL of water. The optical density (OD) of the sample was measured using the spectrophotometer (GENESYS 20, Thermo Scientific, Waltham, MA, USA) at 680 nm. A standard curve was plotted of OD at 680 nm versus the concentration of starch. The OD of the sample was compared with the standard curve to obtain the water-soluble starch content.

Add 200 mg of bread crumb to 15 mL distilled water



Compare OD with standard curve to obtain starch concentration

Figure 3. 2 Water-soluble starch analysis

#### 3.3.4.5 Bread crumb structure

The bread samples were sliced into 2 cm thickness and photographed with a digital camera.

### 3.3.4.6 Color

The bread was sliced into 2 cm thickness and analyzed randomly on areas A, B, C, and D (Figure 3.3). The color of bread crumbs were measured in  $L^*$ ,  $a^*$ , and  $b^*$  values using the Minolta® chroma meter (model CM-600d, Konica Minolta Sensing, Osaka, Japan) in CIELAB scale using D65 illuminant and 10° observer. Four measurements at point A, B, C, and D were done for each replicate. Hue angle and chroma were calculated using Equations (3.2) and (3.3). Please note that Equation (3.2) can only be used if the  $a^*$  and  $b^*$  values are both positive.

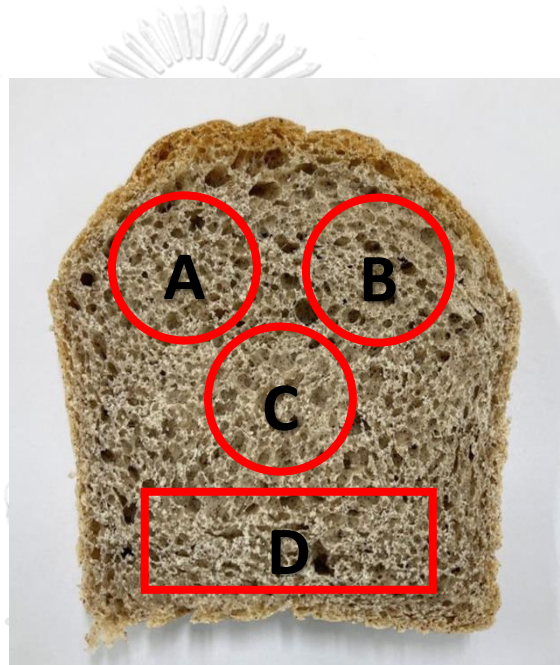


Figure 3. 3 Area of bread crumb that were analyzed for color

$$\text{Hue angle } (^{\circ}) = \arctan (b^*/a^*) \quad \dots(3.2)$$

$$\text{Chroma} = (a^{*2}+b^{*2})^{1/2} \quad \dots(3.3)$$

### 3.3.5 Statistical analysis

The experiment was done in three replicates using a Completely Randomized Design (CRD). The data were analyzed using Analysis of Variance (ANOVA). Duncan's new multiple range test were used to determine the difference among sample means at  $p=0.05$  (Cochran & Cox, 1957).

## CHAPTER 4

### RESULTS AND DISCUSSION

#### 4.1 Banana flour composition

The banana flour used in this study was made from Namwa banana (*Musa acuminata* × *Musa balbisiana*). Proximate analysis shows that the banana flour contained 10.16% moisture, 2.32% crude protein, 0.22% crude fat, 2.64% ash, and 1.27% crude fiber, on a wet basis (Table 4.1). The starch content was 75.87% (wet basis), similar to that found for green banana flour from *Musa cavendishii* (73.8%) and green banana flour from *Musa paradisiaca* (73.40%) (Juarez-Garcia et al., 2006; Tribess et al., 2009). The high starch content proves that bananas have the potential to become the next staple crop. The amylose content was 12.41% of starch. Therefore, the amylopectin content obtained by difference is at 87.59% of starch.

Table 4. 1 Banana flour composition

Composition	Amount
Moisture (% wet basis)	10.16±0.06
Crude protein (% wet basis)	2.32±0.11
Crude fat (% wet basis)	0.22±0.02
Ash (% wet basis)	2.64±0.00
Crude fiber (% wet basis)	1.27±0.01
Starch (% wet basis)	75.87±0.57
Amylose (% of starch)	12.41±0.14

Mean±SD of three replicates

## 4.2 Effects of hydrocolloids on pasting and gelling properties of banana flour

### 4.2.1 Pasting behavior

Pasting properties of the banana flour (control) and banana flour-hydrocolloid mixtures with xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG) at 0.5, 1.0, and 1.5% are shown in Table 4.2. The peak viscosity, breakdown viscosity, final viscosity, setback viscosity and pasting temperature were evidently affected by the type and concentration of hydrocolloids.

Regarding the peak viscosity, the increasing concentration of XG and GA, both of which are anionic, resulted in decreasing viscosity with GA at 1.5% concentration showing the lowest peak viscosity. A decrease in peak viscosity induced by anionic hydrocolloid was also reported by Weber et al. (2009). On the other hand, the addition of HPMC and GG, both of which are non-ionic, resulted in an increase in peak viscosity with increasing concentration with GG at 1.5% showing the highest peak viscosity. The peak viscosity reflects the ability of the starch granules to freely swell before their physical breakdown (Rojas et al., 1999). The increasing peak viscosity suggested that the hydrocolloids did not entrap the starch granules which allowed it to freely swell (Leite et al., 2012). The starch-hydrocolloid system can be portrayed as a biphasic system with the hydrocolloids located in the continuous phase. The starch granules swell during gelatinization to reduce the volume of the accessible phase to the hydrocolloid which increases the concentration of the hydrocolloids which result in an increase in the viscosity of the mixture (Alloncle et al., 1989).

A positive correlation between the peak viscosity and trough viscosity suggested by Singh Sandhu and Singh (2005) is observed in all of the hydrocolloids. The decrease in peak viscosities of XG and GA also resulted in a decrease in trough viscosity. As for HPMC and GG which increased in peak viscosity, the trough viscosity increases as well. A study done by Huaisan et al. (2009) also explained hydrocolloids such as GG and high methoxyl pectin (HMP) resulted in high trough due to increased stability of starch paste.

Table 4. 2 Pasting properties of banana flour with and without added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG) at 0.5, 1.0, and 1.5% concentration

Samples	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown viscosity (cP)	Final viscosity (cP)	Setback viscosity (cP)	Peak time (min)	Pasting temp. (°C)
Control	2437.33±75.79 <sup>at</sup>	2095.00±62.44 <sup>atg</sup>	342.33±18.82 <sup>abcd</sup>	3127.33±82.76 <sup>d</sup>	1032.33±24.90 <sup>bc</sup>	5.64±0.05 <sup>c</sup>	82.15±0.27 <sup>c</sup>
XG0.5%	2698.00±16.52 <sup>c</sup>	2460.66±117.37 <sup>bc</sup>	237.33±101.25 <sup>e</sup>	3392.66±80.00 <sup>c</sup>	932.00±53.77 <sup>coest</sup>	6.28±0.42 <sup>b</sup>	82.18±0.27 <sup>c</sup>
XG1.0%	2330.00±121.65 <sup>te</sup>	2281.33±120.59 <sup>d</sup>	48.66±1.52 <sup>t</sup>	3139.33±153.12 <sup>d</sup>	858.00±35.51 <sup>t</sup>	6.61±0.09 <sup>a</sup>	82.93±0.24 <sup>bc</sup>
XG1.5%	2273.00±25.45 <sup>te</sup>	2206.50±26.16 <sup>oest</sup>	66.50±0.70 <sup>t</sup>	3221.00±192.33 <sup>cd</sup>	1014.50±166.17 <sup>cd</sup>	6.77±0.00 <sup>a</sup>	85.35±0.98 <sup>a</sup>
GA0.5%	2276.33±29.36 <sup>te</sup>	1965.00±35.93 <sup>gh</sup>	311.33±40.37 <sup>bcde</sup>	2933.33±31.39 <sup>st</sup>	968.33±13.42 <sup>coe</sup>	5.70±0.06 <sup>c</sup>	82.76±0.25 <sup>bc</sup>
GA1.0%	2198.33±29.14 <sup>gh</sup>	1936.00±42.67 <sup>gh</sup>	262.33±22.54 <sup>de</sup>	2834.66±65.39 <sup>te</sup>	898.66±45.50 <sup>st</sup>	5.69±0.02 <sup>c</sup>	83.31±0.95 <sup>bc</sup>
GA1.5%	2112.66±19.50 <sup>h</sup>	1838.66±17.24 <sup>h</sup>	274.00±25.51 <sup>de</sup>	2732.66±28.50 <sup>te</sup>	894.00±38.22 <sup>st</sup>	5.67±0.06 <sup>c</sup>	83.16±0.98 <sup>bc</sup>
HPMC0.5%	2477.33±23.45 <sup>de</sup>	2073.33±20.98 <sup>te</sup>	404.00±12.12 <sup>a</sup>	3079.00±15.39 <sup>de</sup>	1005.66±17.61 <sup>cd</sup>	5.74±0.02 <sup>c</sup>	82.21±0.47 <sup>c</sup>
HPMC1.0%	2530.50±38.89 <sup>de</sup>	2236.50±68.58 <sup>oest</sup>	294.00±29.69 <sup>de</sup>	3148.50±68.58 <sup>o</sup>	912.00±0.00 <sup>oest</sup>	5.79±0.01 <sup>c</sup>	82.72±0.03 <sup>oc</sup>
HPMC1.5%	2605.66±19.73 <sup>co</sup>	2308.66±80.03 <sup>co</sup>	297.00±71.14 <sup>coe</sup>	3257.33±36.82 <sup>co</sup>	948.66±67.50 <sup>coest</sup>	5.72±0.04 <sup>c</sup>	82.68±0.42 <sup>oc</sup>
GG0.5%	2615.33±44.65 <sup>cd</sup>	2270.66±27.53 <sup>de</sup>	344.66±35.27 <sup>abcd</sup>	3387.66±63.87 <sup>c</sup>	1117.00±45.57 <sup>b</sup>	5.73±0.06 <sup>c</sup>	82.65±0.35 <sup>bc</sup>
GG1.0%	2891.50±36.06 <sup>b</sup>	2505.00±33.94 <sup>b</sup>	286.50±2.12 <sup>ab</sup>	3723.50±58.68 <sup>b</sup>	1218.50±24.74 <sup>a</sup>	5.88±0.04 <sup>c</sup>	83.77±0.60 <sup>b</sup>
GG1.5%	3163.66±190.84 <sup>a</sup>	2779.33±223.02 <sup>a</sup>	384.33±50.64 <sup>abc</sup>	3994.66±223.78 <sup>a</sup>	1215.33±28.44 <sup>a</sup>	5.87±0.13 <sup>c</sup>	82.26±1.35 <sup>c</sup>

Mean±SD of three replicates

Means within the same column followed by different letters are significantly different ( $p \leq 0.05$ ).

Breakdown viscosity represents the resistance to heat and mechanical shearing of swollen starch granules (Lee et al., 2002). Lower values of breakdown viscosity mean that it is harder for starch granules to break with thermal treatment and shear stress. Addition of XG showed significant decrease in breakdown viscosity ( $p \leq 0.05$ ) at all concentrations but 1.0% resulted in the lowest value. This is in agreement with studies done by Lee et al. (2002) and Christianson et al. (1981) which reported that addition of XG can reduce the breakdown viscosity. The interaction between XG and starch has been suggested to increase the shear stability significantly (Rojas et al., 1999). A similar trend is seen in all of the other hydrocolloids (GA, HPMC, and GG), which has the lowest breakdown viscosity at 1.0% concentration. However, HPMC at 0.5% and GG at 0.5 and 1.5% resulted in an increase in the breakdown viscosity. This increase may be due to the dissociations between starch and hydrocolloids through structure shrinkage of these polymers from the decrease in temperature (Funami, 2005).

As for the final viscosity, the addition of GG with increasing concentration significantly increased the final viscosity ( $p \leq 0.05$ ). This may be due to the thickening effect of guar gum which results in an increase of the viscosity of the continuous phase and the overall suspension (Shahzad et al., 2019). XG at 0.5% also significantly increased the final viscosity ( $p \leq 0.05$ ). However, the other concentrations and the other hydrocolloids either decreased or did not significantly affect the final viscosity ( $p > 0.05$ ). This might be attributed to the presence of lower amounts of starch in the blend due to the replacement with the hydrocolloids (Alamri et al., 2013).

All of the hydrocolloids reduced the setback viscosity when compared with the control except for GG which significantly increased the setback viscosity ( $p \leq 0.05$ ). The increase in setback viscosity suggests that GG facilitated intermolecular bonding or junction zone formation which are the first stage of molecular crystallization or retrogradation (Huaisan et al., 2009). On the contrary, XG provided the greatest reduction in the setback viscosity at 1.0%. The decreasing degree of amylose-amylose interaction, which is the primary factor of short-term retrogradation, was likely to occur due to the competition between hydrocolloids and amylose



molecules to an intermolecular interaction (Leite et al., 2012). The cooling stage causes the amylose chains which were diffused out of the starch granules to retrograde. This phenomenon is the cause of the firming of bread crumbs after the first hours of baking. Therefore, the addition of hydrocolloids that can reduce the setback viscosity can provide anti-staling effects or a delay of crumb firming.

The addition of hydrocolloids, independently of the concentration used, did not significantly affect ( $p>0.05$ ) the time to peak viscosity except for XG which exhibited an increase in this parameter as the concentration increased.

A slight increase in pasting temperature was witnessed in all hydrocolloid-added samples, with addition of XG at 1.5% resulting in the greatest increase. This shift in pasting temperature can be interpreted as a result of lower heating rates and water diffusivities from the increased viscosity from the addition of hydrocolloids (Krüger et al., 2003).

#### **4.2.2 Gelatinization temperature and enthalpy**

Despite the apparent differences observed on the pasting properties of banana flour with the addition of hydrocolloids, it was found that there were no significant differences ( $p>0.05$ ) between gelatinization onset ( $T_o$ ), peak ( $T_p$ ), and conclusion ( $T_c$ ) temperatures of banana flour with and without hydrocolloids detected by using the differential scanning calorimeter (DSC) (Table 4.3). These results were also previously reported by Biliaderis et al. (1997) which found no differences to the gelatinization temperature but found an increase in the enthalpy of gelatinization ( $\Delta H_G$ ) with the addition of xanthan,  $\beta$ -glucan, arabinoxylan, and guar gum to waxy maize and wheat starch. According to Table 4.3, only HPMC at 0.5% caused a significant increase ( $p\leq 0.05$ ) of the  $T_o$  which may be due to the interactions between the starch and hydrocolloid which delayed the starch gelatinization (Rojas et al., 1999).

However, addition of all the hydrocolloids generally resulted in an increase in the  $\Delta H_G$  with XG at 1.0%, GA at 0.5%, HPMC at 1.5% and GG at 1.5% resulting in the highest significant increase in  $\Delta H_G$  ( $p\leq 0.05$ ) which corresponds with

the results previously mentioned by Biliaderis et al. (1997). Liu et al. (2003) also found that the addition of yellow mustard mucilage to wheat and rice starch did not affect the peak temperature but increased the  $\Delta H_G$ . This may be due to the association between hydrocolloids and starch which decreased the mobility of starch chains and resulted in higher thermal energy required for gelatinization (Leite et al., 2012). Another possible explanation for this is the delay of water migration towards the starch granule during the gelatinization process due to the hydrophilic behavior of these hydrocolloids which results in higher energy required for the process (Varela et al., 2016). These results of higher  $\Delta H_G$  corresponded well with the higher pasting temperatures, as obtained using RVA in the previous section (4.2.1), upon addition of hydrocolloids.



Table 4. 3 Gelatinization temperatures and enthalpy of banana flour with and without added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG) at 0.5, 1.0, and 1.5%

Samples	Gelatinization temperature			$\Delta H_G$ (J/g)
	$T_o$ (°C)	$T_p$ (°C) <sup>ns</sup>	$T_c$ (°C) <sup>ns</sup>	
Control	75.73±0.36 <sup>b</sup>	79.67±0.45	84.67±0.76	3.89±0.18 <sup>e</sup>
XG0.5%	75.67±0.48 <sup>b</sup>	79.75±0.45	85.17±0.65	4.66±0.18 <sup>cd</sup>
XG1.0%	75.35±0.15 <sup>b</sup>	79.43±0.14	84.63±0.55	5.35±0.31 <sup>ab</sup>
XG1.5%	75.84±0.11 <sup>ab</sup>	79.80±0.34	84.83±0.61	4.93±0.26 <sup>bc</sup>
GA0.5%	75.77±0.17 <sup>b</sup>	79.86±0.23	85.11±0.66	4.96±0.74 <sup>bc</sup>
GA1.0%	75.61±0.43 <sup>b</sup>	79.70±0.50	85.05±0.84	4.36±0.38 <sup>cde</sup>
GA1.5%	75.64±0.29 <sup>b</sup>	79.69±0.47	85.09±0.51	4.36±0.23 <sup>cde</sup>
HPMC0.5%	76.84±1.17 <sup>a</sup>	79.97±0.10	84.91±0.56	4.86±0.14 <sup>bc</sup>
HPMC1.0%	75.82±0.05 <sup>ab</sup>	79.91±0.45	84.72±1.10	4.73±0.21 <sup>bcd</sup>
HPMC1.5%	75.87±0.05 <sup>ab</sup>	80.02±0.19	85.35±0.78	5.60±0.56 <sup>a</sup>
GG0.5%	75.59±0.26 <sup>b</sup>	79.70±0.41	84.89±0.58	4.46±0.19 <sup>cde</sup>
GG1.0%	75.61±0.59 <sup>b</sup>	79.76±0.49	85.05±0.52	4.08±0.33 <sup>de</sup>
GG1.5%	75.73±0.06 <sup>b</sup>	79.74±0.18	84.98±0.42	5.84±0.11 <sup>a</sup>

Mean±SD of three replicates

Means within the same column followed by different letters are significantly different ( $p \leq 0.05$ ).

<sup>ns</sup> Means within the column are not significantly different ( $p > 0.05$ ).

### 4.2.3 Temperature and enthalpy of amylopectin crystallite melting

During storage, starch molecules of gelatinized starch rearrange and form hydrogen bonds between the starch chains to form a new crystalline structure from an initially amorphous or disordered state through the process of retrogradation. Long-term retrogradation is a result of the recrystallization of amylopectin which takes longer time to retrograde due to its branched structure as compared to amylose which has a linear structure (Fu et al., 2015). Therefore, the higher the enthalpy of retrogradation means that there is higher energy required to melt the amylopectin crystallite which means higher retrogradation.

Table 4.4 presents the enthalpy required to melt the amylopectin crystallites ( $\Delta H_R$ ) of banana flour with and without hydrocolloids. Generally, the addition of hydrocolloids did not cause a significant effect on the  $\Delta H_R$  or starch retrogradation ( $p > 0.05$ ) in all the days of storage. Some hydrocolloids, like GA at 0.5% and GG at 1.5%, even resulted in a significant increase in retrogradation, clearly shown in Day 7 ( $p \leq 0.05$ ). This promotion of retrogradation can be evidence of the association of hydrocolloids with amylopectin molecules in which the hydrocolloids act as an adhesive between starch molecules (BeMiller, 2011).

For Day 10, there were no significant differences between the banana flour with and without the addition of hydrocolloids ( $p > 0.05$ ) except for GG at 1.5% concentration which showed a significant increase in the  $\Delta H_R$  corresponding with the results from Day 7. On the other hand, XG at 1.5% resulted in the lowest  $\Delta H_R$  which is in agreement with a study done by He et al. (2015) showing that XG provided the strongest effect of  $\Delta H_R$  reduction, suggesting that it can significantly prevent retrogradation of the amylopectin molecules. This may be due to the excellent water retention property of XG that increases the viscosity of starch molecules, thereby delaying the mobility rate of the starch chains. This does not prevent the retrogradation process but delays it because we can see that Day 14 showed no significant differences between banana flour with and without the addition of hydrocolloids ( $p > 0.05$ ). This can be an implication that banana flour quickly retrograded and might fully retrograde before 14 days of storage at 4°C. This is

expected because Huang et al. (2019) mentioned that tropical fruit flours, especially that from banana, exhibited a dramatically greater  $\Delta H_R$  than commercial wheat flour which indicates a high propensity of the reassociation of amylopectin molecules of banana flour during storage. However, Roman et al. (2019) suggested that starch retrogradation greatly contributes to higher  $\Delta H_R$  values of flours but that does not necessarily influence the texture during storage negatively.



Table 4. 4 Enthalpy of amylopectin crystallite melting ( $\Delta H_R$ ) of banana flour with and without added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG) at 0.5, 1.0, and 1.5% stored at 4°C for 7, 10, and 14 days

Samples	$\Delta H_R$ (J/g)		
	Day 7	Day 10	Day 14 <sup>ns</sup>
Control	4.08±0.03 <sup>c</sup>	4.70±1.08 <sup>ab</sup>	4.34±0.93
XG0.5%	5.07±0.53 <sup>abc</sup>	4.69±1.29 <sup>ab</sup>	4.64±0.26
XG1.0%	4.55±0.64 <sup>abc</sup>	4.25±0.61 <sup>ab</sup>	5.53±0.13
XG1.5%	5.05±0.17 <sup>abc</sup>	3.51±0.49 <sup>b</sup>	4.93±0.36
GA0.5%	5.15±0.19 <sup>ab</sup>	4.94±1.04 <sup>ab</sup>	4.88±1.13
GA1.0%	4.12±0.51 <sup>bc</sup>	4.69±1.06 <sup>ab</sup>	5.60±1.31
GA1.5%	4.86±0.61 <sup>abc</sup>	4.33±0.31 <sup>ab</sup>	5.48±2.24
HPMC0.5%	4.46±0.31 <sup>abc</sup>	4.66±0.41 <sup>ab</sup>	5.86±1.31
HPMC1.0%	4.38±0.64 <sup>abc</sup>	4.23±0.36 <sup>ab</sup>	5.96±1.55
HPMC1.5%	4.47±0.19 <sup>abc</sup>	5.32±0.41 <sup>ab</sup>	4.70±2.05
GG0.5%	4.50±0.01 <sup>abc</sup>	5.30±0.95 <sup>ab</sup>	4.87±0.88
GG1.0%	4.10±4.35 <sup>bc</sup>	4.72±0.76 <sup>ab</sup>	5.72±2.86
GG1.5%	5.21±0.52 <sup>a</sup>	5.75±0.01 <sup>a</sup>	5.89±0.10

Mean±SD of three replicates

Means within the same column followed by different letters are significantly different ( $p \leq 0.05$ ).

<sup>ns</sup> Means within the column are not significantly different ( $p > 0.05$ )

#### 4.2.4 Gel firmness

The long-term development of ordered structure and starch crystallinity is considered a result of amylopectin retrogradation (Tran et al., 2001). The major limitation of banana flour is that they are highly retrograded resulting in a gel with high hardness when compared with flour from other plant sources (Zhang & Hamaker, 2012). This may be due to a greater ability of banana flour gel to re-crystallize due to longer polymer chains of amylopectin which enabled more crystallization junction zones (Kongolo et al., 2017). Hence, banana flour is still underutilized, particularly on an industrial scale. Firmness of banana flour gel with and without hydrocolloids is shown in Table 4.5. The concentration as well as the type of hydrocolloids posed a major effect on gel firmness.

For freshly prepared gels (Day 0), types and concentrations of hydrocolloids minimally affected the gel firmness. XG at 1.5% showed the lowest firmness while HPMC at 1.0% exhibited the greatest firmness value. However, they were not significantly different from the control ( $p>0.05$ ).

Upon storage, the addition of hydrocolloids seemed to give rise to the changes in gel firmness. The effects are dependent on the type as well as the concentration of the hydrocolloids. XG at 0.5% decreased banana flour gel firmness while at 1.0 and 1.5% concentration XG caused an increase in gel firmness. The increase in hardness may be due to the immobilization of water molecules with the addition of hydrocolloids which results in an increase in the effective concentration of starch (Gałkowska et al., 2014). Moreover, as the concentration increased, the internal bonds may be stronger due to more formation of hydrogen bonds between the hydrocolloids with the dissolved starch polymers leading to greater resistance to structural disintegration which leads to increased hardness (Da Silva Costa et al., 2020).

GA at 1.5% significantly reduced the firmness but the hydrocolloid did not show an effect at lower concentrations. HPMC at 0.5% resulted in lower gel firmness than at higher concentrations. GG greatly decreased the gel firmness at the

concentration of 1.0% but slightly increased or had no effect on the firmness at other concentrations when compared with the control. The decrease in hardness in higher concentrations may be due to the more interactions between amylose and hydrocolloid which inhibited starch retrogradation with a higher degree of crystallinity in amylose (Dangi et al., 2019).

To summarize, XG at 0.5%, GA at 1.5%, HPMC at 0.5%, and GG at 1.0% resulted in the lowest gel firmness. These trends of all of the hydrocolloids are observed in all of the days of storage (Days 7, 10, and 14). Therefore, these results signify the effect of hydrocolloid concentration on the gel firmness as also reported by BeMiller (2011). Additionally, different types of hydrocolloids have different effects on the gel firmness because they are so structurally diverse and possess different functional properties.

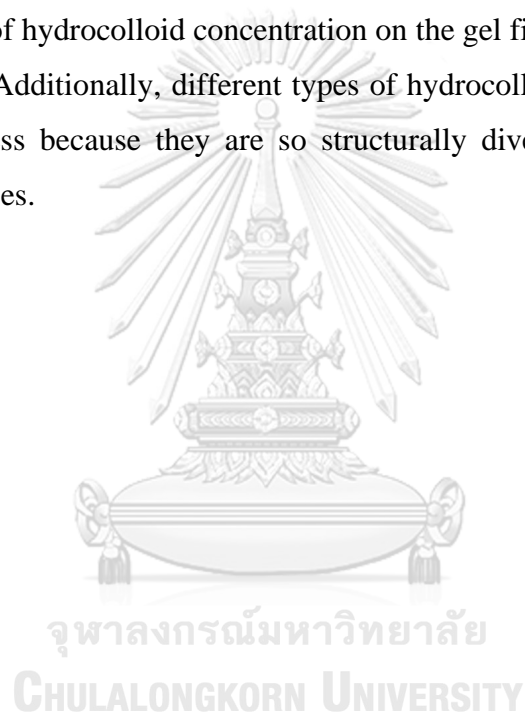




Table 4. 5 Firmness of freshly prepared banana flour gel with added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG) at 0.5, 1.0, and 1.5% and of the gels stored at 4°C for 7, 10, and 14 days

Samples	Gel firmness (N)			
	Day 0	Day 7	Day 10	Day 14
Control	0.18±0.00 <sup>ab</sup>	0.62±0.08 <sup>cde</sup>	0.66±0.17 <sup>bc</sup>	0.78±0.03 <sup>abc</sup>
XG0.5%	0.21±0.00 <sup>ab</sup>	0.57±0.06 <sup>de</sup>	0.58±0.07 <sup>cd</sup>	0.52±0.02 <sup>e</sup>
XG1.0%	0.19±0.00 <sup>ab</sup>	0.81±0.00 <sup>a</sup>	0.82±0.00 <sup>a</sup>	0.83±0.01 <sup>a</sup>
XG1.5%	0.15±0.01 <sup>b</sup>	0.73±0.03 <sup>ab</sup>	0.75±0.02 <sup>ab</sup>	0.79±0.02 <sup>ab</sup>
GA0.5%	0.15±0.01 <sup>ab</sup>	0.69±0.05 <sup>bc</sup>	0.68±0.03 <sup>bc</sup>	0.70±0.04 <sup>cd</sup>
GA1.0%	0.17±0.01 <sup>b</sup>	0.72±0.04 <sup>b</sup>	0.71±0.02 <sup>b</sup>	0.76±0.03 <sup>abc</sup>
GA1.5%	0.17±0.00 <sup>ab</sup>	0.33±0.01 <sup>g</sup>	0.33±0.01 <sup>e</sup>	0.43±0.08 <sup>f</sup>
HPMC0.5%	0.17±0.01 <sup>ab</sup>	0.54±0.01 <sup>e</sup>	0.53±0.00 <sup>d</sup>	0.57±0.01 <sup>e</sup>
HPMC1.0%	0.25±0.15 <sup>a</sup>	0.66±0.05 <sup>bc</sup>	0.68±0.02 <sup>bc</sup>	0.72±0.05 <sup>bc</sup>
HPMC1.5%	0.17±0.01 <sup>ab</sup>	0.69±0.04 <sup>bc</sup>	0.67±0.04 <sup>bc</sup>	0.75±0.04 <sup>bc</sup>
GG0.5%	0.18±0.01 <sup>ab</sup>	0.81±0.06 <sup>a</sup>	0.70±0.04 <sup>b</sup>	0.76±0.06 <sup>abc</sup>
GG1.0%	0.16±0.00 <sup>ab</sup>	0.43±0.00 <sup>f</sup>	0.40±0.04 <sup>e</sup>	0.41±0.01 <sup>f</sup>
GG1.5%	0.18±0.00 <sup>ab</sup>	0.63±0.02 <sup>cd</sup>	0.64±0.01 <sup>bc</sup>	0.65±0.01 <sup>d</sup>

Mean±SD of three replicates

Means within the same column followed by different letters are significantly different ( $p \leq 0.05$ ).

### **4.3 Application of banana flour with selected hydrocolloids as a wheat flour-substitute in bread production**

According to the results from the previous study (4.2.4), XG at 0.5%, GA at 1.5%, HPMC at 0.5%, and GG at 1.0% were selected for the application in bread. Bread without added hydrocolloid was used as a control.

#### **4.3.1 Moisture content and water activity**

The residual moisture in bread can affect the overall texture including the crumb softness and crust crispiness as well as the shelf-life of the bread (Rogers et al., 1988). The relationship between bread staling and moisture content is essentially the connection between the staling rate and moisture loss rate (He & Hoseney, 1990). Past research indicated that the hardening process during storage was due to water loss from water migration from the bread crumb to crust (Piazza & Masi, 1995).

According to Table 4.6, freshly baked breads (Day 0) showed that control has the lowest moisture content which reflects the hydrocolloids' ability to bind with water molecules due to its hydrophilic nature. The interaction between hydrocolloids' hydroxyl groups and water through hydrogen bonding may prevent or delay the diffusion phenomena of moisture from crumb to crust (Tavakoli et al., 2017). The same results were observed by Liu et al. (2020) and Kang et al. (2018) which reported that the addition of hydrocolloids slightly increased the moisture content of bread crumb in freshly baked breads.

No significant differences were observed in the moisture content of all bread samples as the storage time increases on Day 7, 10, and 14 ( $p > 0.05$ ). However, Day 10 bread samples showed that the control breads have the highest moisture content while the addition of hydrocolloids displayed a slight decrease. Kang et al. (2018) showed similar results for their bread samples during Day 10 storage. This may be explained by the moisture migration of the freshly baked control breads to the bread crumb to reach an equilibrium state during storage. On the other hand, breads with hydrocolloids did not show any change in the moisture content due to the

interaction of hydrocolloids with water which changes the water mobility of the system preventing moisture migration.

Table 4. 6 Moisture content of banana flour-substituted bread with and without added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG)

Samples	Moisture Content			
	Day 0	Day 7 <sup>ns</sup>	Day 10	Day 14 <sup>ns</sup>
Control	38.95±0.80 <sup>b</sup>	37.28±0.32	38.96±2.94 <sup>a</sup>	36.04±0.31
XG	39.96±0.80 <sup>ab</sup>	37.00±1.20	37.55±0.47 <sup>ab</sup>	36.63±0.30
GA	40.03±0.15 <sup>ab</sup>	37.14±0.18	35.64±0.58 <sup>b</sup>	35.64±1.02
HPMC	40.83±0.28 <sup>a</sup>	36.19±0.90	36.12±0.41 <sup>b</sup>	35.98±0.78
GG	39.46±0.73 <sup>b</sup>	36.43±1.25	37.24±0.53 <sup>ab</sup>	36.15±0.29

Mean±SD of three replicates

Means within the same column followed by different letters are significantly different ( $p \leq 0.05$ ).

<sup>ns</sup> Means within the column are not significantly different ( $p > 0.05$ ).

Water activity is another important factor that affects the bread quality because it associates with the amount of free water in food products which can be used for physical, chemical, and microbiological changes. Table 4.7 shows the water activity of banana flour-substituted bread with and without hydrocolloids.

For the bread at Day 0, water activity was found to be almost unaffected by hydrocolloid addition. During storage, there was just a slightest difference between the water activity of breads with and without hydrocolloids observed. This may be due to the hydrophilic nature of both hydrocolloids and starch (Rosell et al., 2001).

Table 4. 7 Water activity of banana flour-substituted bread with and without added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG)

Samples	Water activity			
	Day 0 <sup>ns</sup>	Day 7 <sup>ns</sup>	Day 10 <sup>ns</sup>	Day 14 <sup>ns</sup>
Control	0.96±0.00	0.95±0.00	0.95±0.00	0.95±0.00
XG	0.97±0.00	0.96±0.00	0.96±0.00	0.96±0.00
GA	0.97±0.00	0.96±0.00	0.95±0.00	0.95±0.00
HPMC	0.96±0.00	0.96±0.00	0.95±0.00	0.96±0.00
GG	0.96±0.00	0.95±0.00	0.96±0.00	0.96±0.00

Mean±SD of three replicates

<sup>ns</sup> Means within the column are not significantly different ( $p>0.05$ ).

### 4.3.2 Specific loaf volume

Specific loaf volume is one of the important characteristics of bread in determining consumer acceptance and product quality (Das et al., 2015). The specific loaf volume of the bread samples with and without hydrocolloids is shown in Table 4.8.

As for the freshly baked bread samples (Day 0), there were no significant differences ( $p>0.05$ ) in the specific loaf volume of the breads with and without hydrocolloids. On Day 7, GA resulted in the highest specific loaf volume which may be due to its anionic and branched structure that allows it to form a network or aggregate with the gluten in dough (Kang et al., 2018). The result could be an increase in loaf volume and improvement in the texture of bread as reported by Ribotta et al. (2004). However, from the results of Days 10 and 14, the addition of GG resulted in the highest specific loaf volume. Kang et al. (2018) also reported that GG

was the most effective in improving the loaf volume. The mechanism of this improvement to the specific loaf volume of breads is explained by the hydrocolloids ability to influence gluten hydration which facilitates more gas to be trapped in the dough thereby increasing the overall loaf volume (Mikuš et al., 2014; Ribotta et al., 2004).

Table 4. 8 Specific loaf volume of banana flour-substituted bread with and without added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG)

Samples	Specific loaf volume (cm <sup>3</sup> /g)			
	Day 0 <sup>ns</sup>	Day 7	Day 10	Day 14
Control	3.65±0.30	2.73±0.16 <sup>ab</sup>	3.04±0.25 <sup>ab</sup>	3.20±0.31 <sup>ab</sup>
XG	3.23±0.23	2.80±0.41 <sup>ab</sup>	2.82±0.08 <sup>abc</sup>	3.07±0.43 <sup>ab</sup>
GA	3.79±0.39	3.05±0.10 <sup>a</sup>	2.71±0.26 <sup>bc</sup>	3.41±0.28 <sup>ab</sup>
HPMC	3.54±0.35	2.34±0.29 <sup>b</sup>	2.45±0.14 <sup>c</sup>	2.89±0.46 <sup>b</sup>
GG	3.55±0.21	2.64±0.24 <sup>ab</sup>	3.11±0.17 <sup>a</sup>	3.58±0.10 <sup>a</sup>

Mean±SD of three replicates

Means within the same column followed by different letters are significantly different ( $p \leq 0.05$ ).

<sup>ns</sup> Means within the column are not significantly different ( $p > 0.05$ ).

On the other hand, bread samples with the addition of HPMC were not able to retain its volume and has the lowest specific loaf volume when compared with other hydrocolloids during storage seen on Days 7, 10, and 14. McCarthy et al. (2005) also mentioned that HPMC caused a decrease in specific volume of bread. Similarly, Rodriguez-Sandoval et al. (2015) also reported that GG increased the specific loaf volume while GG/HPMC blend did not produce a high specific loaf volume. This

may be due to its linear non-ionic nature which suggests that the branched or anionic properties of other hydrocolloids may play a role in retaining the specific loaf volume.

Moreover, the effects of XG addition are noteworthy to mention due to its consistent decrease in specific loaf volume when compared with the control in freshly prepared breads (Day 0) and all the days of storage (Day 7, 10, and 14). The same results were reported by Demirkesen et al. (2010) which explained that XG makes the dough system too rigid to incorporate gases resulting in low specific volume values. Schober et al. (2005) also mentioned the decrease in loaf volume with increasing XG levels.

#### **4.3.3 Texture profile analysis**

The parameters of the texture profile analysis can be an indication of the quality of food products as well as provide an insight on how samples behave when chewed. In the case of bread samples, the hardening of crumb can indicate the retrogradation of starch and moisture loss (Gray & Bemiller, 2003). Additionally, adhesivity and cohesiveness are attributes that can also indirectly reflect the number of unbound amyloses. Directional migration of amylose which results in adhesivity decline and an increase in cohesiveness can be indicators of starch retrogradation (He et al., 2014). Springiness of crumb in bread reflects its ability to return to its original state after deformation under certain pressure which usually decreases as the bread undergoes staling (Kang et al., 2018; Liu et al., 2020). Gumminess can be calculated from the hardness and cohesiveness values from the bread crumb. Chewiness is the amount of energy required to simulate consumption in which the value is related to hardness and springiness (Liu et al., 2020).

For freshly baked bread samples (Day 0), Table 4.9 shows that the addition of GA resulted in the lowest hardness and gumminess which may be due to its branched anionic structure that hampers the amylose molecular gathering and rearrangement thereby weakening the interaction between amylose molecules (Kulicke et al., 1996). GA has also been reported to exhibit high elasticity and stability in starch system (Singh et al., 2017). However, GA also resulted in the lowest

adhesiveness and highest cohesiveness which suggests the directional migration of amylose molecules or short-term retrogradation. The addition XG significantly resulted in the highest gumminess ( $p \leq 0.05$ ) which was also reported by Vidaurre-Ruiz et al. (2019) who determined the influence of tara gum and XG on the textural properties of starch-based gluten-free dough and bread. The hydrocolloids did not significantly affect the adhesiveness, springiness, and chewiness of bread samples ( $p > 0.05$ ).

Again, at Day 7, the addition of GA resulted in the lowest hardness values. However, the values are not significantly different from the sample without hydrocolloid addition ( $p > 0.05$ ). The hardening of the crumb appeared more significant in the early stages of storage than longer storage which was also mentioned by Kang et al. (2018). However, Table 4.10 shows an increase in all hardness values for all bread samples when compared with the values from Day 0. The addition of HPMC and GG significantly increased the hardness of bread crumbs ( $p \leq 0.05$ ). It was previously reported by that HPMC and GG significantly increased the setback of the system which indicated the addition of these hydrocolloids may promote starch retrogradation (Funami et al., 2005). Other parameters which include cohesiveness, springiness, gumminess, and chewiness showed no significant difference from the control ( $p > 0.05$ ).

For Day 10, the addition of XG and GG showed the lowest values of hardness but values are not significantly different from the control ( $p > 0.05$ ). He et al. (2015) indicated that the addition of XG could prevent the recrystallization of amylopectin or long term retrogradation which causes hardening of bread crumb during this period of storage. On the other hand, the addition of guar gum was reported to delay the staling of bread through a softening effect which is probably caused by inhibition of amylopectin retrogradation as GG prefers to bind with starch (Rodriguez-Sandoval et al., 2015). The addition of GA and HPMC, on the contrary, resulted in significantly higher ( $p \leq 0.05$ ) hardness values than that of the control which can be observed in Table 4.11. Gumminess and chewiness also showed the same trend as hardness which is also observed with a study done by Liu et al. (2020). This shows the ability of the addition of GA to delay retrogradation up to a certain period of time

as seen in Day 7 but promotes it as time exceeds. Moreover, there may be a correlation to the specific volume of HPMC-added samples because smaller loaves are denser and tightly packed crumb structure which can result in higher crumb hardness values (Sabanis & Tzia, 2011).

After 14 days of storage, there are no significant differences ( $p>0.05$ ) in all attributes of all the bread samples which suggest the effects of long-term retrogradation can only be delayed to an extent as shown in Table 4.12. However, all of the hardness values of bread samples with hydrocolloids are lower than that of the control even though it is not significantly different ( $p>0.05$ ). This finding can indicate that the interaction between hydrocolloids and starch can hamper the formation of cross-links between amylopectin molecules even though the effects may be minimal.

It is important to note that there are many factors that can affect the texture profile of bread crumb such as water migration, amylose recrystallization, starch-gluten protein interaction as well as storage conditions (Liu et al., 2020).



Table 4. 9 Texture profile of banana flour-substituted bread with and without added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG) at Day 0

Day 0	Texture Profile						
	Samples	Hardness	Adhesiveness <sup>ns</sup>	Cohesiveness	Springiness <sup>ns</sup>	Gumminess	Chewiness <sup>ns</sup>
Control	893.37±187.59 <sup>a</sup>	-0.75±0.67	0.62±0.24 <sup>b</sup>	0.50±0.03	554.48±81.89 <sup>ab</sup>	284.63±63.40	
XG	896.60±224.41 <sup>a</sup>	-1.70±1.51	0.64±0.01 <sup>ab</sup>	0.50±0.03	578.91±152.30 <sup>a</sup>	294.37±101.82	
GA	590.09±54.56 <sup>b</sup>	-0.15±0.26	0.69±0.02 <sup>a</sup>	0.57±0.02	408.16±20.46 <sup>b</sup>	233.26±20.10	
HPMC	942.05±72.80 <sup>a</sup>	-0.56±0.97	0.51±0.03 <sup>c</sup>	0.51±0.00	486.66±50.95 <sup>ab</sup>	249.16±29.60	
GG	706.57±84.04 <sup>ab</sup>	-0.22±0.19	0.59±0.02 <sup>b</sup>	0.56±0.06	418.69±54.79 <sup>ab</sup>	237.21±57.37	

Mean±SD of three replicates

Means within the same column followed by different letters are significantly different ( $p \leq 0.05$ ).

<sup>ns</sup> Means within the column are not significantly different ( $p > 0.05$ ).

Table 4. 10 Texture profile of banana flour-substituted bread with and without added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG) at Day 7

Day 7	Texture Profile						
	Samples	Hardness	Adhesiveness	Cohesiveness <sup>ns</sup>	Springiness <sup>ns</sup>	Gumminess <sup>ns</sup>	Chewiness <sup>ns</sup>
	Control	1822.31±414.03 <sup>b</sup>	-0.64±1.12 <sup>a</sup>	0.37±0.04	0.42±0.02	664.85±97.05	280.73±44.41
	XG	2006.34±781.91 <sup>b</sup>	-0.84±1.45 <sup>a</sup>	0.39±0.01	0.45±0.04	790.91±266.46	355.15±104.35
	GA	1636.32±270.79 <sup>b</sup>	-0.97±1.57 <sup>a</sup>	0.38±0.05	0.47±0.03	629.73±138.37	293.88±44.85
	HPMC	2937.83±595.74 <sup>a</sup>	-3.71±2.12 <sup>b</sup>	0.24±0.18	0.42±0.03	683.95±520.87	286.88±206.46
	GG	1815.75±255.74 <sup>a</sup>	0±0.00 <sup>a</sup>	0.36±0.00	0.43±0.01	660.91±87.51	284.83±34.40

Mean±SD of three replicates

Means within the same column followed by different letters are significantly different ( $p \leq 0.05$ ).

<sup>ns</sup> Means within the column are not significantly different ( $p > 0.05$ ).

Table 4. 11 Texture profile of banana flour-substituted bread with and without added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG) at Day 10

Day 10	Texture Profile						
	Samples	Hardness	Adhesiveness <sup>ns</sup>	Cohesiveness <sup>ns</sup>	Springiness	Gumminess	Chewiness
Control	1834.28±313.28 <sup>b</sup>	-1.04±0.99	0.33±0.05	0.48±0.01 <sup>ab</sup>	599.23±23.41 <sup>bc</sup>	289.41±19.34 <sup>ab</sup>	
XG	1691.34±79.12 <sup>b</sup>	-0.28±0.49	0.33±0.04	0.44±0.03 <sup>ab</sup>	568.09±81.04 <sup>bc</sup>	253.38±56.69 <sup>ab</sup>	
GA	2447.58±565.26 <sup>a</sup>	-1.29±2.23	0.29±0.04	0.50±0.05 <sup>a</sup>	723.00±139.65 <sup>ab</sup>	368.25±98.58 <sup>a</sup>	
HPMC	2817.3±153.575 <sup>a</sup>	-0.02±0.04	0.31±0.05	0.42±0.01 <sup>b</sup>	883.46±117.37 <sup>a</sup>	374.98±65.07 <sup>a</sup>	
GG	1482.00±270.63 <sup>b</sup>	-0.59±1.02	0.30±0.03	0.44±0.01 <sup>ab</sup>	455.21±88.22 <sup>c</sup>	203.71±45.66 <sup>b</sup>	

Mean±SD of three replicates

Means within the same column followed by different letters are significantly different ( $p \leq 0.05$ ).

<sup>ns</sup> Means within the column are not significantly different ( $p > 0.05$ ).

Table 4. 12 Texture profile of banana flour-substituted bread with and without added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG) at Day 14

Day 14		Texture Profile					
Samples	Hardness <sup>ns</sup>	Adhesiveness <sup>ns</sup>	Cohesiveness <sup>ns</sup>	Springiness <sup>ns</sup>	Gumminess <sup>ns</sup>	Chewiness <sup>ns</sup>	
Control	2474.99±59.71	-2.55±2.54	0.45±0.25	0.39±0.02	1120.61±637.74	456.88±289.25	
XG	2203.89±895.62	-0.00±0.00	0.32±0.02	0.35±0.05	726.77±308.88	258.91±104.52	
GA	2423.50±1254.72	-1.06±1.83	0.29±0.05	0.40±0.01	764.46±543.53	311.14±234.13	
HPMC	1991.68±786.53	-1.35±2.35	0.33±0.02	0.37±0.04	658.00±228.99	249.37±102.52	
GG	1686.99±90.52	-1.60±2.78	0.41±0.13	0.33±0.03	706.65±261.36	240.45±105.34	

Mean±SD of three replicates

Means within the same column followed by different letters are significantly different ( $p \leq 0.05$ ).

<sup>ns</sup> Means within the column are not significantly different ( $p > 0.05$ ).

#### 4.3.4 Water-soluble starch content

Gelatinized starch is known to eventually form crystalline structures (Miles et al., 1985). This crystallite formation is accompanied by increase in rigidity and phase separation between the polymer and solvent through the process called syneresis (Colonna et al., 1992). The crystallites formed lose their solubility and become insoluble in water when compared to their native form (Ghodke, 2009). As time increases, the amount of crystallites increase which results in decrease water-soluble starch. Therefore, a lower value of water-soluble starch content ultimately signifies higher retrogradation.

Table 4.13 represents the water-soluble starch content of bread samples with and without hydrocolloids. Freshly baked breads (Day 0) showed that the addition of HPMC and GG have the highest water-soluble starch but is not significantly different than that of the control ( $p>0.05$ ). On the contrary, the addition of XG and GA significantly reduced the amount of water-soluble starch which indicates a high amount of crystallite formation or retrogradation. The anionic structures of XG and GA may promote crystallite formation which decreases the water-soluble starch content. Maleki et al. (2012) also mentioned that the amount of water-soluble starch showed a significant decrease with XG addition. Moreover, Fu & BeMiller (2017) reported that the presence of XG promoted overall retrogradation in maize starch gel samples.

During storage, it can be seen on Day 7 that the water-soluble starch content of the bread samples with the addition of HPMC and GG became significantly higher than the control ( $p\leq 0.05$ ). Maleki et al. (2012) also mentioned the effect of cellulose derivatives on retaining the amount of water-soluble was more obvious when compared to other hydrocolloids. Furthermore, Ghodke (2009) reported the ability of GG to weaken the starch structure to provoke water distribution as well as decrease in crumb resistance resulting in higher water-soluble starch content. This proves the ability of these hydrocolloids to prevent starch retrogradation which can be seen in values from Days 10 and 14 as well even though the results are no longer significantly different than the control ( $p>0.05$ ). HPMC and GG are both nonionic

which may be the reason it does not promote crystallite formation and may even act as steric hindrance to prevent it.

As for the addition of XG and GA during storage, the water-soluble starch content of bread samples is lower than the control but the values are not significantly different on Day 7 ( $p \leq 0.05$ ). However, it became significantly lower than the control ( $p > 0.05$ ) on Days 10 and 14 which shows the same trend as freshly baked breads.

Table 4. 13 Water-soluble starch content of banana flour-substituted bread with and without added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG)

Samples	Water-soluble starch content (% w/w)			
	Day 0	Day 7	Day 10	Day 14
Control	4.54±1.11 <sup>a</sup>	1.46±0.26 <sup>b</sup>	1.23±0.23 <sup>abc</sup>	1.25±0.20 <sup>a</sup>
XG	2.87±0.29 <sup>b</sup>	1.05±0.34 <sup>b</sup>	0.75±0.10 <sup>c</sup>	0.72±0.10 <sup>b</sup>
GA	4.28±1.23 <sup>b</sup>	1.40±0.05 <sup>b</sup>	1.09±0.08 <sup>bc</sup>	0.73±0.06 <sup>b</sup>
HPMC	5.19±0.30 <sup>a</sup>	1.95±0.14 <sup>a</sup>	1.70±0.60 <sup>a</sup>	1.09±0.28 <sup>a</sup>
GG	5.43±0.52 <sup>a</sup>	2.03±0.35 <sup>a</sup>	1.60±0.06 <sup>ab</sup>	1.09±0.09 <sup>a</sup>

Mean±SD of three replicates

Means within the same column followed by different letters are significantly different ( $p \leq 0.05$ ).

#### 4.3.5 Bread crumb structure

Consumer acceptance of breads is greatly influenced by crumb structure. However, this is completely subjective to a person's preference. Generally, regarding normal wheat bread, fine crumb structure is preferred (Sciarini et al., 2010). According to Figure 4.2, all of the banana-flour substituted bread samples have fairly compact bread crumb structures. The air cells are small and uniformly distributed throughout the whole slice of bread. The addition of all the hydrocolloids seems to have no effect on the crumb structure of bread samples or their effects may be dominated by the functional properties of banana flour. Aziz et al. (2011) previously reported that the partial substitution of banana pseudo-stem flour with wheat flour at 10% level can significantly decrease the physical properties such as specific volume and loaf height of bread samples. Hydrocolloids are usually added to bakery products to improve their shelf life or retard staling by the retention of water (Collar et al., 1999). However, there were no obvious differences seen in the bread crumb structure of bread samples with or without the addition of hydrocolloids at all of the storage days.



Figure 4. 1 Bread crumb structure of banana flour-substituted bread with and without added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG)



#### 4.3.6 Color

Color is another important characteristic for baked product because it can contribute greatly to consumer perception considering that it is visually seen. According to the CIELAB scale,  $L^*$  values signifies lightness with 100=white and 0=black,  $a^*$  values: +ve=red and -ve=green and  $b^*$  values: +ve=yellow and -ve=blue. Hue angle is the common distinction between colors positioned around a color wheel. Chroma may be defined as the strength of the hue or the color intensity.

As seen in Tables 4.14-4.17, the color parameters of banana flour-substituted bread with and without the addition of hydrocolloids are quite similar throughout all the days of storage. The  $L^*$  values or the lightness of bread samples are in the range of 50.69 to 58.33. The  $a^*$  values are in the range between 3.06 to 3.73. As for the  $b^*$  values, it ranges from 9.35 to 11.61. Moreover, the hue angle ranges between 69.77 to 73.64 indicates a yellowish-red hue. The chroma is in the range of 9.91 to 12.20. Although the significant differences of the color parameters will be explained in further detail later, the range of the values shown from all of the tables indicate that the effects of the hydrocolloids are not too drastic or may be dominated by the functional properties of banana flour.

However, the addition of HPMC seems to be able to significantly affect the color parameters of bread samples. For instance, the addition of HPMC resulted in significantly darker crumb color in freshly baked samples and Day 14 storage which was also reported by Sabanis & Tzia (2011) that HPMC-containing breads had significantly darker crumb color.

It is important to note that color of bread depends on many factors such as the physicochemical characteristic of the dough such as water content, pH, reducing sugars and amino acid content as well as the operating conditions during the baking process which include temperature, relative humidity, and modes of heat transfer (Esteller & Lannes, 2008).

The color parameters of freshly baked breads (Day 0) can be seen in Table 4.14. The addition of hydrocolloids have slightly lower L\* values which means that it is darker than the control. The a\* values were not significantly different ( $p>0.05$ ) for all of the bread samples and is in the range of 3.29 to 3.44. The addition of HPMC significantly increased the b\* values while the addition of other hydrocolloids significantly decreased the value when compared with the control ( $p\leq 0.05$ ). As for the hue angle, the same trend is seen with the b\* values with the addition of HPMC increasing the value. The chroma of HPMC-added samples is also the highest but it is not significantly different than that of the control ( $p>0.05$ ).

Table 4. 14 Color values of banana flour-substituted bread with and without added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG) at Day 0

Day 0	CIELAB				
	Sample	L*	a* <sup>ns</sup>	b*	Hue angle (°)
Control	58.33±0.53 <sup>a</sup>	3.40±0.10	10.11±0.14 <sup>b</sup>	71.42±0.54 <sup>b</sup>	10.66±0.14 <sup>a</sup>
XG	56.84±1.21 <sup>ab</sup>	3.29±0.15	9.51±0.19 <sup>c</sup>	70.86±0.63 <sup>bc</sup>	10.06±0.21 <sup>b</sup>
GA	56.46±1.10 <sup>ab</sup>	3.44±0.03	9.36±0.39 <sup>c</sup>	69.77±0.71 <sup>c</sup>	9.98±0.37 <sup>b</sup>
HPMC	55.32±1.35 <sup>b</sup>	3.31±0.15	10.68±0.25 <sup>a</sup>	72.79±0.73 <sup>a</sup>	11.18±0.26 <sup>a</sup>
GG	56.36±1.12 <sup>ab</sup>	3.29±0.21	9.35±0.42 <sup>c</sup>	70.62±0.63 <sup>bc</sup>	9.91±0.46 <sup>b</sup>

Mean±SD of three replicates

Means within the same column followed by different letters are significantly different ( $p\leq 0.05$ ).

<sup>ns</sup> Means within the column are not significantly different ( $p>0.05$ ).

After 7 days of storage (Table 4.15), there were no significant differences shown in the L\* values ( $p>0.05$ ). As for the a\*, b\*, and chroma values, the addition of HPMC resulted in the highest values with significant different to other bread samples ( $p\leq 0.05$ ). However, HPMC-added samples had the lowest hue angle and GA had significantly higher hue angle than all the other bread samples ( $p\leq 0.05$ ).

Table 4. 15 Color values of banana flour-substituted bread with and without added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG) at Day 7

Day 7	CIELAB				
	Sample	L* <sup>ns</sup>	a*	b*	Hue angle (°)
Control	53.57±1.61	3.28±0.02 <sup>b</sup>	10.80±0.09 <sup>b</sup>	73.08±0.19 <sup>b</sup>	11.29±0.09 <sup>b</sup>
XG	52.24±0.93	3.32±0.07 <sup>b</sup>	10.86±0.20 <sup>b</sup>	73.01±0.11 <sup>b</sup>	11.36±0.21 <sup>b</sup>
GA	50.93±0.96	3.18±0.08 <sup>b</sup>	10.85±0.11 <sup>b</sup>	73.64±0.23 <sup>a</sup>	11.31±0.13 <sup>b</sup>
HPMC	50.69±1.16	3.56±0.10 <sup>a</sup>	11.49±0.27 <sup>a</sup>	72.79±0.16 <sup>b</sup>	12.03±0.28 <sup>a</sup>
GG	51.67±3.60	3.36±0.17 <sup>b</sup>	10.93±0.30 <sup>b</sup>	72.90±0.45 <sup>b</sup>	11.44±0.34 <sup>b</sup>

Mean±SD of three replicates

Means within the same column followed by different letters are significantly different ( $p\leq 0.05$ ).

<sup>ns</sup> Means within the column are not significantly different ( $p>0.05$ ).

The color values of bread samples after 10 days of storage are presented in Table 4.16. The control bread resulted in the highest L\* values with significant difference to other samples with hydrocolloid addition which shows a similar trend to freshly baked breads ( $p \leq 0.05$ ). The control also had the highest hue angle and HPMC-added samples had the lowest hue angle with significant difference when compared to other bread samples ( $p \leq 0.05$ ). However, there were no significant differences observed in the a\*, b\*, and chroma values of all the samples ( $p > 0.05$ ).

Table 4. 16 Color values of banana flour-substituted bread with and without added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG) at Day 10

Day 10	CIELAB				
	Sample	L*	a* <sup>ns</sup>	b* <sup>ns</sup>	Hue angle (°)
Control	57.01±1.12 <sup>a</sup>	3.22±0.13	11.22±1.44	73.51±1.02 <sup>a</sup>	11.69±1.42
XG	52.95±1.13 <sup>b</sup>	3.73±0.83	11.61±2.01	72.42±0.63 <sup>ab</sup>	12.20±2.17
GA	54.00±0.71 <sup>b</sup>	3.26±0.16	10.58±0.44	72.86±0.16 <sup>ab</sup>	11.07±0.47
HPMC	52.13±0.54 <sup>b</sup>	3.59±0.12	11.11±0.18	72.09±0.33 <sup>b</sup>	11.68±0.20
GG	54.21±1.66 <sup>b</sup>	3.18±0.05	10.51±0.17	73.19±0.05 <sup>ab</sup>	10.99±0.18

Mean±SD of three replicates

Means within the same column followed by different letters are significantly different ( $p \leq 0.05$ ).

<sup>ns</sup> Means within the column are not significantly different ( $p > 0.05$ ).

After 14 days of storage, the color values of bread samples seem to show a different trend when compared with other storage days and are shown in Table 4.17. The addition of HPMC resulted in the lowest L\* value again ( $p \leq 0.05$ ). However, the addition of GG resulted in the highest L\* value with significant difference to other samples ( $p \leq 0.05$ ). GG-added samples also had the lowest a\*, b\*, and chroma values and highest hue angle ( $p \leq 0.05$ ). On the other hand, the control showed the highest b\* and chroma values ( $p \leq 0.05$ ).

Table 4. 17 Color values of banana flour-substituted bread with and without added xanthan gum (XG), gum arabic (GA), hydroxypropyl methylcellulose (HPMC), and guar gum (GG) at Day 14

Day 14	CIELAB				
	Sample	L*	a*	b*	Hue angle (°)
Control	53.90±0.08 <sup>ab</sup>	3.48±0.17 <sup>a</sup>	10.56±0.44 <sup>a</sup>	71.73±0.42 <sup>b</sup>	11.12±0.47 <sup>a</sup>
XG	55.25±2.07 <sup>ab</sup>	3.37±0.25 <sup>a</sup>	10.27±0.30 <sup>ab</sup>	71.85±0.79 <sup>ab</sup>	10.81±0.37 <sup>ab</sup>
GA	53.40±0.58 <sup>ab</sup>	3.40±0.10 <sup>a</sup>	9.99±0.37 <sup>ab</sup>	71.17±0.18 <sup>b</sup>	10.55±0.38 <sup>ab</sup>
HPMC	52.88±0.81 <sup>b</sup>	3.40±0.01 <sup>a</sup>	9.98±0.15 <sup>ab</sup>	71.17±0.23 <sup>b</sup>	10.55±0.14 <sup>ab</sup>
GG	55.70±1.76 <sup>a</sup>	3.06±0.09 <sup>b</sup>	9.86±0.25 <sup>b</sup>	72.75±0.70 <sup>a</sup>	10.32±0.24 <sup>b</sup>

Mean±SD of three replicates

Means within the same column followed by different letters are significantly different ( $p \leq 0.05$ ).

<sup>ns</sup> Means within the column are not significantly different ( $p > 0.05$ ).

## CHAPTER 5

### CONCLUSION

Addition of different hydrocolloids provided different effects on pasting properties of banana flour. XG and GA reduced the peak viscosity while HPMC and GG increased it. All of the hydrocolloids reduced the setback viscosity which proved their potential to retard short-term retrogradation except for GG which resulted in an increase in the setback viscosity. Although hydrocolloid addition posed no significant effect on the gelatinization temperature, it was shown to increase gelatinization enthalpy. Regarding retrogradation, the hydrocolloids used in this study did not show the retrogradation-retarding effect. This may be due to that banana starch is highly retrograded as it seemed to become fully retrograded after 7 days of storage. According to the gel firmness, the optimum concentration of the hydrocolloids with the least firmness is XG at 0.5%, GA at 1.5%, HPMC at 0.5%, and GG at 1.0% during storage. Other concentrations may cause an increase in firmness or have no effect at all.

The application of bread shows that the different hydrocolloids have different effects on different attributes concerning bread quality. HPMC showed the highest significance when it comes to moisture retention. As for specific loaf volume, HPMC-added bread samples resulted in the lowest specific loaf volume during storage while GA and GG had the highest specific loaf volume. Regarding the texture profile, the addition of GA resulted in the lowest hardness and gumminess for freshly baked breads. During storage, XG and GG showed lowest hardness while GA and HPMC increased the hardness. As for water-soluble starch content, HPMC resulted in the highest values throughout all the days which prove its ability to prevent retrogradation. Nonetheless, the addition of hydrocolloids did not seem to affect the bread crumb structure and resulted in a compact structure with small and uniform distribution of air cells. The same is seen with the colors of the bread samples in which some hydrocolloids may affect some color parameters but the range of results are not too drastic that show obvious differences.

To summarize, the hydrocolloids used in this study were found to modify pasting properties and short-term retrogradation of banana flour to some extent. In spite of that, the hydrocolloids showed no effect on long-term retrogradation. However, hydrocolloids could influence starch gelatinization and retrogradation by changing the intermolecular or intramolecular forces. Therefore, different hydrocolloids at different concentrations can affect pasting behavior and gelling properties of banana flour in various ways thereby affecting the different attributes of product quality. This research serves as a guide to select the optimum type and concentration of hydrocolloid to suit the different characteristics of banana flour required for specific purposes.





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**PUBLICATION** Herman, R. & Mahawanich, T. (2021). Pasting, gelatinization, and retrogradation of banana flour as affected by hydrocolloid incorporation. Full paper in the Proceedings of the Food Innovation Asia Conference 2021 (FIAC2021), 17-18 June 2021