EFFECTS OF SOLUBLE AND INSOLUBLE FIBRES ON PASTING AND RETROGRADATION OF WHEAT FLOUR AND QUALITY OF BREAD

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ผลของเส้นใยที่ละลายน้ำได้และละลายน้ำไม่ได้ต่อการเกิดเพสต์และรีโทรเกรเดชันของแป้งสาลี
และคุณภาพของขนมปัง

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อาราตี บัททา : ผลของเส้นใยที่ละลายน้ำได้และละลายน้ำไม่ได้ต่อการเกิดเพสต์และรีโทรเกรเดชันของแป้งสาลีและคุณภาพของขนมปัง.
(EFFECTS OF SOLUBLE AND INSOLUBLE FIBRES ON PASTING AND RETROGRADATION OF WHEAT FLOUR AND QUALITY OF BREAD) อ.ที่ปรึกษาหลัก : ธนจันทร์ มหาวนิช, อ.ที่ปรึกษาร่วม : ศิริมา พ่วงประพันธ์

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของเส้นใยต่อการเกิดเพสต์และการเกิดรีโทรเกรเดชันทั้งในระยะสั้นและระยะยาวของสตาร์ชในแป้งสาลี และเพื่อศึกษาคุณภาพของขนมปังที่เติมเส้นใย โดยศึกษาผลของการเติมเส้นใยที่ละลายน้ำได้ 2 ชนิด ได้แก่ ทารากัม (TG) และแคปปา-คารา จีแนน (CAR) และเส้นใยที่ละลายน้ำไม่ได้ 1 ชนิด ได้แก่ เซลลูโลส (CEL) แปรความเข้มข้นของเส้นใยเป็น 3 ระดับ ได้แก่ 1.0, 1.5 และ 2.0% สำหรับพฤติกรรมการเกิดเพสต์พบว่าการเติมเส้นใยที่ละลายน้ำได้ทำให้ peak viscosity, trough viscosity, breakdown viscosity และ final viscosity มีค่าเพิ่มขึ้น ในขณะที่ setback viscosity และ pasting temperature มีค่าลดลง ในขณะที่การเติมเส้นใยที่ละลายน้ำไม่ได้มีผลค่อนข้างน้อยต่อสมบัติการเกิดเพสต์ของแป้งสาลี ในด้านการเกิดเจลาติไนเซชันของสตาร์ช พบว่าการเติมเส้นใยไม่มีผลต่ออุณหภูมิเจลาติไนเซชันมากนัก แต่มีผลทำให้เอนทาลปีของการเกิดเจลาติไนเซชัน (ΔH_G) มีค่าลดลง ในการติดตามการเกิดรีโทรเกรเดชันในระยะยาว ในงานวิจัยนี้ได้วิเคราะห์เอนทาลปีของการเกิดรีโทรเกรเดชัน (ΔH_R) ของตัวอย่างที่เก็บรักษาไว้ที่อุณหภูมิ 4 องศาเซลเซียส เป็นเวลา 6, 10 และ 14 วัน พบว่าตัวอย่างที่เติมเส้นใยส่วนใหญ่มี ΔH_R ลดลง ยกเว้นตัวอย่างที่เติม TG ซึ่งมี ΔH_R เพิ่มขึ้น ในด้านความแน่นเนื้อของเจลพบว่าการเติมเส้นใยทำให้เจลที่เตรียมเสร็จใหม่ๆ มีความแน่นเนื้อมากขึ้น โดยตัวอย่างที่เติม CAR มีความแน่นเนื้อสูงสุด อย่างไรก็ตามในระหว่างการเก็บรักษา พบว่าตัวอย่างเจลที่เติมเส้นใยมีการเพิ่มขึ้นของความแน่นเนื้อที่ช้ากว่าเมื่อเทียบกับตัวอย่างควบคุม ณ วันที่ 14 ของการเก็บรักษา พบว่าเจลทุกตัวอย่าง ยกเว้นตัวอย่างที่เติม TG เข้มข้น 1.5 และ 2.0% มีความแน่นเนื้อของเจลต่ำกว่าตัวอย่างควบคุมอย่างมีนัยสำคัญ ($p \leq 0.05$) จากผลการทดลองที่ได้นี้ จึงคัดเลือกความเข้มข้นของเส้นใยที่เหมาะสม (1.0% TG, 1.5% CAR และ 2.0% CEL) เพื่อนำไปใช้ในขนมปังและติดตามคุณภาพของขนมปังที่ผลิตเสร็จใหม่ๆ (วันที่ 0) และระหว่างการเก็บรักษาไว้ที่อุณหภูมิห้องเป็นระยะเวลา 6, 10 และ 14 วัน ในช่วงแรกของการเก็บรักษา พบว่าเนื้อในของขนมปังทุกตัวอย่างมีปริมาณความชื้นที่ใกล้เคียงกัน อย่างไรก็ตาม เมื่อระยะเวลาการเก็บรักษาเพิ่มขึ้น (≥ 10 วัน) การเติมเส้นใยที่ละลายน้ำได้และละลายน้ำไม่ได้สามารถช่วยรักษาความชื้นไว้ได้ ในด้านปริมาตรจำเพาะของก้อนขนมปัง เป็นที่น่าสนใจว่าขนมปังที่เติม CEL มีปริมาตรจำเพาะสูงที่สุด ในขณะที่การเติม CAR ทำให้ขนมปังที่ได้มีปริมาตรจำเพาะต่ำที่สุด สำหรับสมบัติด้านเนื้อสัมผัส ขนมปังที่เติมเส้นใยทั้งที่ละลายน้ำได้และละลายน้ำไม่ได้มีความแข็งต่ำกว่าตัวอย่างควบคุม การที่ขนมปังมีค่า hardness, gumminess และ chewiness ลดลง และมีค่า springiness และ cohesiveness ที่เพิ่มขึ้น แสดงให้เห็นว่าเส้นใยสามารถช่วยรักษาคุณภาพของขนมปังได้ ขนมปังที่เติมเส้นใยมีรูอากาศที่มีขนาดเล็กและกระจายตัวอย่างสม่ำเสมอเมื่อเทียบกับตัวอย่างควบคุม ขนมปังที่ได้มีเนื้อในสีเหลืองและเปลือกนอกสีส้ม-เหลือง โดยไม่มีการเปลี่ยนแปลงของสีในระหว่างการเก็บรักษา จึงสรุปได้ว่าการใช้เส้นใยที่ละลายน้ำได้และละลายน้ำไม่ได้มีผลดีต่อคุณภาพของขนมปังที่ผลิตเสร็จใหม่ๆ และคุณภาพในระหว่างการเก็บรักษา

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

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This study aimed to investigate the effect of selected fibres on pasting as well as short-term and long-term retrogradation of starch in wheat flour and to investigate the quality of fibre-added white pan breads. Two soluble fibres, tara gum (TG) and k-carrageenan (CAR), and one insoluble fibre, cellulose (CEL), were added to wheat flour at different levels (1.0, 1.5 and 2.0%). Regarding pasting behaviour, addition of soluble fibres was found to cause an increase in peak, trough, breakdown, and final viscosity. Meanwhile the addition tended to decrease setback viscosity and pasting temperature. In contrast, insoluble fibre addition posed a minimal effect on the pasting properties of wheat flour. Regarding starch gelatinisation, all fibres minimally affected gelatinisation temperature but did reduce the gelatinisation enthalpy (ΔH_G). To monitor long-term retrogradation, enthalpy of retrogradation (ΔH_R) of the samples stored for 6, 10 and 14 days was observed. It was found that most of the fibre reduced in ΔH_R while TG at all concentrations caused an increase. As of gel firmness, fibre addition was shown to increase firmness of the freshly prepared flour gel, with CAR samples exhibited the highest firmness. However, during storage, the fibre-added samples demonstrated a slower increase in gel firmness than the control. At 14 days of storage (4°C), all gel samples, except 1.5% TG and 2.0% TG, possessed significantly lower gel firmness as compared to the control ($p \leq 0.05$). Based on the above study, the optimum concentration (1.0% TG, 1.5% CAR and 2.0% CEL) were selected and added in white pan breads to investigate quality of freshly prepared bread (Day 0) and of those stored at room temperature for 6, 10 and 14 days. At the beginning of the storage, moisture content of all breadcrumbs was similar in value. However, during prolonged storage ($\geq$10 days), both soluble and insoluble fibres were found to help retain moisture. As to specific loaf volume, CEL bread interestingly displayed the highest value while CAR resulted in a bread with the lowest specific loaf volume. In terms of textural property, both soluble and insoluble fibres significantly demonstrated lower hardness than the control. The decrease in hardness, gumminess and chewiness and an increase in springiness and cohesiveness signified that the fibres play a role in maintaining quality of the breads. All fibre-added breads had smaller and evenly distributed air cells when compared to the control. All the breadcrumbs were yellow in colour, with orange-yellow bread crust. Both crumb and crust colour demonstrated minimal changes during storage. In summary, both soluble and insoluble fibres proved to have a positive effect on quality of the freshly baked as well as the stored breads.

Field of Study:	Food Science and Technology	Student's Signature ....
Academic Year:	2020	Advisor's Signature ....
		Co-advisor's Signature ....

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

It has long been recognized about the health benefits of fibre intake. Consumer health concern, together with willingness to pay for a 'healthy food' have urged the food manufacturers to develop various fibre-fortified recipes. However, fibre fortification inevitably modifies the total composition of blend formulation and the fibres added may also interact with other ingredients or flour components. This results in limitations in its content appliable to achieve desired quality of the final products.

Fibres can be classified into two types based on their solubility. Soluble fibres, as the name implies, are those that dissolve in water and turn into gel-like matter. They help relieve constipation and protect the colon muscle. Insoluble fibres, on the other hand, are those that do not dissolve in water. They stimulate gastrointestinal tract and increase bowel movement. Fibre fortification have been reported to alter rheological property of food systems and properties of the final products (Naruenartwongsakul et al., 2004; Yildiz et al., 2013).

Starchy foods, like bread, are staple in many cultures and suitable for fortifying with fibres. Additives that can interact with starch and/or water have been reported to affect gelatinisation and retrogradation of starch, and this, in turn, exerts an influence on the properties of the starch and flour during processing as well as the quality and stability of the final product (De Bondt et al., 2020; Mahawanich, 2013; Qiu et al., 2017).

The objective of this study was therefore to investigate the effects of addition of soluble fibres, namely tara gum (non-ionic) and κ-carrageenan (ionic), and insoluble fibre cellulose on pasting property as well as short-term and long-term retrogradation of wheat flour and on baking and keeping quality of white pan bread.

CHAPTER 2

LITERATURE REVIEW

2.1 Starch

2.1.1 Structure

Starch is the principal storehouse of carbohydrate in plants and the main source of calories for foods. Starch comprises about 60-70% in wheat flour. Wheat starch has a bimodal granule-size distribution which consists of large, lenticular-shaped granules (A-granules) which covers 75% by weight and small, round granules (B-granules) that covers 25% by weight (Maningat & Seib, 2010; Singh et al., 2010; Vamadevan & Bertoft, 2020). Some authors even explained about C-type granules in wheat starch (Singh et al., 2010).

Starch is a mixture of two polymers of glucose namely amylose and amylopectin. Amylose is a linear chain of α-glucose monomers joined by 1,4-glycosidic bonds. Amylopectin is a branched chain of α-glucose monomers joined by 1,4- and 1,6-glycosidic bonds. The 1,6-glycosidic bonds form the links which make branches. The clusters of amylopectin are branched and double-helical (Donald, 2004; Singh et al., 2010; Van Hung et al., 2006). Wheat starch generally contains 25% amylose and 75% amylopectin.

Under optical microscope, starch granules show multiple concentric layers which form the growth rings that rise from the hilum towards the surface. When starch granules are examined under polarised microscope, it shows birefringence, or the so-called Maltese cross, which implies that crystalline order is present in starch granules. In each granule, the amylose and amylopectin molecules are arranged radially creating alternating layers of crystalline and amorphous regions (Figure 2.1). The linear amylose together with the clustered double helix branches of amylopectin can arrange themselves with the formation of hydrogen bonding, resulting in ordered crystalline structure. The branching areas of amylopectin molecules causes structural hindrance, resulting in the formation of less dense

amorphous regions (Ratnayake & Jackson, 2008; Shevkani et al., 2017; Van Hung et al., 2006).
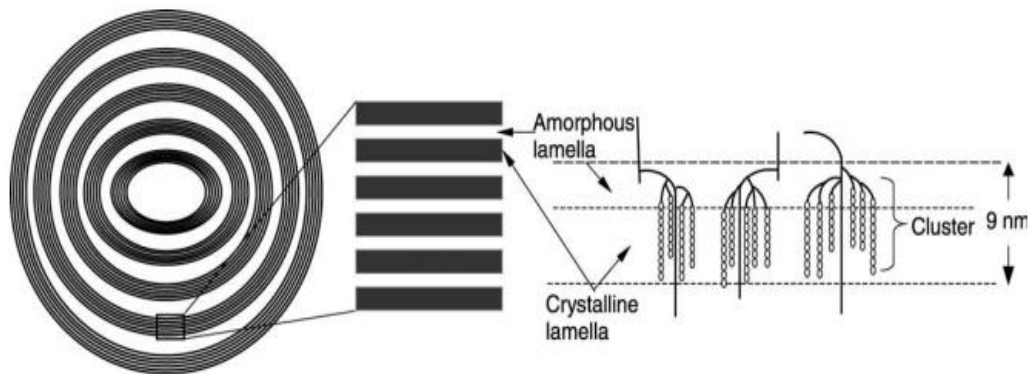
Figure 2. 1 Radially arranged alternate amorphous and crystalline structure of starch

Source: Tester et al. (2004)

The functionality of starch depends on its ability to absorb water and swell, which affects gelatinisation and pasting properties when heating and leads to retrogradation when heated starch is cooled. The location of amylose in starch granules relates to structural properties that are involved in swelling of starch, gelatinisation and pasting properties. Amylose and lipids retard these processes whereas amylopectin contributes to it. Therefore, viscous gel formation and thermal properties appear to be influenced by amylose and amylopectin ratio (Singh et al., 2010). The difference in this ratio also causes difference in structure of granules, quality and physiochemical properties of final products (Van Hung et al., 2006). To succeed in application of starch, changes of starch during heating and cooling of starch should be fully understood (Copeland et al., 2009).

2.1.2 Gelatinisation

Starch is insoluble in cold water due to crystalline structure resulted from the hydrogen bonding among the straight chain of starch molecules. When heat is supplied, the hydrogen bonds are broken, disrupting the crystalline structure and cause the granules to swell causing the loss of birefrienge. The friction induced by the swollen starch granules results in an increase in paste viscosity. This irreversible

swelling of starch granules is termed gelatinisation. Different starches have different gelatinisation temperatures. For wheat starch, the gelatinisation temperature is about 60°C (Suresh & Shamsher, 2013).

        2.1.3 Retrogradation

Upon cooling, the gelatinised paste is formed into a gel. Due to their linear structure, amylose molecules start to align themselves and form hydrogen bond together within a matter of minutes to hour. Steric hindrance resulting from the branched structure of amylopectin molecules causes them to retrograde at a slower rate than amylose. Thereby, amylopectin takes from hours to a few days to recrystallise. This ongoing tendency of starch molecules to re-align into crystalline structure, with the release of hydrated water and formation of hydrogen bond is called retrogradation. This property is of great importance for the processing and storage of starchy products (Copeland et al., 2009; Qiu et al., 2017). Figure 2.2 demonstrated the fate of starch during gelatinisation and retrogradation.
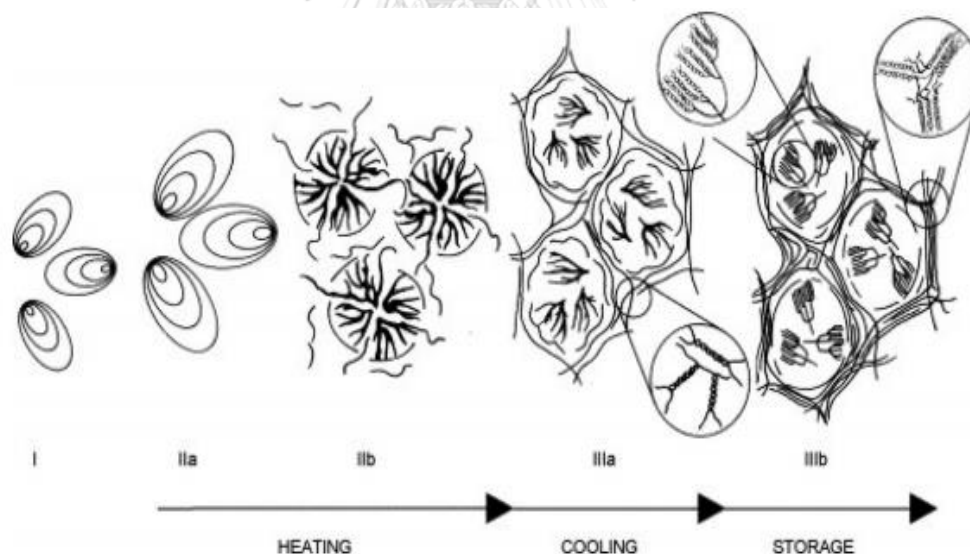
Figure 2. 2 Gelatinisation and retrogradation process in starch-water system, (I) native starch granules, (IIa) swelling of granules, (IIb) amylose leaching, (IIIa) amylose retrogradation, and (IIIb) amylopectin retrogradation

Source: (Wang et al., 2015)

2.2 Wheat

Wheat is among the main staple crops of the world, ranking after rice and maize (Shewry et al., 2002) . Although the crop is successfully grown in the middle latitudes (between 30°N to 60°N and 27°S to 40°S), it can be cultivated throughout the world. The global production of wheat rose to over 765 million metric tons in the 2019/2020 production year, which was 30 million metric tons greater than the previous year (Shahbandeh, 2020). Its prominence among other grains and cereals is due to its cultivation, adaptability, and high yield and wheat is easy to store and transport and is a non-perishable food.

Wheat flour is simply made by grinding wheat grains. The flour can be categorised according to the components being ground. Wheat white flour contains endosperm only. When some of the germ and bran are included, the product obtained is called brown flour while whole grain flour is made by grinding endosperm, germ, and bran. Wheat flour is the most common flour used in many food products like breads, noodles, pasta, cookies, biscuits and other bakeries (Wang et al., 2020). One of the reasons behind its popularity is its unique viscoelasticity which other flours lack. The viscoelastic dough comes with the ability to retain gas from which leavened food products can be made. Wheat flour contains about 9% moisture, 2% fat, 0.8% fibre, 0.7% ash (Ocheme et al., 2018). The protein content is different among different types of wheat flour. Bread flour contains about 13-14% protein while all-purpose and cake flours have lower protein contents of 12% and 7.5-9%, respectively (Suresh & Shamsher, 2013).

Wheat proteins are classified into four fractions based on their solubility. Albumins are soluble in water while globulins are soluble in dilute salt solution. Gliadins are soluble in aqueous alcohol and glutenins are soluble in acid or alkali (Goesaert et al., 2005). Glutenin includes low and high molecular weight subunits that are connected mainly by intermolecular disulfide bonds, hydrophobic interactions, and some other forces. Gliadin, on the other hand, includes intermediate and low molecular weight subunits which are linked by intramolecular disulfide bonds. The side chain of cysteine participates in intra and interchain disulfide bridges in the

formation of tertiary structure (Roongthongsri, 2008). Glutenin is responsible for dough elasticity whereas gliadin is responsible for dough viscosity. Gluten is formed through the cross-linking of glutenins and gliadin which results in disulfide bonded network. Therefore, both glutenin and gliadin have their role in providing viscoelasticity and gas retention ability to the dough while globulins and albumins only have their roles in nutritional aspects (Belitz et al., 2009; Call et al., 2020).

About 2% of lipids are also present in wheat flour (Ocheme et al., 2018; Van Der Borght et al., 2005). Most of the triglycerides are stored in the germ and only a small amount is stored in the aleurone layer. Phospholipids and glycolipids are found in the endosperm. Linoleic acid is the fatty acid that is found in high amount in the wheat flour. Polar lipids were reported to positively affect the volume of baked products while non-polar lipids usually show negative effect. Even being in small amount, lipids were found to affect the baking quality (Belitz et al., 2009).

The major polysaccharides of wheat flour other than starch are dietary fibres. These include cellulose, arabinoxylans, pentosans, β-glucans and glucofructans. The pentosans of wheat flour are water soluble and can absorb more water to form viscous paste. Water-insoluble cellulose and hemicellulose are also present in minor amount, with higher amount in outer part of the kernel. β-Glucan with its mucous characteristics can increase viscosity of the flour paste. Sugars are present in small amount as monosaccharides, disacchharides and trisacchharides. These smaller saccharides have their role in carbon dioxide production during dough fermentation and contribute to brown colour to the crust.

2.3 Dietary Fibres

2.3.1 Definition

Dietary fibres include dietary components which are indigestible in the human upper gastrointestinal tract (Wen et al., 2020). Hipsley (1953) was the first to use the term ″dietary fibre″ to describe non-digestible plant cell wall components which was later adopted by other researchers and connected it with nutrition and healthy perspectives. In 1999, the American Association of Cereal Chemists came upon with the revised definition of dietary fibres that stated "Dietary fibre is the

edible part of plant or analogous carbohydrates that are resistant to digestion or absorption in human small intestine with complete or partially fermentation in large intestine (DeVries, 2003) (cited). Dietary fibres mainly include plant structural polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibres promote beneficial physiological effects like laxation, blood cholesterol attenuation, and blood glucose attenuation (DeVries, 2003; Kongrit, 2003).

2.3.2 Classification

Fibres are usually non-starch polysaccharides that include cellulose, hemicellulose, pectin, gums and mucilages, fructooligosaccharides as inulin, and heteropolymers like arabinoxylans and arabinogalactans, and even it includes lignin, which is not carbohydrate, but it closely associates with polysaccharides in the plant cell wall. Cellulose, hemicellulose, and lignin are the components of plant cell wall while gums and mucilages are formed in specialised secretory plant cells. Based on the definition, analogous carbohydrates include chemically modified cellulose derivatives (e. g. methylcellulose), indigestible dextrins and resistant starch, are therefore categorised as dietary fibres. Waxes, suberin, cutin and tannins are also found chemically cross-linked with dietary fibre polysachharides (DeVries, 2003). Based on their solubility in water, dietary fibres are classified into two types which are insoluble fibres and soluble fibres (Dhingra et al., 2012; Raman & Doble, 2015).

2.3.2.1 Insoluble fibres

Insoluble fibres mainly consist of hemicellulose, cellulose and lignin and cannot dissolve in either warm or hot water. They are less fermentable and assume the role as bulking and texturizing agents. They are found in natural sources as wheat bran, whole grains, cereals, as well as seeds and skins of many fruits and vegetables.

The insoluble fibre used in the current study was cellulose. It is the major component of plant cell wall. Cellulose molecule is unbranched linear chain of several thousand glucose units linked together by β-1,4 glycosidic linkages (Figure 2.3). It is insoluble in strong alkali and can be hydrolyzed by strong acid. Cellulose is

not digested to any extent by the enzymes of the human gastrointestinal tract and remains as a bulk (Dhingra et al., 2012; Murray, 2009; Prosvirnikov et al., 2017).

Although cellulose is not soluble in water, it can swell upon dispersing in water. Naturally obtained cellulose contains impurities. To remove the impurities, cellulose is traditionally extracted from the plant sources by alkali or acid. Then, it is submitted to high temperature and pressure after which it is bleached and washed thoroughly to get isolated purified cellulose (Pappu et al., 2015). Commercial purified celluloses can be obtained in different fibre length. Fibre length poses an effect on the cellulose functional properties like water absorption capacity and textural properties. According to Goldstein et al. (2010), it was reported that 40 μm fibre demonstrated better water absorption capacity than 200 μm fibre did. Sorption properties are enhanced as the surface area is increased (Prosvirnikov et al., 2017). This property is responsible for desirable functional performance in bread such as texturizing, gel formation, crumb structure stabilizing and volume building.
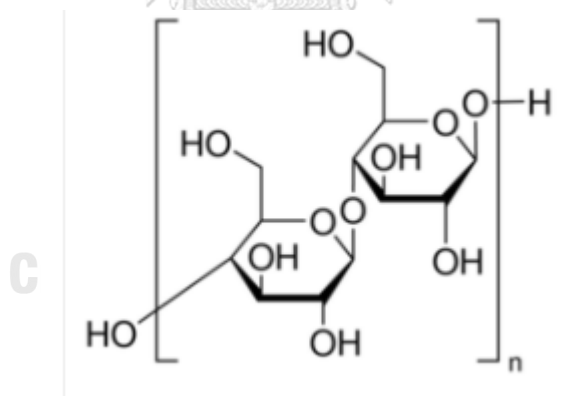
Figure 2. 3 Molecular structure of cellulose

With rising awareness about fibre intake, cellulose has become one of the most popular food additives. Adding cellulose to food allows an increase in bulk and fibre content without major impact on flavour. Along with fibre addition, it is also calorie-reducer, thickener, emulsifier, and anti-caking agent (Yildiz et al., 2013).

2.3.2.2 Soluble fibres

As the name indicates, they are soluble in water with high water absorption capacity. When consumed, they evade digestion of the human gastrointestinal tract but as they reach the colon, they are fermented by the bacteria in the intestine. Soluble fibres can be found naturally in various sources, such as dried beans, oats, oat bran, rice bran, barley, citrus fruits, apples, strawberries, peas, and potatoes. Soluble fibres are obtained from different sources, including plants, seaweeds and bacteria (Salehi, 2019). They are generally highly branched polysaccharides, composed of less common monosaccharide units. Two soluble fibres were used in the current study, namely tara gum and κ-carrageenan.

Tara gum is obtained from the endosperm of *Caesalpinia spinosa* which belongs to the Fabaceae family and is native to Peru. Tara gum consists of high molecular weight polysaccharides composed mainly of galactomannans. Its molecular structure resembles to that of guar gum and locust bean gum. The linear main chain of (1-4)-β-D-mannopyranose is units attached by (1-6) linkages with α-D-galactopyranose units, with mannose-to-galactose ratio of 3:1 which is the in-between of guar gum (2:1) and locust bean gum (4:1) (Figure 2.4). It is essentially non-ionic polysaccharide (Santos et al., 2019; Vidaurre-Ruiz et al., 2019; Wu et al., 2018).
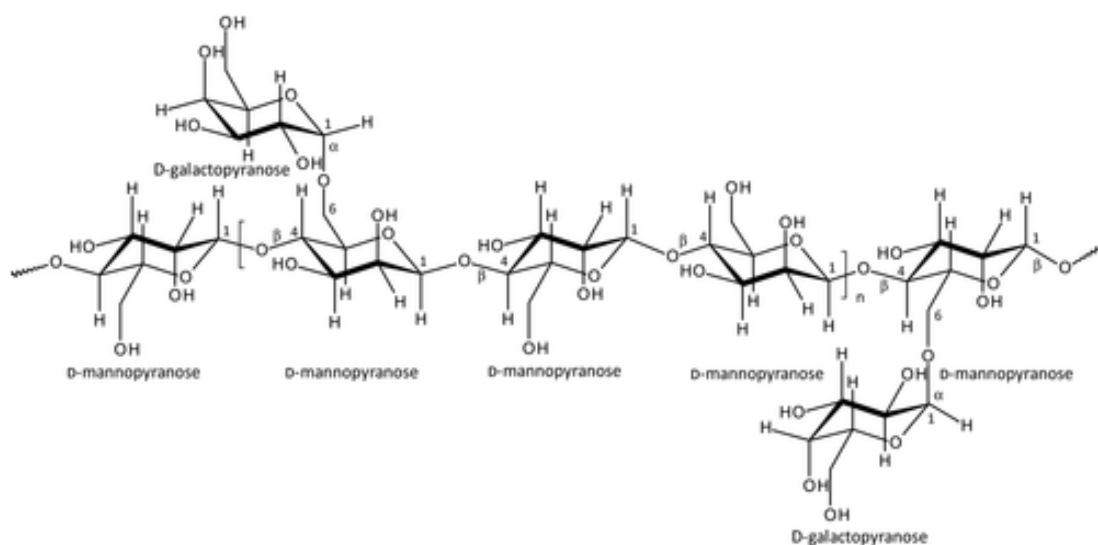
Figure 2. 4 Molecular structure of tara gum

Because of its unique structure which falls between guar gum and locust bean gum, tara gum can be used in food industry in place of guar gum and locust bean gum. Tara gum solution is less viscous than guar gum but more viscous than locust bean gum of same concentration. Tara gum has more advantages in comparison to guar gum. It is tasteless and odourless while guar gum has characteristic unpleasant odour and taste. Tara gum solution is smooth and soft in texture whereas guar gum has slimy texture. Despite that, the current use of tara gum in food industry is still limited. In recent years, the price of guar gum and locust bean gum has increased sharply due to the increasing demand so taking in consideration cheaper price and short growth cycle of tara gum, it is a matter of interest to many research (Huamaní-Meléndez et al., 2020; Santos et al., 2019). Due to its solubility and ability to withstand high temperature, tara gum may find its potential for use in food industry. Tara gum was reported to enhance the quality of dairy products as frozen yogurt and ice cream as it helps to prevent syneresis (BeMiller, 2019).

Carrageenan is extracted from red seaweed called Irish moss (*Chondrus crispus*). It consists of complex polysaccharides made up of repeating galactose units and 3,6-anhydrogalactose, with different levels of sulfate ester. Three classes have found their use in food industry, namely κ-, ι- and λ-carrageenan, which differ in the number of sulfate groups. κ-carrageenan has sulfate ester content of 25-30%. As a result of the negative charge of the sulfate group, all carrageenan is anionic. Structure of κ-carrageenan is illustrated in Figure 2.5.
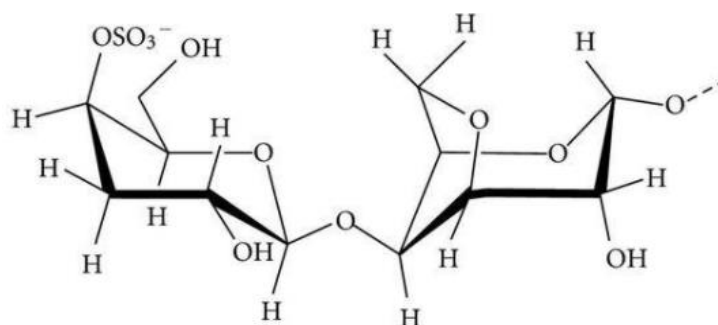
Figure 2. 5 Molecular structure of κ-carrageenan

Carrageenan also exhibits health benefits and biocompatibility. Studies showed that they are more operative in lowering cholesterol and blood pressure than other fibre sources (Jiménez-Escrig & Sánchez-Muniz, 2000; Raman & Doble, 2015). Carrageenan is frequently used as gelling and thickening agent in dairy desserts because they have synergism with milk casein (Huang et al., 2007).

2.3.3 Dietary fibres in food industry

Apart from the health benefits, dietary fibres can be added in food to achieve the desired quality. It depends upon how fibres interact with starch and other components of food. In starch-based foods, the interaction between starch and fibres may improve texture and moisture retention capacity of the products. Dietary fibres have been added to products like noodles, dairy products, beverages, breads and cookies (Han et al., 2017). With regards to breads, fibre addition can improve bread volume, crumb porosity and sensory acceptance. Soluble fibres can be used in frozen dough to prevent gluten damage and large ice crystal formation. They can be used to delay starch retrogradation as well (Rosell et al., 2001; Yildiz et al., 2013).

2.3.4 Effect of fibres on starch gelatinisation and pasting

Fibres, when added to flour or starch systems, were reported to modify gelatinisation behaviour (Qiu et al., 2017). Fibres provide a variety of effects on viscosities of starch during pasting (Alam et al., 2009).The extent of the effect depends on type and concentration of the fibre used and its interactions with starch and other components of the flour (Bárcenas et al., 2009; Wang et al., 2015). Different fibres and different starches produced an assortment of effects on pasting viscosities (Shi & BeMiller, 2002).

Alam et al. (2009) investigated the effect of guar gum, xanthan gum, gum arabic, carboxymethyl cellulose and gum tragacanth on gelatinisation and pasting behaviour of hard wheat flour. Gelatinisation temperature was found to decrease upon adding guar gum and xanthan gum. The author proposed that xanthan gum and guar gum absorbed little water. This resulted in more water available for starch hence a lower gelatinisation temperature. Guar gum and xanthan gum produced a flour paste with increased peak viscosity as compared to the other hydrocolloids. In

the case of breakdown viscosity, the paste with gum arabic showed the least breakdown which means that the system was more stable to heat and shear. As to setback, xanthan gum resulted in the lowest setback viscosity, implying the lowest retrogradation upon cooling of the flour paste.

2.3.5 Effect of fibres on starch retrogradation

According to Wang et al. (2015), fibres can either increase, decrease or even have no effect on starch retrogradation. In general, fibres tend to accelerate short-term retrogradation and decelerate long-term retrogradation as they retard amylose-amylose interactions and amylopectin recrystallisation (BeMiller, 2011). However, inconsistency was found regarding the effect of fibres on retrogradation process. For instance, Luo et al. (2017) investigated the effects of inulin fortification on retrogradation of wheat starch and reported that inulin inhibited amylose retrogradation but enhanced amylopectin retrogradation during long-term storage.

In the case of gluten-free flour as rice flour, fibres are added as additive to prevent retrogradation as they help increase the viscoelastic property of dough (Vidaurre-Ruiz et al., 2019). According to Chen et al. (2015), when pullulan was added to rice starch, it inhibited both short-term and long term retrogradation. The author proposed that this decrease in retrogradation might be due to a couple of reasons. Firstly, owing to the competition for available water between starch and fibre, the rice starch may not be fully gelatinised. This may decrease the starch chain mobility and decrease further crystallisation of starch molecules (De Bondt et al., 2020). Lack of retrogradation has been well recognised in products containing ungelatinised starch such as cookies. Secondly, the interaction between fibre and leached amylose may subside the extent of amylose-amylose interactions (Ma et al., 2019).

According to Funami et al. (2008), an increase in retrogradation was demonstrated in wheat starch added with 0.5% of either locust bean gum, konjac glucomannan, guar gum or tara gum in comparison with the control. The authors suggested that fibre-amylopectin association may reduce molecular mobility of starch, hence facilitated retrogradation.

2.4 Bread

Bread has been consumed by people in various cultures for a long time. Different types of bread are found in different parts of world. Breads are usually characterized either by their physical appearance such as flat bread, pan bread, baguette, or by the composition of which they are made such as banana bread, rye bread, sorghum bread.

The main component of bread is wheat flour due to the viscoelastic property of its gluten. Nevertheless, bread can be made from different cereals, grains, pulses, and legumes. Various ingredients and additives are also added in flour to improve baking and organoleptic properties. White pan bread will be focused on this review.

2.4.1 Bread ingredients

The main ingredients for making white pan bread are wheat flour, yeast, water, sugar, salt, and fat. Other ingredients may also be added to improve colour, flavour, nutrition or shelf-life (Reed & Nagodawithana, 1991).

Wheat flour contains glutenin and gliadin which upon kneading with water yield the viscoelastic gluten that provides elasticity to the dough that could trap air bubbles and later gives the desirable structure to the bread. For bread, in general, stronger gluten is needed which results in bread with better quality.

Yeast is a biological leavener. Baker's yeast or *Saccharomyces cerevisiae* is the common yeast used in baking. Yeast uses sugar and produces carbon dioxide and alcohol during the fermentation process. Carbon dioxide is the leavening gas that expands the dough and gives typical texture to the bread. Alcohol produced gives typical fermentation flavour to bread (Roongthongsri, 2008). Yeast produces both volatile and non-volatile compounds which are responsible for the flavour of bread (Cauvain & Young, 2007).

Water is used to disperse the dough ingredients and it participates in gluten formation and starch gelatinisation. The amount of water required to produce a

dough with desirable properties depends on many factors such as type of flour, presence of other ingredients, dough preparation method and the equipment used to prepare the dough. The pH and hardness of water is also important factors for proper bread making.

Salt is another important ingredient in bread making. It helps improve gluten network and preserves elasticity. It acts as a stabilizer on the action of yeast during proofing so that carbon dioxide is not produced too fast before developing flavour. Salt improves volume, appearance of bread and crispiness of crust.

Regarding the fat used in bread recipe, either oil, butter or shortening can be used. Fat makes the crumb softer, increases loaf volume and adds flavour.

Sugar is essential in bread making as it is the substrate of yeast. During fermentation, sugar is converted into carbon dioxide and alcohol by the action of yeast. Sugar provides sweetness that has pronounced effect on flavour. It also develops brown colour on the crust due to caramelisation (Larsen, 2019). Sugar also increases hygroscopicity and softens the crumb as well as retards staling.

2.4.2 Baking methods

Even though different methods could be used to make bread, the common aim is to convert wheat flour into palatable, soft, and aerated product. White pan bread can be made by different techniques, for instance, straight dough method, sponge dough method, no-time dough development and mechanical dough development. Among which some are discussed below.

The straight dough method may be the simplest way of making bread. It takes less time and effort. It is a single fermentation process where all ingredients are mixed at same time and the dough is mixed until gluten fully develops. It takes 3 to 4 h to make bread by this method. The fermentation is done after punching the dough to remove the gas inside. After the fermentation for 2 to 3 h, the fermented dough is punched again, then divided into equal weights and fermented again for 15-20 min. Finally, the dough is moulded into bread pans and kept for final proofing before baking in an oven. The straight dough method gives chewy, strong bread with

open crumb grain. The reason behind using straight dough method is to obtain good quality bread even in short time in comparison to other methods (Gisslen, 2012; Sievert et al., 2000).

The sponge and dough method produces bread with good flavour and aroma with softer crumb and texture than the straight dough method. Even the shelf-life of the bread is longer, this method requires more time, effort, and space. It is a two-stage process. In this method, a part of the ingredients (about 65%) is mixed first and allowed to ferment for 3 to 4 h. After fermentation, the sponge obtained is mixed with the remaining ingredients. The dough is fermented for a second time for 20 min and then baked in an oven (Cauvain, 2015; Sievert et al., 2000). Liquid fermentation process is also same as this method, but water is used instead of sponge.

No-time dough method or short-time or rapid processing method is the method in which the dough is fermented for a very short period. The dough is then divided, proofed, and baked. It is a time-saving method. This method is usually carried out in small bakeries. Flavour development is of a lesser degree than other methods (Cauvain, 2015).

2.4.3 Changes in physico-chemical properties of dough and bread during preparation steps

2.4.3.1 Mixing

This first step involves the transformation of flour of dough through Shear force, deformation, and extension. Many physical and chemical parameters are changed. The mechanical force is applied to hydrated flour and other particles continuously (Autio & Laurikainen, 1997). When water is added, it is absorbed by starch and protein in wheat flour. Gliadin and glutenin are formed into gluten network which traps air bubbles and provides viscoelasticity to the dough (Rosell, 2011). Starch in wheat flour affects water absorption, swelling, gelatinisation and retrogradation properties. It affects softness of fresh bread and hardness of stored bread (Cauvain, 2012).

2.4.3.2 Fermentation

Normally, dough fermentation is done in two steps. During the first fermentation, the dough starts to rise due to the formation of carbon dioxide. Sugars in the recipe as well as those from the breakdown of starch via the action of α- and β-amylases indigenous to wheat flour are used by the yeast, with the production of carbon dioxide and alcohol (Cauvain, 2012; Rosell, 2011). The gluten helps retains the gas in the dough structure. Small bubbles with thick gluten wall are more stable than large bubbles with thin gluten wall. According to Błaszczak et al. (2004), a slight increase in temperature during fermentation causes swelling of starch granules, leading to granule deformation and amylose leaching. During the second fermentation, alcohols, esters, and acids are produced which contribute to flavour and aroma of the bread.

2.4.3.3 Punching, moulding, and proofing

Punching helps to release the gas and get the dough exposed to oxygen for proper fermentation and maintains an even temperature. During moulding, the dough is divided and sheeted. Placing the dough with the seam down to bread pan ensures that the gas will not escape from the loaf. Proofing is done to allow the dough to rest and rise for a final time before subjected to baking. During this step, yeast continues to ferment and produce gas.

2.4.3.4 Baking

Once done with proofing, the dough is baked in the oven where heat is transferred from the outside to the inside. Heat causes the gas and air to expand, resulting in an increase in loafs volume referred to as oven spring. As the temperature inside increases to 45°C, 95% of yeast are killed (Reed & Nagodawithana, 1991). At around 65°C, starch gelatinises, and starch granules begin to swell. Starch in the crumb fully gelatinises while incomplete gelatinisation occurs in the crust due to limited moisture. This process also depends on other ingredients present in the dough (Cauvain, 2012). With the usual oven temperature of 200°C, Maillard reaction and sugar caramelisation take place, prominently in the crust, to give the characteristic brown colour and sweet nutty caramelised flavor.

2.4.3.5 Cooling and storage

The moment, when the bread is taken out from the oven, the crumb is still very soft. Upon cooling, the linear amylose molecules quickly realign to form crystalline structure. This retrogradation of amylose is, in fact, considered desirable because it gives the initial firmness and stability to the bread structure. During storage, the branched amylopectin slowly retrogrades, and this is among various factors contributing to the staling of bread.

2.4.4 Bread staling

Bread is unstable product which undergoes various physical, chemical, and sensory changes during storage. Bread staling is a major problem for bread manufacturers as well as for consumers. It is a complex phenomenon, characterizing by the changes in moistness, hardness, texture, and flavour of the bread (Gray & Bemiller, 2003; Pateras, 2007; Purhagen et al., 2012).

Starch has been regarded to have a major role in bread staling. Gray & Bemiller (2003) reported that the X-ray diffraction patterns of fresh bread and freshly gelatinised wheat starch were similar while that of stale bread resembled to that of retrograded starch. This implies the relationship between bread staling and starch retrogradation. This is in contradiction to the earlier study which proposed that there was no linkage between bread staling and starch recrystallisation (Dragsdorf & Varriano, 1980) and also breads stored at the temperature above 21°C, starch recrystallisation was not prominent (Colwell et al., 1969; Gray & Bemiller, 2003).

Besides starch, other non-starch components also pose an effect on bread staling (Pateras, 2007). Salehifar et al. (2010) reported that bread with higher protein content staled at a slower rate than bread with lower protein content. Pentosans, the non-starch polysaccharides, were also suggested as the key component to inhibit starch recrystallisation. This was due to their high water affinity and also ability to interact with both amylose and amylopectin (Gray & Bemiller, 2003; Pateras, 2007). When small amounts of pentosans (0.5 to 2%) were added to bread

dough, it could produce moderate increase in Loaf volume as well as help to reduce bread firmness.

Lipids in bread comes naturally from wheat flour or from the added fat required by the recipe. Wheat lipids could form complexes with amylose which was proposed to inhibit bread staling. The shortening added could act as a barrier to prevent moisture migration from the crumb to the crust. The anti-firming effect of lipids was demonstrated (Gray & Bemiller, 2003; Pareyt et al., 2011).

2.5 Fibres in bread-making

Dietary fibres have been recognized for its contribution to health and well-being, reduction of the risk of heart disease, prevention against diabetes and some types of cancer, as well as reduction of blood cholesterol and constipation. Fibre intake of people in general is usually below the recommended level. Fibre fortification to bread, one of the most common staple foods, may be a means to meet the requirement of fibre intake. Addition of fibres enhances the nutritional quality of bread but, at the same time, inevitably poses an effect on certain properties of the bread.

2.5.1 Effect of fibres on physico-chemical properties of bread

Several researchers have explored the use of fibres in baked products (Kohajdová & Karovičová, 2008; Seguchi et al., 2007). For example, Sangnark et al. (2004) indicated that fibre fortification can modify loaf volume, loaf firmness, crumb hardness and springiness of bread but the extent of modification depended on the type of fibre. Gómez et al. (2003) reported a significant and negative effect of fibre addition on loaf volume and texture of bread.

Rosell et al. (2001) reported that carrageenan could be used as texture improver as it reduced firmness and increased specific volume of the bread. Contrastingly, Turabi et al. (2008) reported that, κ-carrageenan was not effective in maintaining the required structure of gluten-free cake made from rice flour. Fibres were found to alter texture and consistency of bread (Kurek & Wyrwisz, 2015). Hardness and springiness were reported to be affected by fibre addition.

Seguchi et al. (2007) investigated the effect of cellulose particle size (6-650 µm) on bread properties. It was found that cellulose with a particle size of less than 154 µm yielded a bread with greater loaf height than those with larger particle size. It was reported that Barbari bread exhibited improved physical properties when added with carboxymethyl cellulose (CMC), hydroxypropylmethyl cellulose (HPMC), guar gum and xanthan gum. Specific volume of the breads was found to increase significantly, with the greatest specific volume belonged to the bread added with HPMC. Regarding moisture content, the CMC-added bread exhibited the highest moisture content, by the one added with HPMC. For the crumb softness, all fibre-added bread had softer crumb as compared to the control, with those added with cellulose derivatives possessing the softest crumb (Maleki & Milani, 2013).

Vidaurre-Ruiz et al. (2019) investigated the effect of tara gum and xanthan gum on properties of gluten-free bread made from corn starch and potato starch. The breads added with tara gum could retain less water than those added with xanthan gum. Regarding texture, the greatest hardness was reported for the tara gum-added bread made from potato starch.

2.5.2 Effect of fibres on keeping quality of bread

Palyvoda et al. (2016) reported that carob gum, tara gum and guar gum, at concentration of 0.2 to 0.5% of wheat flour, demonstrated antistaling effect on bread during the first two days of storage. Tara gum and carob gum at 0.5% concentration were found to be more effective in retaining bread freshness and reducing crumb fragility. This was proposed to be due to their ability in absorbing moisture and forming complexes with amylose.

Almeida et al. (2013) examined the effect of different fibres, namely wheat bran (WB), RS-2 type corn resistant starch (RS) and locust bean gum (LBG), on properties of wheat flour bread. It was observed that WB and LBG helped retaining moisture of the bread crumb during 7-day storage. However, fibre addition might pose an effect on bread properties. It was reported that WB addition caused a reduction in specific loaf volume and an increase in both crumb chroma and hue angle while RS had a minimal effect on bread quality.

In a study of Hager et al. (2011), gluten-free bread samples were added with either 5.6% oat β-glucan or 9% inulin while wheat bread samples were added with either 2.6% oat β-glucan or 6.8% inulin. It was found that addition of oat β-glucan reduced crumb hardness of gluten-free bread but increased crumb hardness of wheat bread. When inulin was added to gluten-free and wheat breads, crumb hardness as well as rate of staling were found to increase.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Calcium propionate (Chemipan, Bangkok, Thailand)

Cellulose fibre, 20 μm, Sigmacell^{TM} (Sigma Aldrich, St. Louis, MO, USA)

Instant dried yeast, Bruggeman^{TM} (Algist Bruggeman, Ghent, Belgium)

Iodised salt, Tipp^{TM} (Saha Pathanapibul, Bangkok, Thailand)

Refined sugar, Mitr Phol^{TM} (Mitr Phol Sugar, Bangkok, Thailand)

Shortening, Cream Topp$^®$ (Three Top Chemical & Foods, Bangkok, Thailand)

Tara gum (Chemipan, Bangkok, Thailand)

Wheat flour (unbleached bread flour), Shuttle^{TM} Brand (Thai Flour Mill Industry, Samut Prakan, Thailand)

κ-Carrageenan (Chemipan, Bangkok, Thailand)

3.2 Instruments

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

Food mixer, model CHEF XL (Kenwood, Kittisit, Pathum Thani, Bangkok)

Hot air oven, model 600 (Memmert, Schwabach, Germany)

Infrared food oven, model PL-6 (Kluay Num Thai Kitchen, Bangkok, Thailand)

Rapid visco analyzer (RVA), model RVA-4, (Newport Scientific, Warriewood, NSW, Australia)

Shaking water bath, model SW23 (Julabo labortechnik, Seelbach, Germany)

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

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

UV-Vis spectrophotometer, GENESYS™ 10 (Thermo Fischer Scientific, Waltham, MA, USA)

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

3.3 Methods

3.3.1 Composition of wheat flour

3.3.1.1 Proximate composition

Moisture, crude protein, crude fat, ash and crude fibre contents were determined according to the AOAC (1990) standard methods. Moisture content was determined using air oven method at 105°C. Nitrogen content was determined using the Kjeldahl method and crude protein content was then calculated from the nitrogen content using a conversion factor of 5.70. Crude fat content was determined by using Soxhlet extraction. Ash content was determined by dry ashing at 550°C. Crude fibre was obtained by treating the sample with 5% sulfuric acid and 5% sodium hydroxide.

3.3.1.2 Starch content

Starch content of wheat flour was determined using the amyloglucosidase/α-amylase method according to AOAC (2007).

3.3.1.3 Amylose content

Amylose content was determined using amperometric titration method as described by Gibson et al. (1997) and Takeda et al. (1987) with some modifications.

3.3.2 Effect of soluble and insoluble fibres on pasting behaviour, gelatinisation and retrogradation of wheat flour

Two soluble fibres, tara gum (TG) and κ-carrageenan (CAR) and one insoluble fibre, cellulose (CEL), were added at 1.0, 1.5, and 2.0% of wheat. Wheat flour without added fibre was set as a control. The flour samples were determined for the following properties:

3.3.2.1 Pasting properties

Pasting properties of the flour samples were determined using an RVA (model RVA-4, Newport Scientific, Warriewood, NSW, Australia) following the modified method described elsewhere (Bao, 2008).

Each fibre at a designated concentration was firstly dispersed in 25 mL distilled water by being magnetically stirred for 30 min (1200 rpm for 20 min and 1500 rpm for 10 min) at room temperature (25°C). Then the fibre dispersion was transferred to the RVA canister in which 3 g of wheat flour was added. The mixture was thoroughly stirred using the RVA paddle and the canister was then loaded into the machine. The starting temperature was set at 50°C followed by 13 min test profile according to Table 3.1. The peak, trough, breakdown, final and setback viscosities along with peak time and pasting temperature were obtained from the RVA curve, using Thermocline for Windows software (Newport Scientific, Warriewood, NSW, Australia).

Table 3. 1 Testing profile for RVA experiments

Stage	Temperature/speed	Time
1	50°C	0 min, 0 s
2	960 rpm	0 min, 0 s
3	160 rpm	0 min, 10 s
4	50°C	1 min, 0 s
5	95°C	4 min, 42 s
6	95°C	7 min, 12 s
7	50°C	11 min, 0 s
End of test		13 min, 0 s
Time between readings		4 s

3.3.2.2 Gelatinisation temperature and enthalpy

Gelatinisation of the samples was monitored using a DSC (Diamond DSC®, Perkin Elmer, Waltham, MA, USA) following a previously described method (Karim et al., 2008). Firstly, the required amount of fibre was dispersed in distilled water using magnetic stirrer for a total mixing time of 30 min (1200 rpm for 20 min and 1500 rpm for 10 min). The exact amount (approximately 3 mg) of wheat flour was weighed into 60 µL-capacity stainless steel DSC pan (Perkin Elmer, Waltham, MA, USA) after which 12 µL of fibre dispersion was added. The pan was hermetically sealed, reweighed, and allowed to equilibrate overnight before analysis. For gelatinisation experiment, the pan was heated from 30 to 100°C at the rate of 10°C/min. An empty pan was used as a reference. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures and enthalpy of gelatinisation (ΔH_G) were obtained from the DSC curve using Pyris software (Perkin Elmer, Waltham, MA, USA).

3.3.2.3 Temperature and enthalpy of amylopectin crystallite melting

The gelatinised sample obtained from the experiment in 3.3.2.2 was stored at 4°C for 6, 10, and 14 days. At each storage day, the sample was removed from refrigerator and equilibrated for 1 h and rescanned from 10 to 100°C using DSC following a previously described method (Karim et al., 2008). T_o, T_p, and T_c and enthalpy for melting of amylopectin crystallites (ΔH_R) were obtained from the DSC curve using Pyris software (Perkin Elmer, Waltham, MA, USA).

3.3.2.4 Gel firmness

Gel samples were prepared according to the modification in earlier study (Muadklay & Charoenrein, 2008). To prepare a gel, each concentration of fibre was firstly added to distilled water and stirred using a magnetic stirrer for a total time of 30 min (1200 rpm for 20 min and 1500 rpm for 10 min). Then, 12 g of wheat flour were added to 40 mL of fibre dispersion and manually stirred for 15 min. The mixture was heated in a water bath at 95°C for 60 min with continuous stirring and then transferred to a silicone mould. The samples were allowed to set at room temperature (25°C) for 60 min. The freshly prepared gels (Day 0) were subjected to

firmness measurement while the rest of the gels were stored at 4°C for 6, 10, and 14 days. The stored gel samples were equilibrated at room temperature (25°C) before subjecting to firmness measurement.

To evaluate gel firmness, each gel sample was cut into a piece with an exact dimension of 20×20×10 mm. Gel firmness was measured using a texture analyzer (model TA-XT2i, Stable Micro Systems, Surrey, UK). A puncture test was carried out using a stainless-steel spherical probe (P/0.25S) at a pre-test speed of 1 mm/s, test speed of 10 mm/s and 50% strain deformation. The penetration curve was analysed using Exponent Lite software (Stable Micro Systems, Surrey, UK). Gel firmness was defined as the maximum force needed to obtain the specified deformation.

For each fibre, the concentration that resulted in a decrease in retrogradation was selected and used in bread formulation in Section 3.3.3.

3.3.3 Effects of soluble and insoluble fibres on baking and keeping quality of white pan bread

The basic formulation for white pan bread included 500 g wheat flour, 5 g dried yeast, 25 g sugar, 7.5 g salt, 25 g shortening, 300 g water, and 1.5 g calcium propionate (as a mold inhibitor). As selected from Section 3.3.2, the fibres used in this part were 1.0% TG, 1.5% CAR, and 2.0% CEL by weight of flour.

The breads were made by straight dough method following Chondee (2003) with some modifications. Bread without added fibre was used as a control. Firstly, the dry ingredients (wheat flour, dried yeast, calcium propionate, and fibre) were sifted and transferred to a Kenwood mixing bowl. Sugar and salt were separately dissolved in water and then added to the dry ingredients. The mixture was mixed using a food mixer (model CHEF XL, Kenwood, Kittisit, Pathum Thani, Bangkok) attached with a dough hook using a speed of 3 for 2 min. Shortening was added to the flour mixture and mixed at a speed of 6 for 8 min.

The dough obtained was punched, hand kneaded, and fermented at 32°C for 60 min. After that, the risen dough was punched again to remove gas and

divided into 250 g pieces. The divided dough pieces were rounded and rested in a baking tray at 32°C for 30 min. Once rested, the dough was hand-kneaded, sheeted by rolling pin, rolled into log shape, and kept for final proofing at 32°C for 60 min. Finally, the dough was baked in the pre-heated oven at 200°C for 30 min. The baked breads were removed from the pan and cooled at room temperature for 1 h, then packed in the polyethylene bags. The freshly baked breads were analysed in the same day (Day 0) while the rest of the bread samples were stored at room temperature and analysed for the following properties on Days 6, 10 and 14.

3.3.3.1 Moisture content

Moisture content of the bread samples was analysed using air oven method according to AACC (2000) .

3.3.3.2 Water activity

Water activity was analysed using AquaLab^{TM} water activity meter (series 3TE, Decagon Devices, Pullman, WA, USA) at 25°C.

3.3.3.3 Specific loaf volume

Specific loaf volume was determined using rapeseed displacement method according to Bárcenas and Rosell (2006). A container large enough to accommodate the loaf was first over-filled with rapeseeds and the rapeseeds were made even to the rim of the container by scraping with a straight edged spatula. The rapeseeds in the container were then transferred to a measuring cylinder to determine the container volume (V_1). Next, the bread loaf was placed inside the same container and over-filled with rapeseeds. The rapeseeds were scraped to make them even to the rim. The bread loaf was removed, and the rapeseeds were determined for the volume (V_2). The difference between V_1 and V_2 was designated as the loaf volume. Specific loaf volume was calculated by dividing the loaf volume with the loaf weight.

3.3.3.4 Crumb texture

The texture analyzer (model TA-XT2i, Stable Micro Systems, Surrey, UK) equipped with 1 kg load cell was used to measure the crumb texture.

Each bread crumb was sliced by using a bread knife from the midsection of the bread loaf into a cube of 30×30×30 mm. Texture profile analysis (TPA) was carried out in which the bread cube was compressed using a P/100 aluminum compression platen (100 mm diameter) at a test speed of 1 mm/s until achieving 70% strain deformation. Waiting time of 5 s was allowed before making the second compression. The data were analysed by Exponent Lite Express software (Stable Micro Systems, Surrey, UK). Hardness, cohesiveness, springiness, adhesiveness, gumminess, and chewiness were determined from the TPA curve.

3.3.3.5 Water soluble starch content

Water soluble starch content was used as an index of starch crystalinity. The content was determined according to the method described by Shaikh et al. (2007). Two hundred mg of bread crumb were placed in a centrifuge tube, after which 15 ml of distilled water were added. The mixture was then shaken at 25°C for 20 min. The slurry obtained was centrifuged at 1000×g for 10 min. Ten ml of the supernatant were treated with 2 ml of standard iodine solution which was prepared by dissolving 2 g of iodine and 20 mg of potassium iodide in 100 ml of distilled water. A UV-Vis spectrophotometer (GENESYS^{TM} 10, Thermo Fischer Scientific, Waltham, MA, USA) was used to measure the optical density at 680 nm. Concentration of starch was obtained from a standard curve.

3.3.3.6 Bread crumb structure

Bread loaf was sliced across into 2 cm-thick pieces and photographs of bread crumb were taken using a digital camera.

3.3.3.7 Colour

Colour of the crumb and crust were measured using a spectrophotometer (model CM-600d, Konica Minolta Sensing, Osaka, Japan). Bread loaf was sliced across into 2 cm-thick pieces. The crumb was measured at four different positions (A-D) while the crust was measured at two different positions (E and F) (Figure 3.1). The measurement was done in CIELAB system. L*, a*, b*, hue angle, chroma and colour difference (ΔE*) were determined.
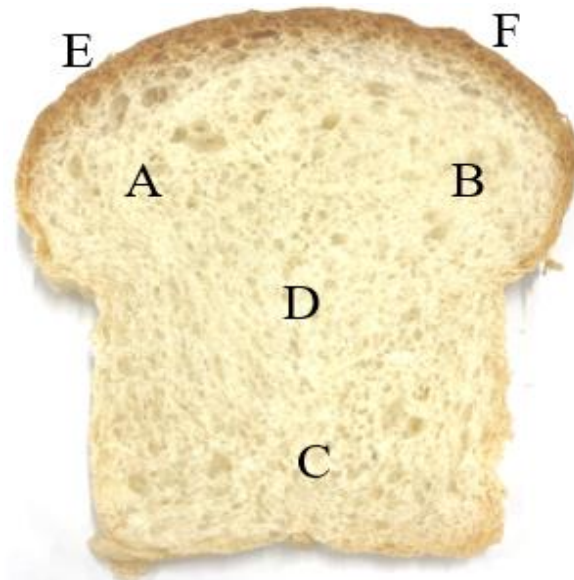
Figure 3. 1 Positions at which colour measurement was done on bread crumb (A, B, C, D) and crust (E, F)

3.3.4 Statistical analysis

All experiments were done in three replicates using a completely randomised design (CRD). Data were analysed using Analysis of Variance (ANOVA). Difference among the means was determined by Duncan's new multiple range test (Cochran & Cox, 1957) at p=0.05 using IBM SPSS Statistics Version 22 for Windows (IBM, Armonk, NY, USA).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Compositions of wheat flour

The compositions of wheat flour used in this study is shown in Table 4.1.

Table 4. 1 Compositions of wheat flour

Compositions	Content
Proximate composition	
Moisture (% wb)	11.41 ± 0.03
Crude protein (% wb)	16.91 ± 0.03
Crude fat (% wb)	0.87 ± 0.08
Ash (% wb)	0.59 ± 0.00
Crude fibre (% wb)	0.21 ± 0.00
Others	
Starch (% wb)	73.03 ± 0.86
Amylose (% of starch)	23.87 ± 0.38

4.2 Effect of soluble and insoluble fibres on pasting behaviour, gelatinisation and retrogradation of wheat flour

4.2.1 Pasting properties

The RVA curves of wheat flour added with 1.0, 1.5, and 2.0% fibres as well as that of the control were shown in Figures 4.1-4.3. The pasting properties are summarised in Table 4.2. It was clear that pasting behaviour of wheat flour was affected by fibre type and concentration.

Regarding the peak viscosity, it was observed that the soluble fibres, TG and CAR, induced a significant increase in peak viscosity from that of the control ($p \leq 0.05$), with TG demonstrating the greatest peak viscosity-increasing effect. Peak viscosity explains the ability of starch granule swelling before their physical breakdown (Rojas et al., 1999). Higher swelling capacity results higher peak viscosity

(Yildiz et al., 2013). The viscosity-increasing ability of TG may be due to that when the gum was dispersed in water, the galactomannan assumed a sheet-like structure in a continuous phase that did not trap starch granules and thus allowed the granule swelling freely, which in turn, resulted in higher peak viscosity. This phenomenon was also demonstrated in guar gum, which is also a galactomannan (Vidaurre-Ruiz et al., 2019). As for CAR, an ionic gum which contains negatively charged sulfate groups, charge repulsion might be responsible for its ability to increase the peak viscosity. The repulsion between like charges inhibits interchain associations. This leads to more water absorption causing greater swelling of granules (Liu et al., 1999). On the other hand, peak viscosity was not affected by the addition of the insoluble fibre CEL, except at the highest addition level of 2.0% in which a significant increase in peak viscosity from that of the control was observed. This result resembles with the another study which demonstrated that water insoluble fibres, like carboxymethyl cellulose, microcrystalline cellulose, and alkaline soluble fibrous cellulose, could increase dynamic viscosity of gelatinised starch but the effect was smaller when compared to water soluble fibres (Kohyama & Nishinari, 1992). However, the result of our study is in contrast to previous report of Goldstein et al. (2010) who found that cellulose of 40 μm and 200 μm particle size caused a decrease in peak and final viscosity of wheat flour. This discrepancy is probably due to the difference in the particle size of CEL used.

Trough viscosity is the minimum viscosity of starch/flour paste obtained when hold at maximum temperature (95°C). It is affected by starch granule swelling and amylose leaching (Yildiz et al., 2013). Regarding TG- and CAR-added samples, the trough viscosity was found to be greater than the control but that of the CEL-containing samples was not significantly different from the control ($p > 0.05$). The higher trough viscosity of those samples added with soluble fibres could probably be due to the viscosity contributing by the fibres.

In general, breakdown viscosity is an index that represents the starch granules disruption and explains the stability of paste. Low breakdown indicates the ability of the starch paste to withstand heat and shear. It was found that breakdown viscosity of TG and CAR-added samples was greater than that of the control while

that of the samples containing CEL was not significantly different from the control (p>0.05). The increase in breakdown viscosity upon adding TG and CAR implied that the paste cannot withstand heat well during cooking indicating low thermal stability and poor shear resistance of the pastes. The result is in agreement with Alam et al. (2009) who investigated the effects of guar gum, carboxymethyl cellulose, and xanthan gum on pasting properties of wheat flour. The authors reported that guar gum caused the highest increase in breakdown viscosity. It was explained that addition of hydrocolloids induced greater degree of granule rupture.

Setback viscosity explains the reassociation of amylose molecules during cooling of starch paste. It indicates short-term retrogradation (Luo et al., 2017). From this study, it was found that 1.0% TG, 1.5% CAR and 2.0% CEL exhibited a significant decrease in setback (p≤0.05) in comparison to the control. This low setback could be due to the competition between fibres and amylose chains which disturbs and weakens the regular amylose-amylose interaction. For TG and CAR, it was found that setback decreased up to a certain fibre concentration after which it became increasing again. Alike, Luo et al. (2017) who previously reported that when inulin was added to wheat starch, it inhibited initial retrogradation as the setback value decreased initially but became increasing with increasing inulin concentration.

For final viscosity, TG at all concentrations showed a significant increase in final viscosity from the control (p≤0.05). In the case of CAR, all samples exhibited greater final viscosity than control where highest concentration (2.0%) caused a significant increase (p≤0.05) while CEL was found to pose no effect on final viscosity. The increase in final viscosity upon adding soluble fibres should be due to their ability to increase the viscosity of the continuous phase (da Silva Costa et al., 2020).

With respect to pasting temperature, a decreasing trend was manifested by TG and CAR addition while CEL incorporation did not affect pasting temperature. Decreased pasting temperature indicates free swelling of starch granules. In this result soluble fibres especially TG, did not restrict the granule swelling which obviously decreased the pasting temperature. Regarding peak time, it is the time required for the

peak viscosity to occur. It is related with the duration of swelling of granules until rupture. It was seen that the fibres, in general, posed a minimal effect on peak time.
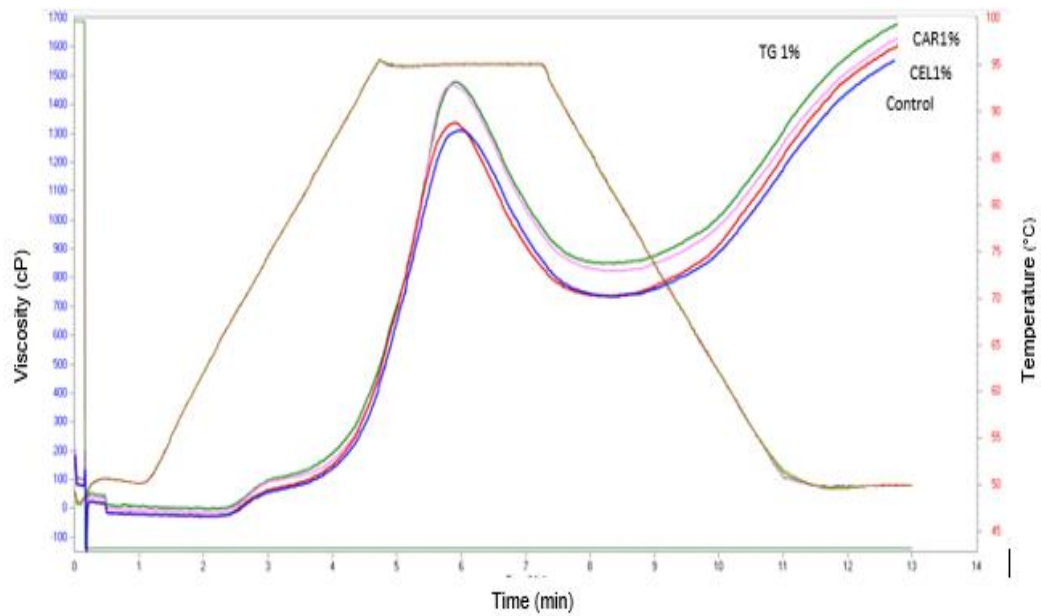
Figure 4. 1 RVA curves of wheat flour (control 0%) and wheat flour added with tara gum (TG), κ-carrageenan (CAR) and cellulose (CEL) at 1.0% level
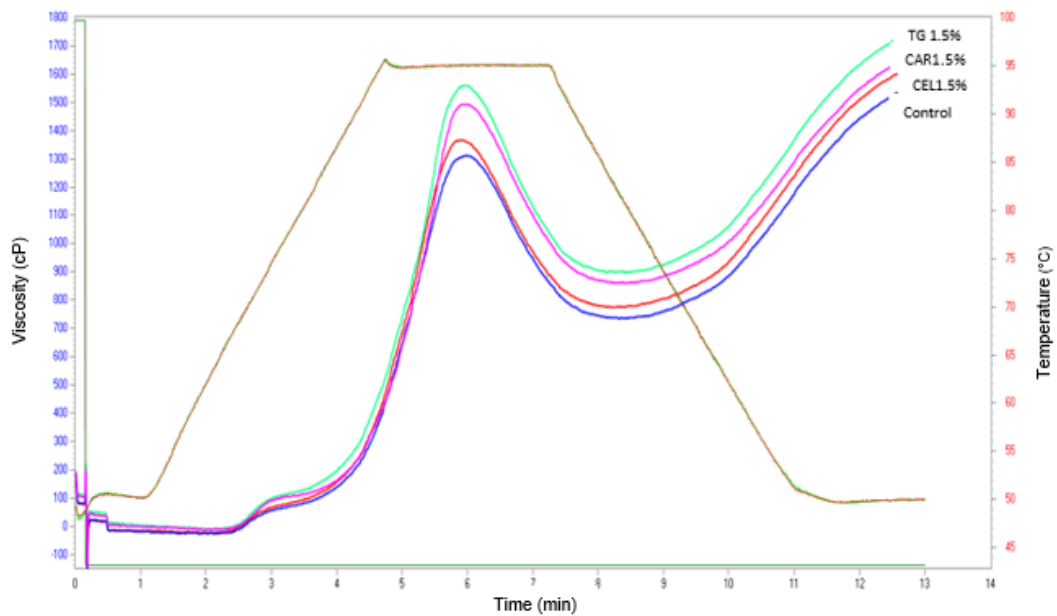
Figure 4. 2 RVA curves of wheat flour (control 0%) and wheat flour added with tara gum (TG), κ-carrageenan (CAR) and cellulose (CEL) at 1.5% level
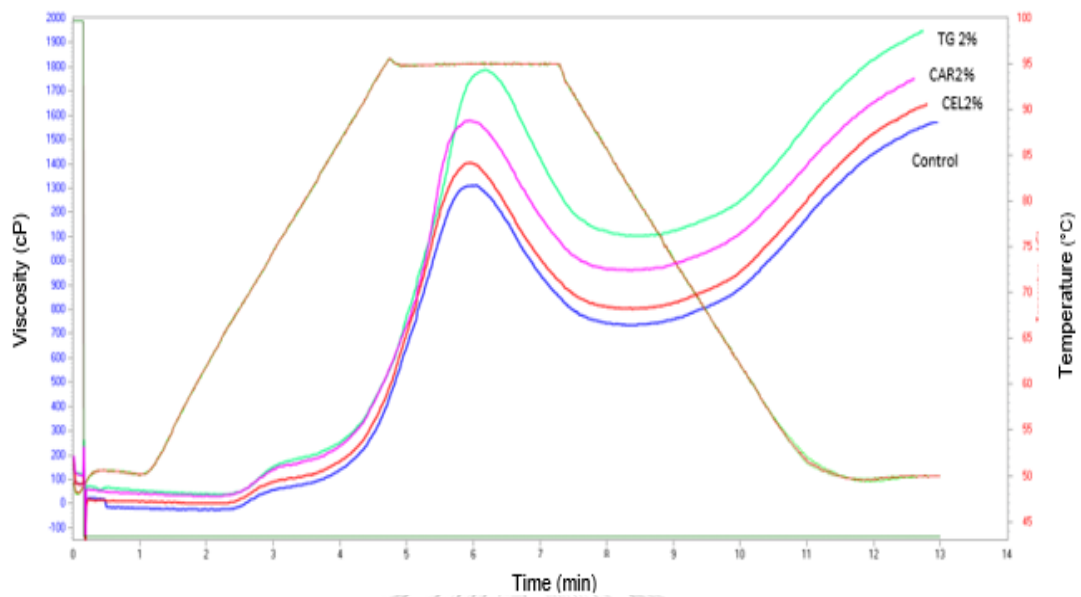
Figure 4. 3 RVA curves of wheat flour (control 0%) and wheat flour added with tara gum (TG), κ-carrageenan (CAR) and cellulose (CEL) at 2.0% level

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Table 4. 2 Pasting properties of wheat flour (WF) added with tara gum (TG), κ-carrageenan (CAR), and cellulose (CEL) at 1.0, 1.5, and 2.0% levels

Samples	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown viscosity (cP)	Final viscosity (cP)	Setback viscosity (cP)	Peak time (min)	Pasting temperature (°C)
WF (Control 0%)	1350.67±35.47 [f]	762.00±48.22 [e]	588.67±15.28 [d]	1632.00±44.03 [d]	870.00±4.36 [ab]	5.94±0.08 [bc]	88.40±0.43 [a]
WF+TG 1.0%	1463.00±25.12 [c]	860.33±30.29 [c]	603.67±25.38 [cd]	1695.67±27.57 [c]	835.33±13.58 [c]	5.96±0.08 [bc]	88.23±0.94 [a]
WF+TG 1.5%	1569.67±19.58 [b]	933.67±36.07 [b]	635.00±23.77 [bc]	1784.67±27.30 [b]	851.00±19.70 [bc]	6.06±0.10 [ab]	77.03±9.80 [bc]
WF+TG 2.0%	1752.00±31.00 [a]	1069.67±41.40 [a]	682.33±24.11 [a]	1940.00±31.10 [a]	870.33 ±20.55 [ab]	6.12±0.06 [a]	70.65±0.61 [c]
WF+CAR 1.0%	1422.33±14.47 [d]	772.67±11.72 [e]	650.67±24.11 [ab]	1643.33±4.04 [d]	870.67±15.70 [ab]	5.87±0.08 [c]	88.35±0.48 [a]
WF+CAR 1.5%	1481.33±13.20 [c]	841.33±19.01 [cd]	640.00±6.24 [bc]	1669.00±20.07 [cd]	827.67±2.52 [c]	5.92±0.09 [c]	82.02±10.48 [ab]
WF+CAR 2.0%	1555.33±30.44 [b]	917.33±39.83 [b]	638.00±20.00 [bc]	1766.00±35.79 [b]	848.67±22.74 [bc]	5.90±0.04 [c]	70.15±0.40 [c]
WF+CEL 1.0%	1351.67±13.66 [f]	742.67±9.29 [e]	608.00±12.17 [cd]	1628.67±10.97 [d]	886.00±17.35 [a]	5.88±0.06 [c]	87.67±0.65 [a]
WF+CEL 1.5%	1369.00±13.77 [ef]	754.00±30.41 [e]	615.00±24.52 [bcd]	1630.33±29.74 [d]	876.33±3.06 [ab]	5.87±0.06 [c]	87.48±0.67 [a]
WF+CEL 2.0%	1393.33±22.94 [de]	798.00±9.85 [de]	595.33±13.66 [d]	1652.67±26.86 [cd]	854.67±17.10 [bc]	5.98±0.03 [bc]	88.58±0.33 [a]

Mean ± SD of three replicates

Means in the same column with different superscript letter differ significantly at p=0.05.

4.2.2 Gelatinisation

A typical thermogram of gelatinisation obtained from DSC is shown in Figure 4.4. The gelatinisation temperature and enthalpy of wheat flour and wheat flour added with fibres were shown in Table 4.3.
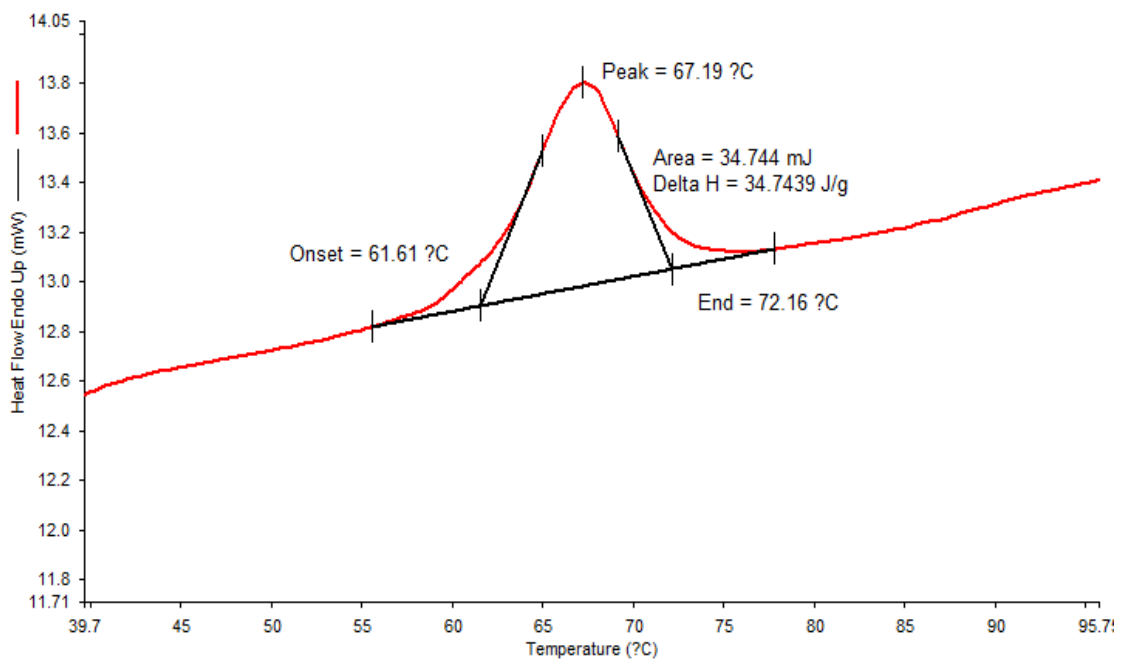
Figure 4. 4 A typical DSC thermogram of wheat flour sample showing an endothermic transition associated with starch gelatinisation

It was observed that fibre addition posed a nominal effect on gelatinisation temperature. The transition temperature ranged from 60.68-61.87°C for onset temperature (T_o), 66.78-67.78°C for peak temperature (T_p) and 71.83-73.28°C for conclusion temperature (T_c). Gelatinisation temperature of all samples varied within a narrow range and were not significantly different (p>0.05) compared to the control. This result is in consistent with a previous report by Rojas et al. (1999) who reported that addition of 0.5 and 1% of guar gum, pectin, alginate, xanthan gum , κ-carrageenan, and hydroxypropylmethyl cellulose posed no significant effect on gelatinisation temperature as compared to the control wheat flour. Biliaderis et al. (1997) also reported that addition of 1% of xanthan gum, guar gum, oat β-glucan, and arabinoxylan had no effect on the gelatinisation temperature of wheat starch.

Table 4. 3 Temperature and enthalpy (ΔH_G) of gelatinisation of wheat flour (WF) added with tara gum (TG), κ -carrageenan (CAR), and cellulose (CEL) at 1.0, 1.5, and 2.0% levels

Samples	Onset temperature (T_o) (°C)	Peak temperature (T_p) (°C)	Conclusion temperature (T_c) (°C)	ΔH_G (J/g)
WF (control 0%)	61.29±0.01 [ab]	66.78± 0.11[c]	72.17 ± 0.10 [ab]	12.10 ± 0.42 [a]
WF+TG 1.0%	61.57±0.83 [ab]	67.12 ±0.12 [bc]	72.96 ± 1.03 [ab]	9.76 ± 1.54 [b]
WF+TG 1.5%	61.41±0.01 [ab]	66.78 ± 0.12 [c]	71.88 ± 0.40 [b]	10.24 ± 0.79 [b]
WF+TG 2.0%	60.68±0.74 [b]	67.37 ± 0.47 [ab]	72.99 ± 0.70 [ab]	10.39 ± 1.31 [ab]
WF+CAR 1.0%	61.24±0.04 [ab]	67.28 ± 0.12 [abc]	73.19 ± 0.47 [a]	7.85 ± 0.26 [c]
WF+CAR 1.5%	61.87±0.20 [a]	67.28 ± 0.12 [abc]	72.75 ± 0.25 [ab]	7.32 ± 0.32 [c]
WF+CAR 2.0%	61.51±0.70 [ab]	67.78 ± 0.12 [a]	73.28 ± 0.15 [a]	7.40 ± 0.51 [c]
WF+CEL 1.0%	61.25±0.37 [ab]	66.78 ± 0.36 [c]	71.91 ± 0.04 [b]	10.56 ± 0.48 [ab]
WF+CEL 1.5%	61.57±0.06 [ab]	67.19 ± 0.00 [bc]	71.83 ± 0.47 [b]	10.65 ± 0.48 [ab]
WF+CEL 2.0%	61.76±0.04 [ab]	67.02 ± 0.24 [b]	72.39 ± 0.40 [ab]	10.39 ± 0.35 [ab]

Mean ± SD of three replicates

Means in the same column with different superscript letter differ significantly at p=0.05.

For the gelatinisation enthalpy, it was found that control wheat sample exhibited the highest gelatinisation enthalpy (ΔH_G) (12.10 J/g). Addition of fibres, particularly the soluble ones, caused a decrease in ΔH_G. The significant decrease ($p \leq 0.05$) was obtained in the case of CAR for all three concentrations. This is in consistent with the study of Tang et al. (2013) where gelatinisation enthalpy became decreasing when xanthan gum was added to rice starch. Rojas et al. (1999) also reported that addition of 0.5 and 1.0% of guar gum, pectin, alginate, xanthan gum, κ-carrageenan, and hydroxypropylmethyl cellulose to wheat flour caused overall decrease in enthalpy of gelatinisation. Šubarić et al. (2011) proposed that soluble fibres reduced water availability, thus causing partial gelatinisation of starch granules. Moreover, starch-fibre interaction may also play a role in reducing ΔH_G.

4.2.3 Retrogradation (amylopectin crystallite melting)

Due to the branched structure of amylopectin, it retrogrades at a slower rate than the linear amylose extending from days to weeks. Recrystallisation of

amylopectin thus signifies long-term retrogradation which occurs during storage of starchy products (Luo et al., 2017). During the storage periods, the amylopectin molecules re-associate with amylose or amylopectin chains by the formation of double helices (Huang et al., 2019). The enthalpy of retrogradation (ΔH_R) serves as a quantitative measure of the energy required to melt the reassociated amylopectin (Singh et al., 2010). In this study, DSC was used to monitor the retrogradation of gelatinised wheat flour systems stored at 4°C at the storage time of 6, 10 and 14 days. A typical DSC curve of melting of amylopectin crystallites is illustrated in Figure 4.5 and the temperature and enthalpy required to melt recrystallised amylopectin are shown in Table 4.4.
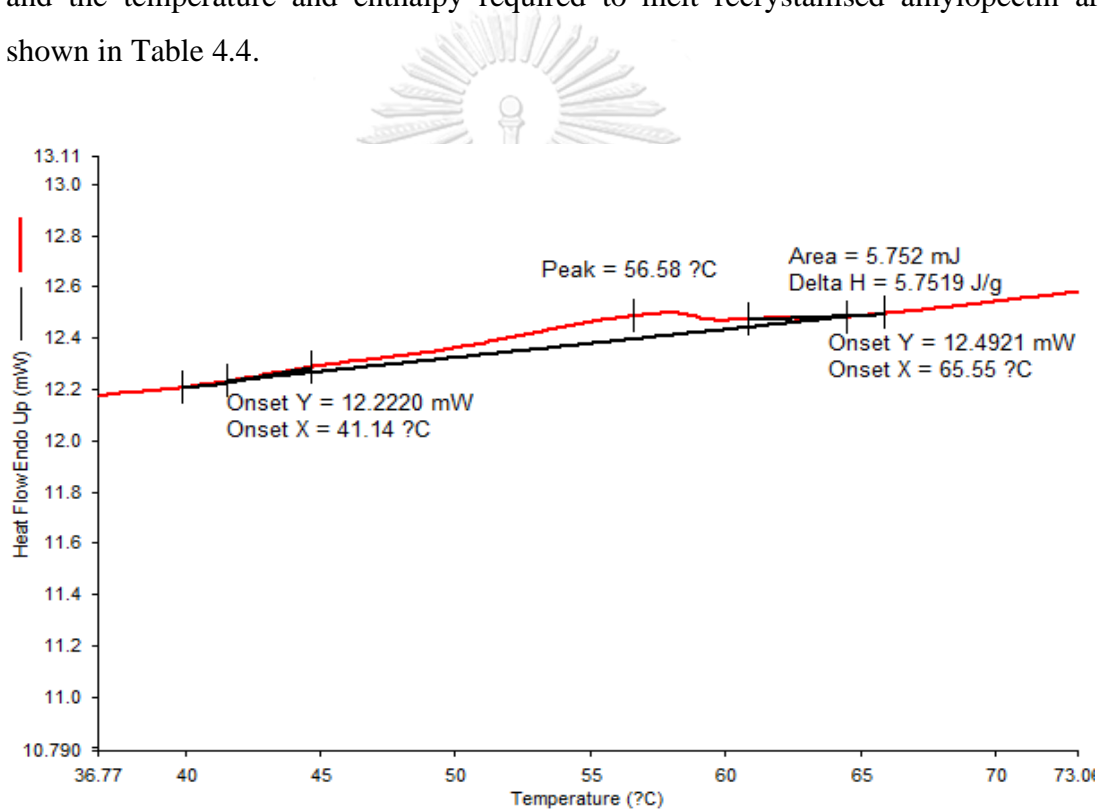
Figure 4. 5 A typical DSC thermogram of wheat flour sample showing an endothermic transition associated with amylopectin crystallite melting

The melting endotherm for the control and fibre-added samples were found in the range of 40-70°C. There was no significant difference (p>0.05) in the T_o, T_p, T_c between the control and fibre-added samples. Peak temperature of amylopectin crystallite melting of all samples was in a range of 51-57°C.

It was found that fibre addition did not pose a significant effect on retarding amylopectin recrystallisation since ΔH_R of the fibre-added samples were not significantly different from that of the control (p>0.05). Anyhow, a decreasing trend in ΔH_R could be noticed in the CAR- and CEL-added samples. We speculate that if added at a higher amount, both fibres may have retrogradation-retarding effect.

Various fibres, particularly the soluble ones, have been reported to slow down short-term and long-term retrogradation (Chen et al., 2015; Ma et al., 2019). This inhibition might be due to the competition for available water molecules between starch and fibre during crystallisation. This helps to decrease retrogradation as water would be bound in the system (Tang et al., 2013). According to Luo et al. (2017), inulin was shown to decrease the retrogradation enthalpy as compared to the control. The authors reported that inulin could retard short-term and long-term retrogradation when added in the amount less than 15% of wheat starch. It was hypothesized that starch may not be fully gelatinised in the presence of fibres, which may decrease starch mobility and further restricts interaction between amylopectin chains leading to decreased recrystallisation of starch molecules (Ma et al., 2019; Tang et al., 2013).

Table 4. 4 Tempetrature and enthalpy of amylopectin crystalline melting (ΔH$_R$) of wheat flour (WF) added with tara gum (TG), κ -carrageenan (CAR), and cellulose (CEL) at 1.0, 1.5, and 2.0% levels and stored at 4°C for 6, 10, and 14 days

Samples	Onset temperature (T$_o$) (°C)			Peak temperature (T$_p$) (°C)			Conclusion temperature (T$_c$) (°C)			ΔH$_R$ (J/g)		
	Day 6 ns	Day 10 ns	Day 14	Day 6	Day 10	Day 14	Day 6 ns	Day 10	Day 14	Day 6	Day 10	Day 14
WF (control 0%)	40.61 ±1.91	40.94 ±0.31	41.19 ±0.67[ab]	54.93 ±0.62[a]	56.55 ±0.95[a]	57.25 ±0.05[a]	62.50 ±0.73	63.62 ±0.09[a]	64.66 ±0.21[ab]	1.05 ±0.01[abc]	1.42 ±0.27[abc]	1.45 ±0.06[ab]
WF+TG 1.0%	41.36 ±0.57	41.82 ±0.84	42.21 ±0.48[ab]	52.19 ±0.61[ab]	54.13 ±0.59[ab]	56.08 ±0.44[ab]	62.29 ±1.60	61.28 ±0.13[ab]	61.95 ±0.65[b]	1.26 ±0.36[abc]	1.64 ±0.27[ab]	1.67 ±0.45[ab]
WF+TG 1.5%	40.67 ±1.82	41.37 ±1.43	41.85 ±0.05a[b]	53.67 ±0.33[a]	55.30 ±0.07[ab]	56.75 ±0.12[ab]	64.05 ±0.19	62.30 ±0.05[a]	63.40 ±0.29[ab]	1.35 ±0.56[ab]	1.71 ±0.25[a]	1.84 ±0.24[ab]
WF+TG 2.0%	41.13 ±0.31	40.91 ±0.47	41.99 ±0.88[ab]	55.13 ±0.04[a]	55.95 ±1.05[ab]	57.66 ±0.12[a]	62.58 ±0.18	62.92 ±0.10[a]	64.47 ±1.50[ab]	1.57 ±0.18[a]	1.84 ±0.34[a]	2.12 ±0.14[a]
WF+CAR 1.0%	42.71 ±0.36	41.68 ±0.38	42.90 ±0.50[ab]	52.24 ±0.17[ab]	54.37 ±0.47[ab]	56.58 ±0.70[ab]	60.74 ±1.86	62.56 ±0.21[a]	63.51 ±0.81[ab]	0.83 ±0.31[bc]	1.81 ±0.03[a]	0.92 ±0.22[b]
WF+CAR 1.5%	41.58 ±0.47	42.68 ±0.38	41.76 ±0.76[ab]	51.99 ±0.29[b]	55.12 ±0.11[ab]	56.91 ±0.46[ab]	59.87 ±0.64	61.65 ±0.37[ab]	63.75 ±0.27[ab]	0.64 ±0.11[c]	1.15 ±0.84[bc]	1.31 ±0.00[ab]
WF+CAR 2.0%	41.82 ±0.49	41.16 ±0.72	40.90 ±0.18[b]	52.14 ±0.09[ab]	55.29 ±0.01[ab]	55.91 ±0.71[ab]	59.62 ±0.41	61.56 ±0.10[ab]	63.07 ±0.28[ab]	0.92 ±0.08[abc]	1.60 ±0.56[ab]	1.78 ±0.25[ab]
WF+CEL 1.0%	41.51 ±0.18	40.11 ±0.35	44.11 ±0.19[a]	53.60 ±0.20[a]	55.12 ±0.59[ab]	56.50 ±0.12[ab]	59.99 ±0.12	62.31 ±0.18[a]	64.49 ±0.17[ab]	0.67 ±0.10[c]	1.35 ±0.13[abc]	1.03 ±0.53[b]
WF+CEL 1.5%	41.75 ±0.56	40.91 ±0.33	41.83 ±0.20[ab]	53.58 ±1.39[a]	54.86 ±0.24[ab]	56.82 ±0.35[ab]	61.20 ±0.91	62.12 ±0.42[a]	66.38 ±0.96[ab]	0.82 ±0.10[bc]	1.35 ±0.28[abc]	1.41 ±0.3[ab]
WF+CEL 2.0%	42.58 ±0.36	41.05 ±0.36	40.20 ±0.49[b]	54.76 ±3.27[a]	54.88 ±0.01[ab]	57.24 ±0.01[a]	63.05 ±0.08	62.75 ±0.34[a]	63.88 ±0.21[ab]	0.84 ±0.08[bc]	0.96 ±0.12[c]	0.99 ±0.99[b]

Mean ± SD of three replicates

Means within the same column followed by different superscripts letter differ significantly at p=0.05

ns Means within the column were not significantly different at p=0.05.

4.2.4 Gel firmness

Gel firmness is a key parameter to estimate retrogradation capacity as the firmness is induced by the increase in crystalline structure (Qiu et al., 2017). Upon storage, the firmness of gel continues to increase due to the recrystallisation of amylopectin (Ai & Jane, 2015). Firmness of the gel samples is summarised in Table 4.5

Table 4. 5 Firmness of gel from wheat flour (WF) added with tara gum (TG), κ - carrageenan (CAR), and cellulose (CEL) at 1.0, 1.5, and 2.0% during storage at 4°C

Samples	Gel firmness (N)			
	Day 0	Day 6	Day 10	Day 14
WF (control 0%)	0.09 ± 0.00 [de]	0.27 ± 0.02 [b]	0.34 ± 0.02 [b]	0.37 ± 0.02 [a]
WF+TG 1.0%	0.08 ± 0.00 [e]	0.20 ± 0.00 [d]	0.25 ± 0.01 [c]	0.27 ± 0.01 [b]
WF+TG 1.5%	0.10 ± 0.00 [de]	0.27 ± 0.02 [b]	0.34 ± 0.03 [b]	0.36 ± 0.03 [a]
WF+TG 2.0%	0.12 ± 0.00 [bc]	0.35 ± 0.02 [a]	0.41 ± 0.03 [a]	0.40 ± 0.04 [a]
WF+CAR 1.0%	0.10 ± 0.01 [cd]	0.22 ± 0.02 [cd]	0.23 ± 0.01 [c]	0.25 ± 0.02 [b]
WF+CAR 1.5%	0.12 ± 0.01 [b]	0.20 ± 0.01 [d]	0.24 ± 0.02 [c]	0.26 ± 0.02 [b]
WF+CAR 2.0%	0.16 ± 0.00 [a]	0.20 ± 0.01 [d]	0.25 ± 0.02 [c]	0.26 ± 0.01 [b]
WF+CEL 1.0%	0.11 ± 0.02 [bcd]	0.26 ± 0.05 [bc]	0.28 ± 0.05 [c]	0.29 ± 0.04 [b]
WF+CEL 1.5%	0.09 ± 0.01 [de]	0.20 ± 0.03 [d]	0.24 ± 0.04 [c]	0.26 ± 0.04 [b]
WF+CEL 2.0%	0.08 ± 0.02 [e]	0.14 ± 0.02 [e]	0.17 ± 0.03 [d]	0.19 ± 0.03 [c]

Mean ± SD of three replicates

Means in the same column with different superscript letter differ significantly at p=0.05.

At Day 0, higher concentrations of TG and CAR did affect gel firmness while that of the CEL-containing samples was not different from the control (p>0.05). Firmness of all gel samples continued to increase up to 10 days of storage and became level off after that. At each storage time, most of the fibre-containing gels tended to have lower firmness than the control. This is consistent with the ΔH_R discussed above. In general, it was seen that soluble fibres tended to decrease short-term and long-term retrogradation.

For this part, the concentration that resulted in a decrease in retrogradation was selected for each fibre and used in bread formulation in the next section. Fibre type and concentration chosen were 1.0% TG, 1.5% CAR, and 2.0% CEL by weight of flour.

4.3 Effects of soluble and insoluble fibres on baking and keeping quality of white pan bread

In this part, white pan bread was prepared from wheat flour added with 1.0% TG, 1.5% CAR, and 2.0% CEL. The freshly baked breads (Day 0) and those stored at room temperature (25°C) for 6, 10, and 14 days were subjected to property analysis, as compared to the control wheat bread.

4.3.1 Moisture content

Moisture content of food is an important parameter that determines the bread quality and stability. Bread crumb is generally higher in moisture content than the outer crust. This leads to moisture migration from crumb to crust during cooling and storage (Sharadanant & Khan, 2003). This, in turn, poses an effect on starch retrogradation and staling during storage of bread. Moisture content of the bread samples are presented in Table 4.6.

Table 4. 6 Moisture content of breads added with 1.0% tara gum (TG), 1.5% κ-carrageenan (CAR), and 2.0% cellulose (CEL) during storage at 25°C

Bread samples	Moisture content (% wb)			
	Day 0 [ns]	Day 6 [ns]	Day 10	Day 14
WF (control 0%)	37.67 ± 1.53	36.09 ± 0.16	32.27 ± 0.47 [b]	31.33 ± 0.71[c]
WF+TG 1.0%	37.33 ± 2.52	36.43± 0.50	33.74 ± 0.84 [a]	34.33 ± 0.45[a]
WF+CAR 1.5%	36.67 ± 3.06	36.02 ± 0.33	33.94 ± 0.91 [a]	33.39 ± 0.31[ab]
WF+CEL 2.0%	36.33 ± 2.52	36.07 ± 0.14	32.81 ± 0.44 [ab]	33.07 ± 0.56 [b]

Mean ± SD of three replicates

Means in the same column with different superscript letter differ significantly at p=0.05.

[ns] Means in the same column were not significantly different at p=0.05.

Moisture content of the freshly baked breads were not significantly different (p>0.05) from the control. Upon storage, a slight decrease in moisture content was observed in all bread samples. This is due to the moisture transfer from the crumb towards the crust and from the crust to the surrounding atmosphere (Ghodke, 2009; Ho et al., 2014). On Day 6, all the samples were not significantly different (p>0.05) in moisture content. However, at Day 10, a decrease was observed in control sample and this decrease continued further to reach 31.33% on Day 14. This decrease was significantly different (p≤0.05) from the fibre-added breads. It was seen that TG-containing bread was the most efficient in retaining moisture. However, all fibre-added breads demonstrated higher moisture content than the control. This result is in agreement with the study of Movahed et al. (2014). In another study by Guarda et al. (2004), breads with added carrageenan, xanthan, hydroxypropylmethyl cellulose, and alginate at 0.5 and 1.0% levels showed lower moisture loss proving that breads with added hydrocolloid retained more water than the control. Similar reports was observed by Ghodke (2009) where addition of 0.25-1.0% guar gum to wheat *chapatti* (Indian unleavened bread) retained higher moisture content up to 3 days of storage. High water holding capacity of fibres is also considered a factor delaying long term retrogradation and staling.

4.3.2 Water activity

Water activity of the bread samples are shown in Table 4.7. Water activity of freshly baked breads were similar (0.97) in the control and fibre-added breads. It was agreeable with the results of Sahraiyan et al. (2013), in which guar and cress seed gums had no effect on water activity of rice-wheat composite bread. Liu et al. (2017) also reported that konjac glucomannan, hydroxypropylmethyl cellulose, apple pectin, and gum arabic did not affect water activity of wheat potato-wheat breads.

It was obvious that with increasing storage time, water activity became decreasing. The reduction in the water activity was in accordance with the decrease in moisture content (Section 4.3.1). It was observed that the water activity was not affected by the addition of soluble and insoluble fibres

Table 4. 7 Water activity of breads added with 1.0% tara gum (TG), 1.5% κ-carrageenan (CAR), and 2.0% cellulose (CEL) during storage at 25°C

Bread samples	Water activity			
	Day 0[ns]	Day 6[ns]	Day 10[ns]	Day 14[ns]
WF (control 0%)	0.97 ± 0.00	0.96 ± 0.01	0.95 ± 0.01	0.94 ± 0.00
WF+TG 1.0%	0.97 ± 0.00	0.96 ± 0.00	0.96 ± 0.01	0.95 ± 0.00
WF+CAR 1.5%	0.97 ± 0.00	0.96 ± 0.00	0.96 ± 0.00	0.95 ± 0.01
WF+CEL 2.0%	0.97 ± 0.00	0.96 ± 0.01	0.96 ± 0.01	0.95 ± 0.01

Mean $\pm$ SD of three replicates

Means in the same column with different superscript letter differ significantly at p=0.05.

[ns] Means in the same column were not significantly different at p=0.05.

4.3.3 Specific loaf volume

Specific loaf volume reflected the gas retention capacity of the bread, and it affects consumer's acceptance. Appearance of the bread samples was depicted in Figure 4.6 and the specific loaf volume was shown in Table 4.8.

CEL   TG   CAR   Control

Figure 4. 6 Appearance of the bread samples. From left to right: cellulose (CEL)-added bread, tara gum (TG)-added bread, κ -carrageenan (CAR)-added bread, and control bread

Table 4. 8 Specific loaf volume of breads added with 1.0% tara gum (TG), 1.5% κ-carrageenan (CAR), and 2.0% cellulose (CEL) during storage at 25°C

Bread samples	Specific loaf volume (cm³/g)			
	Day 0	Day 6	Day 10	Day 14
WF (control 0%)	5.20 ± 1.34^b	5.12 ± 0.14^b	5.01 ± 0.05^c	4.87 ± 0.07^a
WF+TG 1.0%	5.37 ± 0.17^b	5.35 ± 0.17^b	5.28 ± 0.19^b	5.08 ± 0.27^a
WF+CAR 1.5%	4.80 ± 0.64^c	4.78 ± 0.53^c	4.67 ± 0.15^d	4.57 ± 0.06^b
WF+CEL 2.0%	6.05 ± 0.12^a	6.01 ± 0.12^a	5.86 ± 0.06^a	5.12 ± 0.11^a

Mean ± SD of three replicates

Means in the same column with different superscript letter differ significantly at p=0.05.

It was observed that specific loaf volume differed among the bread samples. Interestingly, CEL demonstrated the highest specific loaf volume. Cellulose and plant fibres have been known to pose a negative impact on bread volume. In this study, we speculate that the high specific loaf volume of the CEL-containing bread may be due to the small particle size of cellulose (20 μm) which did not interfere with the formation of dough network.

For the soluble fibres, TG-containing bread had slightly higher specific volume (5.37 cm³/g) than the control (5.20 cm³/g), but these were not significantly different (p>0.05). CAR-containing bread, on the other hand, demonstrated the lowest specific loaf volume (4.80 cm³/g). This is in agreement with Mandala (2005) who found that, at the concentration greater than 0.1%, ionic gum (xanthan gum) caused a reduction in loaf volume. Rubel et al. (2015) explained that addition of soluble fibres to bread could result in gluten dilution effect which reduced gas retention ability of the dough and gave rise to a lower loaf volume. Sivam et al. (2010) proposed that the high-water affinity of soluble fibre resulted in less water available for the gluten network development. This causes underdeveloped gluten network and reduces the loaf volume. In contrast, it reported that breads with 1% soluble fibres had comparable loaf volume to the control whereas insoluble fibres caused a reduction in loaf volume (Dalgetty & Baik, 2006). Bouaziz et al. (2020) explained that fibre addition to bread could either increase or decrease the loaf volume, depending on the fibre source and supplementation level.

During storage, all bread samples demonstrated a decreasing specific loaf volume. On Day 10 of storage, CEL-added bread continued to bear higher specific loaf volume while CAR-added bread still had the lowest specific loaf volume. On Day 14, CEL-containing bread had a reduction in specific loaf volume to the value that was not significantly different (p>0.05) from the TG-added bread and the control.

4.3.4 Texture profile
4.3.4.1 Hardness

Crumb hardness is an important parameter to monitor staling of bread. Hardness is the peak force that occurs during first compression (Sahin & Sumnu, 2006). Hardness of the crumb samples are shown in Table 4.9. For the freshly baked bread (Day 0), the control was significantly (p≤0.05) higher in hardness than the fibre-added breads while TG- and CEL-added breads possessed lower crumb hardness than the CAR-added bread.

Table 4. 9 Hardness of breads added with 1.0% tara gum (TG), 1.5% κ-carrageenan (CAR), and 2.0% cellulose (CEL) during storage at 25°C

Bread samples	Hardness (g_f)			
	Day 0	Day 6	Day 10	Day 14
WF (control 0%)	274.62 ± 5.17^a	678.71 ± 5.04^a	781.68 ± 2.53^a	845.81 ± 2.66^a
WF+TG 1.0%	167.15 ± 3.74^c	513.81 ± 2.23^d	683.00 ± 1.53^c	757.00 ± 4.47^d
WF+CAR 1.5%	200.81 ± 2.53^b	566.59 ± 3.77^b	746.00 ± 2.07^b	820.00 ± 1.51^b
WF+CEL 2.0%	164.30 ± 2.38^c	550.16 ± 1.99^c	622.38 ± 1.45^d	786.32 ± 4.06^c

Mean ± SD of three replicates

Means in the same column with different superscript letter differ significantly at p=0.05.

The reduction in crumb hardness of the fibre-added breads could be attributed to the moisture retention capacity of fibres as well as the gas retention capacity of the dough which produced a bread with greater specific loaf volume. According to Guarda et al. (2004), when hydrocolloids were added in starchy products, the weakening effect on the starch structure led to better water retention and

distribution causing reduced hardness. Regarding TG, the softening effect initiated by galactomannans inhibited amylopectin retrogradation, together with its hydrophilic nature, led to reduced hardness (Kohajdová & Karovičová, 2009; Shalini & Laxmi, 2007). During storage, an increase in crumb hardness was observed in all samples. The control possessed the highest hardness throughout the storage period. However, the hardness of all samples seemed to increase at a similar rate.

4.3.4.2 Springiness

Springiness shows how well the product returns to its original shape after the force was removed. It is also another parameter to determine degree of staling in bread (Lauková et al., 2017). Springiness of the bread samples are illustrated in Table 4.10. It was found that all fibre-containing bread had more springiness than the control (p≤0.05). This may be due to the indigenous springiness of each fibre. Springiness has been demonstrated by various gums. For example, Samutsri & Thimtuad, 2018 reported that Yanang (*Tiliacora triandra*) gum helped enhancing springiness of waxy rice flour gel (Samutsri & Thimtuad, 2018).

Table 4. 10 Springiness of breads added with 1.0% tara gum (TG), 1.5% κ-carrageenan (CAR), and 2.0% cellulose (CEL) during storage at 25°C

Bread samples	Springiness			
	Day 0	Day 6 [ns]	Day 10 [ns]	Day 14
WF (control 0%)	0.42 ± 0.01^b	0.39 ± 0.02	0.37 ± 0.01	0.37 ± 0.01^b
WF+TG 1.0%	0.45 ± 0.01^a	0.42 ± 0.02	0.37 ± 0.01	0.39 ± 0.01^a
WF+CAR 1.5%	0.44 ± 0.02^{ab}	0.40 ± 0.00	0.37 ± 0.01	0.38 ± 0.01^{ab}
WF+CEL 2.0%	0.45 ± 0.00^a	0.40 ± 0.03	0.37 ± 0.03	0.39 ± 0.01^a

Mean ± SD of three replicates

Means in the same column with different superscript letter differ significantly at p=0.05.

[ns] Means in the same column were not significantly different at p=0.05.

With increasing storage time, it was observed that springiness decreased upto Day 10 and remained relatively constant after that. This result is in

agreement with Ho et al. (2014) who reported a decrease in springiness of composite breads made of banana pseudo stem flour and wheat flour with added xanthan gum and sodium carboxymethyl cellulose during a 3-day storage.

4.3.4.3 Cohesiveness

Cohesiveness represents the ability of the matrix of a material to hold together. Cohesiveness of the bread sample are shown in Table 4.11. For freshly baked bread, the control bread had the lowest cohesiveness compared to the fibre-added breads. Cohesiveness of the CAR-added bread was not significantly different (p>0.05) from control while TG- and CEL-added breads had significantly higher cohesiveness than the control.

With increasing storage time, cohesiveness of all bread samples decreased. The decrease in cohesiveness was more pronounced during the first 6 days of storage as it became levelled off after that. This result is in agreement with Ho et al. (2014). Lauková et al. (2017) reported that cohesiveness of bread increased upon addition of cellulose.

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Table 4. 11 Cohesiveness of breads added with 1.0% tara gum (TG), 1.5% κ-carrageenan (CAR), and 2.0% cellulose (CEL) during storage at 25°C

Bread samples	Cohesiveness			
	Day 0	Day 6	Day 10	Day 14
WF (control 0%)	0.69 ± 0.04^{c}	0.49 ± 0.04^{b}	0.45 ± 0.03^{b}	0.45 ± 0.02^{c}
WF+TG 1.0%	0.77 ± 0.15^{a}	0.56 ± 0.03^{a}	0.49 ± 0.40^{a}	0.48 ± 0.01^{ab}
WF+CAR 1.5%	0.72 ± 0.10^{bc}	0.55 ± 0.02^{a}	0.47 ± 0.01^{a}	0.48 ± 0.02^{ab}
WF+CEL 2.0%	0.75 ± 0.01^{ab}	0.55 ± 0.01^{a}	0.52 ± 0.01^{a}	0.49 ± 0.03^{a}

Mean ± SD of three replicates

Means in the same column with different superscript letter differ significantly at p=0.05.

[ns] Means in the same column were not significantly different at p=0.05.

4.3.4.4 Adhesiveness

Adhesiveness is measured as the negative force area for the first compression that explains the energy required to remove the plunger away from the food (Sahin & Sumnu, 2006). From this study, it was found that fibre addition posed no effect on adhesiveness of the bread samples since all samples were similar in adhesiveness throughout the storage period (Table 4.12).

Table 4. 12 Adhesiveness of breads added with 1.0% tara gum (TG), 1.5% κ-carrageenan (CAR), and 2.0% cellulose (CEL) during storage at 25°C

Bread samples	Adhesiveness			
	Day 0 [ns]	Day 6 [ns]	Day 10 [ns]	Day 14 [ns]
WF (control 0%)	-0.24 ± 0.27	-1.86 ± 0.13	-2.13 ± 0.25	-2.58 ± 1.67
WF+TG 1.0%	-0.19 ± 0.06	-1.18 ± 0.70	-1.89 ± 1.34	-2.03 ± 0.25
WF+CAR 1.5%	-0.12 ± 0.10	-1.52 ± 1.21	-0.73 ± 0.33	-3.24 ± 0.03
WF+CEL 2.0%	-0.29 ± 0.50	-1.45 ± 0.07	-1.32 ± 0.04	-1.90 ± 0.02

Mean ± SD of three replicates

Means in the same column with different superscript letter differ significantly at p=0.05.

[ns] Means in the same column were not significantly different at p=0.05.

4.3.4.5 Gumminess

Gumminess is the force required to disintegrate any material. Both the chewiness and gumminess are the secondary parameters. Gumminess of the bread samples were presented in Table 4.13.

Table 4. 13 Gumminess of breads added with 1.0% tara gum (TG), 1.5% κ-carrageenan (CAR), and 2.0% cellulose (CEL) during storage at 25°C

Bread samples	Gumminess (g_f)			
	Day 0	Day 6	Day 10 [ns]	Day 14
WF (control 0%)	189.57 ± 12.53^a	332.63 ± 25.62^a	349.14 ± 23.69	383.40 ± 16.45^{ab}
WF+TG 1.0%	128.13 ± 3.02^c	289.48 ± 16.71^b	334.69 ± 27.64	360.78 ± 6.81^b
WF+CAR 1.5%	144.59 ± 3.08^b	313.46 ± 11.09^{ab}	353.00 ± 4.97	393.57 ± 14.86^a
WF+CEL 2.0%	122.68 ± 2.27^c	304.42 ± 3.44^{ab}	325.72 ± 4.35	387.96 ± 21.34^{ab}

Mean ± SD of three replicates

Means in the same column with different superscript letters differ significantly at p=0.05.

[ns] Means in the same column were not significantly different at p=0.05.

The addition of fibres induced a decrease in gumminess. As gumminess is the product of hardness and cohesiveness, the results of gumminess correlated with the results of hardness. For the freshly baked bread (Day 0), the control had the highest gumminess (189.57 g_f), whereas CEL-added bread had lower gumminess (122.68 g_f). With increasing storage time, gumminess of each sample became increasing. However, after Day 6, all samples were similar in their gumminess.

4.3.4.6 Chewiness

According to Sahin and Sumnu (2006), chewiness is the energy required to chew a solid food until it could be swallowed. Chewiness is the product of hardness, springiness, and cohesiveness. It depends mostly on hardness rather than springiness and cohesiveness. Chewiness of the bread samples were shown in Table 4.14.

Regarding the freshly baked bread samples (Day 0), the control

had the highest chewiness (79.66 g$_f$), while lowest chewiness was observed in CEL-added sample (55.21 g$_f$). However, there was no significant difference (p>0.05) in chewiness among the stored samples.

Table 4. 14 Chewiness of breads added with 1.0% tara gum (TG), 1.5% κ-carrageenan (CAR), and 2.0% cellulose (CEL) during storage at 25°C

Bread samples	Chewiness (g$_f$)			
	Day 0	Day 6 [ns]	Day 10 [ns]	Day 14 [ns]
WF (control 0%)	79.66 ± 6.47[a]	128.35 ± 4.73	128.19 ± 12.74	141.88 ± 7.96
WF+TG 1.0%	57.64 ± 0.29[bc]	122.41 ± 5.91	123.74 ± 8.84	141.93 ± 4.77
WF+CAR 1.5%	63.15 ± 2.86[b]	125.38 ± 4.44	129.45 ± 3.48	150.81 ± 5.25
WF+CEL 2.0%	55.21± 1.02[c]	121.77 ± 8.12	121.52 ± 7.85	151.36 ± 10.19

Mean ± SD of three replicates

Means in the same column with different superscript letters differ significantly at p=0.05.

[ns] Means in the same column were not significantly different at p=0.05.

4.3.5 Water-soluble starch content

Water-soluble starch content is used to indicate the degree of starch crystallinity. Upon retrogradation, starch molecule interacts with other starch molecules via hydrogen bond, resulted in a more ordered crystalline structure with lowered solubility. Water-soluble starch content of the bread samples was depicted in Table 4.15.

It can be observed that TG-, CAR- and CEL-added freshly baked breads (Day 0) had higher water-soluble starch content than the control. However, there was no significant difference (p>0.05) among the samples.

Table 4. 15 Water-soluble starch content of breads added with 1.0% tara gum (TG), 1.5% κ-carrageenan (CAR), and 2.0% cellulose (CEL) during storage at 25°C

Bread samples	Water-soluble starch content (%)			
	Day 0 [ns]	Day 6 [ns]	Day 10	Day 14 [ns]
WF (control 0%)	6.36 ± 0.11	2.25 ± 0.32	2.02 ± 0.52^b	0.98 ± 0.18
WF+TG 1.0%	7.48 ± 0.95	2.53 ± 0.44	2.26 ± 0.55^{ab}	1.07 ± 0.52
WF+CAR 1.5%	7.92 ± 0.47	2.76 ± 0.38	2.74 ± 0.18^a	1.26 ± 0.39
WF+CEL 2.0%	7.85 ± 2.53	2.79 ± 0.60	2.25 ± 0.11^{ab}	1.00 ± 0.39

Mean $\pm$ SD of three replicates

Means in the same column with different superscript letters differ significantly at p=0.05.

[ns] Means in the same column were not significantly different at p=0.05.

Upon storage, water-soluble starch content of all the samples were found to decrease drastically. This reduction of water-soluble starch content is due to the ongoing recrystallisation of starch molecules. The result was in good agreement with the study done by Sidhu et al. (1997) which reported that water-soluble starch content of khaboos bread decreased during storage. At Day 10, the control had the lowest water-soluble starch content, but with no significant difference (p>0.05) from those of TG- and CEL-added breads. The similar trend was seen on Day 14 with no significant difference (p>0.05) among the samples.

From the above results, it could be said that fibres showed a tendency to help reducing starch retrogradation even though the amount used in this study did not produce a significant difference from the control. According to the study done by Ghodke (2009), when 0.75% of guar gum was added to wheat *chapatti* dough, water-soluble starch content was found to be higher as compared to the control at the third day of storage, signifying the retrogradation-retarding effect of guar gum.

4.3.6 Crumb structure

Characteristics of the air cells correlate with texture and loaf volume of bread. If cell wall is thin and finer with regular uniform cell, it forms soft and springy bread texture whereas thick-walled and coarse structure results in harder

texture (Hager & Arendt, 2013). In this study, the crumb air cells of all bread samples were small and evenly distributed throughout the crumb matrix and seemed similar in all samples (Figure 4.7). Mezaize et al. (2009) studied the effect of hydrocolloids on crumb structure and reported that addition of 2.3% of hydroxypropylmethyl cellulose helped increase the number of air cells in gluten-free breads. In another study done by Turabi et al. (2010), it was reported that xanthan addition had no effect on shape of the air cells but caused an increase in number of air cells.

Control   TG   CAR   CEL
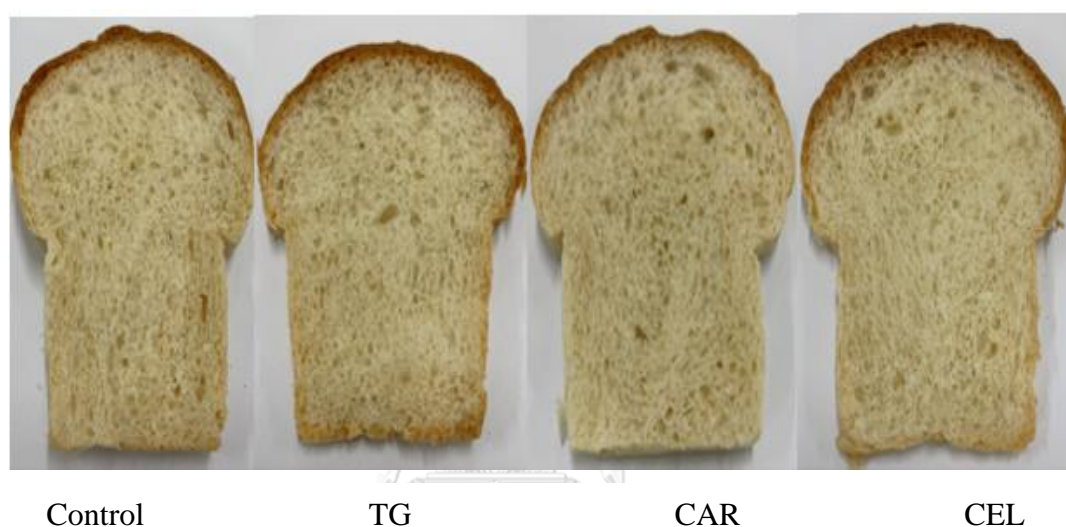
Figure 4. 7 Crumb structure of the bread samples. From left to right: control, tara gum (TG)-added bread, κ-carrageenan (CAR)-added bread, and cellulose (CEL)-added bread

4.3.7 Colour

Colour is one of the significant parameters that determines the acceptability of consumer on observing the appearance of the product. Colour parameters of the bread samples are shown in Tables 4.16-4.19.

For the fresh bread crumb (Table 4.16), the highest value of L* was observed in CEL-added breads with lowest L* was demonstrated by CAR-added samples. L* of the TG-added bread and the control were not significantly different (p>0.05). Rosell et al. (2009) reported that cellulose was of highest lightness among different commercial fibres obtained from different sources. In our study probably more concentration (2%) of CEL was used in comparison to other fibres (1% TG and

1.5% CAR) which tended to increase lightness as well.

The hue angle for the control and fibre added fresh breadcrumbs were approximately 90°. This hue angle represented that all breadcrumbs were yellow in color. No significant difference was observed by addition of fibres. For chroma, it showed that addition of fibres, especially TG and CAR significantly reduced the colour intensity compared to the control. Colour difference (ΔE^*) was more in CEL, followed by TG while less in CAR added breads.

During baking, the colour of bread crust is influenced by Maillard reaction and caramelisation. Maillard reaction produces brown pigment due to the reaction between reducing sugar and compound containing free amino group while caramelisation involves breakdown and polymerisation of sugars under high temperature (Ni et al., 2020; Sivam et al., 2010). Alike bread crumb, the bread crust also showed higher L^* for CEL-added samples whereas control showed the lowest L^* whereas TG- and CAR-added breads had slightly darker crust than CEL. The hue angle was approximately 60° that represents orange- yellow colour and were not significantly different ($p>0.05$) among all bread crusts. The chroma of bread crust was highest for CEL-containing bread while control, TG- and CAR- added breads were not significantly different ($p>0.05$).

The bread crumb and crust samples exhibited minimal changes in colour attributes during storage.

Table 4. 16 CIELAB colour parameters of breads added with 1.0% tara gum (TG), 1.5% κ-carrageenan (CAR), and 2.0% cellulose (CEL) at Day 0

Bread samples	Crumb				Crust			
	L^*	Hue angle [ns]	Chroma	ΔE^*	L^*	Hue angle [ns]	Chroma	ΔE^*
WF (control 0%)	72.40±1.32[ab]	89.47±0.12	14.59±0.61[a]		47.47±1.81[b]	60.40±1.84	30.61±0.92[b]	
WF+TG 1.0%	72.25±3.60[ab]	89.36±0.15	13.17±0.63[b]	3.07±1.52[ab]	48.62±1.15[ab]	61.31±0.19	32.26±064[b]	2.22±0.87[b]
WF+CAR 1.5%	71.54±0.26[b]	89.56±0.21	13.47±0.44[b]	1.41±0.50[b]	49.01±1.20[ab]	62.03±0.90	32.23±0.19[b]	2.53±0.88[b]
WF+CEL 2.0%	76.01±0.79[a]	89.58±0.24	13.73±0.55[ab]	3.73±0.77[a]	51.49±1.91[a]	62.62±1.25	35.31±1.30[a]	6.36±2.23[a]

Mean ± SD of three replicates

Means in the same column with different superscript letters differ significantly at p=0.05.

[ns] Means in the same column were not significantly different at p=0.05.

Table 4. 17 CIELAB colour parameters of breads added with 1.0% tara gum (TG), 1.5% κ-carrageenan (CAR), and 2.0% cellulose (CEL) during storage at 25°C for 6 days

Bread samples	Crumb				Crust			
	$L*^{ns}$	Hue angle ^{ns}	Chroma ^{ns}	$\Delta E*$	$L*^{ns}$	Hue angle ^{ns}	Chroma	$\Delta E*^{ns}$
WF (control 0%)	69.20±0.47	89.42±0.50	13.05±0.37		51.04±0.93	61.45±0.50	32.12 ±1.63^b	
WF+TG 1.0%	69.98±0.56	88.97±0.47	12.95±0.24	0.55±0.19^b	49.66±1.00	61.63±1.60	34.27±1.20^{ab}	4.33±1.26
WF+CAR 1.5%	68.64±0.24	88.71±0.61	13.34±0.99	1.05±0.23^b	50.21±0.41	61.95±0.29	34.23±0.22^a	3.23±0.41
WF+CEL 2.0%	70.49±2.47	89.20±0.40	13.07±0.67	2.34±0.77^a	49.68±0.57	61.19±0.18	35.97±0.34^a	5.28±0.62

Mean ± SD of three replicates

Means in the same column with different superscript letters differ significantly at p=0.05.

^{ns} Means in the same column were not significantly different at p=0.05.

57

Table 4. 18 CIELAB colour parameters of breads added with 1.0% tara gum (TG), 1.5% κ-carrageenan (CAR), and 2.0% cellulose (CEL) during storage at 25°C for 10 days

Bread samples	Crumb				Crust			
	L^*	Hue angle [ns]	Chroma	ΔE^* [ns]	L^*	Hue angle	Chroma [ns]	ΔE^* [ns]
WF (control 0%)	71.76±1.26[a]	89.30±0.18	14.57±0.37[a]		52.22±2.83[a]	66.59±0.97[a]	35.50±0.45	
WF+TG 1.0%	71.88±0.71[a]	89.44±0.17	13.71±0.41[ab]	1.96±0.33	49.18±2.73[ab]	63.65±1.35[b]	36.26±1.40	6.36±2.05
WF+CAR 1.5%	69.44±1.15[ab]	89.37±0.22	13.66±0.35[ab]	2.53±1.08	51.09±0.60[a]	65.68±0.58[ab]	35.10±1.58	3.72±0.47
WF+CEL 2.0%	70.90±1.38[a]	89.49±0.8	13.34±0.14[ab]	3.14±1.31	48.57±3.51[b]	64.73±1.69[ab]	36.70±1.16	6.06±3.75

Mean ± SD of three replicates

Means in the same column with different superscript letters differ significantly at p=0.05.

[ns] Means in the same column were not significantly different at p=0.05.

Table 4. 19 CIELAB colour parameters of breads added with 1.0% tara gum (TG), 1.5% κ-carrageenan (CAR), and 2.0% cellulose (CEL) during storage at 25°C for 14 days

Bread samples	Crumb				Crust			
	L^*	Hue angle [ns]	Chroma [ns]	ΔE^* [ns]	L^*	Hue angle [ns]	Chroma	ΔE^* [ns]
WF (control 0%)	67.87±2.77[b]	89.00±0.74	14.08±1.39		52.17±5.00[a]	67.90±2.37	36.82±1.16[b]	
WF+TG 1.0%	70.13±0.53[ab]	89.23±0.45	14.02±0.27	2.27±0.53	49.09±1.96[b]	64.44±1.19	38.56±1.20[a]	6.94±1.19
WF+CAR 1.5%	71.77±2.58[ab]	89.58±0.51	14.05±0.60	3.96±2.51	52.41±2.27[a]	66.87±0.93	38.11±1.23[a]	3.62±0.90
WF+CEL 2.0%	72.39±1.81[a]	89.71±0.23	13.62±0.25	4.55±1.81	49.89±5.26[ab]	65.58±2.44	37.94±0.92[ab]	5.58±3.86

Mean ± SD of three replicates

Means in the same column with different superscript letters differ significantly at p=0.05.

[ns] Means in the same column were not significantly different at p=0.05.

CHAPTER 5

CONCLUSION

Addition of soluble fibres, namely TG and CAR, were shown to pose an effect on pasting properties, as well as gelatinisation enthalpy. TG and CAR addition generally increased peak, trough, breakdown, and final viscosity while decreased setback viscosity and pasting temperature. Both soluble fibres decreased enthalpy of gelatinisation. As for starch retrogradation, a decreasing trend in enthalpy of amylopectin crystallite melting was shown upon addition of CAR, particularly at early stage of storage. Addition of CAR also slowed down the increment in gel firmness during storage. Addition of CEL, the insoluble fibre, placed a slightest effect on pasting behaviour but could slightly decrease the enthalpy of melting of amylopectin crystallite. By observing the setback viscosity, retrogradation enthalpy and gel firmness, the optimum concentrations of each fibre to reduce retrogradation was 1.0, 1.5 and 2.0% for TG, CAR and CEL, respectively.

On application of the optimum concentration of TG, CAR and CEL fibres to pan bread, it was found that both soluble and insoluble fibres had no significant effect on moisture content and water activity for the freshly prepared breads as compared to the control. With increasing storage time, it was observed that both soluble and insoluble fibres could retain more moisture, particularly for TG. Regarding specific loaf volume, CEL interestingly produced a bread with the highest specific loaf volume while CAR caused a decrease in the volume. In terms of textural property, both soluble and insoluble fibres significantly reduced hardness as compared to the control bread which could be due to the water retention ability of the fibres. This could prove that these fibres can reduce starch retrogradation and bread staling during storage. Addition of fibres resulted in a bread with decreasing hardness, gumminess, and chewiness whereas springiness and cohesiveness showed an increasing trend which could be desirable property in maintaining quality of bread. Regarding water-soluble starch content, fibre-added breads was higher in value. However, the difference was not significant. Adding fibres to breads did not pose any obvious

changes in the crumb structure. All the bread samples had crumb with smaller and evenly distributed air cells throughout the crumb matrix. All the breadcrumbs were yellow in colour whereas orange yellow colour was observed in the crusts.

Both soluble and insoluble fibres proved to have a positive effect on quality of the freshly baked and stored bread. From this study, it was demonstrated that addition of fibres had retained moisture during long-term storage, that may help to retard staling and decrease crumb hardness.

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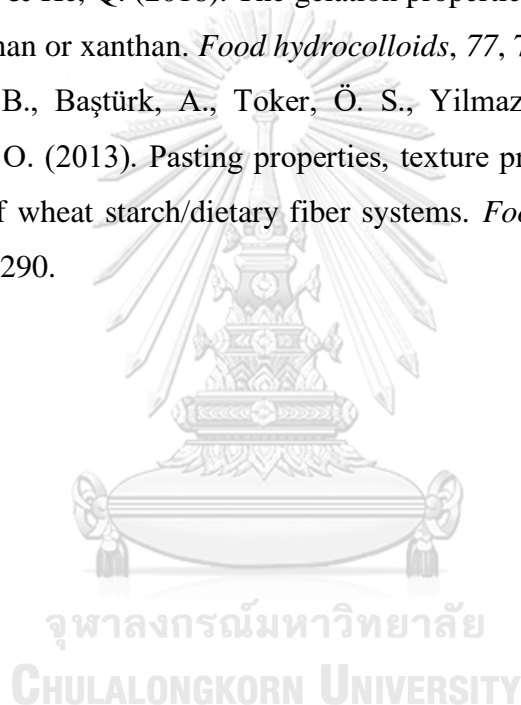
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