Application of Dietary Fibre from Agricultural Products in Bread

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ABSTRACT

APPLICATION OF DIETARY FIBRE FROM AGRICULTURAL PRODUCTS IN BREAD

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This thesis investigated the chemical, structural, and bioactivity characteristics of fibres from sunflower stalk pith (SSP). The sunflower fibres and yellow mustard gum (YMG) were applied in bread making. The yield of sodium hexametaphosphate-extractable pectin (SEP) and residue fraction (RF) were 20.93% and 71.10%, respectively. The high galacturonic content in chemical analysis and the characteristic peaks in FT-IR analysis confirmed the identity of SEP as pectin. The inhibitory effect of SEP/TP complexes against human salivary α-amylase were affected by both the composition and concentration of inhibitors. SEP had no significant inhibitory effect against α-amylase, but significant inhibitory effect was found in SEP/TP complexes. The dough properties were modified by three fibres (SSP, RF, YMG) in different manners at three substitution levels (6%, 9%, 12%). The specific volume reduction and crumb hardening effects were observed in all the fibre-substituted bread. In conclusion, YMG-TP bread had least influence on the bread quality.

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LIST OF ABBREVIATIONS

ATR	Attenuated total reflectance
BU	Brabender unit
C	Catechin
CCNFSDU	The Codex Commission on Nutrition and Foods for Special Dietary Uses
CMC	Carboxymethylcellulose
DATEM	Diacetyl tartaric ester of monoglycerides
DDT	Dough development time
DE	Degree of esterification
DF	Dietary fibre
DM	Degree of methylation
Dw	Dry weight base
EC	Epicatechin
ECG	Epicatechin gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
FT-IR	Fourier transform infrared spectroscopy
Gal	Galactose
GCG	Gallocatechin gallate
GI	Glycemic index
GMS	Glycerol monostearte
HPAEC	High-performance anion exchange

	chromatography
HPMC	Hydroxypropylmethylcellulose
IDF	Insoluble dietary fibre
LDL	Low-density lipoprotein
LMP	Low-methoxyl pectin
MTI	Mixing tolerance index
MC	Methylcellulose
OHC	Oil holding capacity
PAD	Pulsed amperometric detector
PBS	Phosphate buffered saline
RF	Residue fraction
RGI	Rhamnogalacturonans type I
RGII	Rhamnogalacturonans type II
SDF	Soluble dietary fibre
SEP	Sodium hexametaphosphate-extractable pectin
SH	Thiol bond
SHMP	Sodium hexametaphosphate
SS	Disulphide bond
SSL	Sodium stearoyl lactylate
SSP	Sunflower stalk pith powder
SW	Swelling
TP	Tea polyphenol
TTB	Time to breakdown

WA	Water absorption
WHC	Water holding capacity
YMG	Yellow mustard gum

Chapter 1 Introduction

Recently, with the the prevalence of chronic disorders in western and developed countries, people are concerned about the nutritional value and health benefits of food products. As shown in epidemiological studies, obesity and certain cancers were significantly reduced by increasing consumption of dietary fibre (Slavin, 2005). Dietary fibre (DF) refers to the edible part from plants and analogous carbohydrates, which is indigestible in the human small intestine and fermentable in the large intestine. It mainly consists of cellulose, hemicellulose, lignin, gums, and pectins (Stear, 1990). Varying based on the fibre source and processing methods, the functional properties of fibre includes laxation, regulation of blood cholesterol and blood glucose, reduction of the risk of chronic disorders, e.g., coronary heart disease, diabetes, obesity and certain forms of cancer (Bingham et al., 2003; Goff, Repin, Fabek, El Khoury, & Gidley, 2018).

Sunflower (*Helianthus annuus L*) is widely grown around the world as an important oilseed crop (Seiler & Gulya Jr, 2016). After removal of sunflower seed, the lack of processing methods on the residue fractions of the sunflower results in environmental pollution. The major component in the residue is DF. Specifically, the sunflower head residue (without oilseed) and stalks have fibre contents of 47.6% and 66.2%, respectively (Marechal & Rigal, 1999). Up to date, the residues are mainly used in the manufacturing industry and environmental protection

industry (Hesami, Zilouei, Karimi, & Asadinezhad, 2015; Kaymakci, Ayrilmis, Ozdemir, & Gulec, 2013; Ruiz et al., 2013; Sharma, Kalra, & Kocher, 2004; Sun & Shi, 1998). Thus, there is a great potential to apply the residue parts in the food industry.

The yellow mustard seed is one of most important annual oil seed crops, which is ranked after soybean, sunflower, groundnut and cottonseed (Marcone, Yada, Aroonkamonsri & Kakadu, 1997). As this oil crop is grown primarily for condiment production, the function of outer seed coat has been underestimated. The major polysaccharides in the outer seed coat of yellow mustard seed are rhamnose, arabinose, galactose, glucose, xylose, mannose, and galacturonic acid (Vose, 1974; Cui, Eskin, & Biliaderis, 1993). The physical, chemical, and structural characteristic of yellow mustard gum (YMG) has been well investigated in our previous studies (Cui & Eskin, 1993; Cui, Eskin & Biliaderis, 1995; Cui, Eskin, Biliaderis & Marat, 1996; Wu, Cui, Eskin & Goff, 2009; Wu, Cui, Eskin, Goff & Nikiforuk, 2011; Wu, Eskin, Cui & Pokharel, 2015). Potent anti-cancer effect and antioxidant activities of YMG were reported by Eskin et al., (2007) and Wu et al., (2016) (Eskin, Raju & Bird, 2007; Wu, Hui, Eskin & Cui, 2016). With excellent rheological properties and emulsification properties, YMG is a potential hydrocolloid that could be used in food industry (Cui et al., 1996; Wu et al., 2015).

As tea is one of the most popular beverages all around the world, tea leaf, and bud (from *camellia sinensis*) has gained attention from researchers. Based on the manufacturing processes,

teas are classified into green tea (nonfermented), oolong tea (semi-fermented), and black tea (fermented). With inactive of polyphenol oxidase in initial process and less fermentation, green tea preserves the native overall composition and chemical structure of its phenolics. Catechins are the major components of polyphenols within the green tea, which mainly includes (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epigallocaechin-3-gallate (EGCG) (Xing, Zhang, Qi, Tsao & Mine, 2019). Up to now, extensive research has been conducted on its antioxidant activities (Von Gadow, Joubert & Hansmann, 1997; Sakanaka,Tachibana &Okada, 2005; Atoui, Mansouri, Boskou & Kefalas, 2005). Health benefits such as reducing the risk of diabetes, obesity, cardiocasular disease, and cancer are reported (Xing et al., 2019). With the increasing concerning on health problems, attempts have been made to use the tea extracts in the production of health promoting food, such as meat products and bakery products (Lu, Lee, Mau & Lin, 2010; Pasrija, Ezhilarasi, Indrani & Anandharamakrishman, 2015; Tang, Kerry, Sheehan, Buckly & Morrissey, 2001).

Bread is a staple food that is commonly made from wheat flour in western countries. With the increasing interests in functional foods with health benefits, research has been conducted on the incorporation of DF into bread. The most common DF used in bread making are field pea hull, sugar beet fibre, wheat bran, rice bran, whole grain rye, apple fibre, psyllium husk, pseudo-cereals, corn bran, oat bran, carob fibre, flaxseed fibre, peanut hull, and hydrocolloids (Anil,

2002; Kupper, 2005). The addition of DF leads to different dough properties, such as higher water absorption, mixing tolerance, and tenacity, and reduced extensibility (Angioloni & Collar, 2008; Gómez, Ronda, Blanco, Caballero, & Apesteguía, 2003). Consequently, the detrimental effects on bread such as reduced loaf volume, harder crumb, and darkening effects are observed (Wang, Rosell, & De Barber, 2002).

The objectives of this study were to: (1) study the chemical and structural characteristics of sunflower stalk pith (SSP), sodium hexametaphosphate-extractable pectin (SEP) and residue fraction (RF); (2) investigate the inhibitory effects of SEP without and with the presence of tea polyphenols (TP) against the human salivary α-amylase; (3) determine the impacts of both type and concentration of SSP, RF, as well as yellow mustard gum (YMG, included in due to limited amount of SEP) without and with TP addition on the dough properties; (4) evaluate the bread quality with added SSP, RF, as well as YMG without and with presence of TP.

Chapter 2 Literature review

2.1 Dietary fibre

2.1.1 Definition and classification

In 1953, the term of dietary fibre (DF) first appeared in the observation publication of Eben Hipsley (Hipsley, 1953). DF encompasses a vast range of substances, including purified and semi-purified carbohydrates, resistant starch, as well as plant cell wall materials (Guillon & Champ, 2000). The only common point shared by those substances is that they are not digestible by the human endogenous enzymes.

To give a better definition, the 27th session of the Codex commission on Nutrition and Foods for Special Dietary Uses (CCNFSDU) defined DF as carbohydrates that are unhydrolyzable by endogenous enzymes and are comprised of three or more monomeric units (McCleary & Cox, 2017). At the same time, dietary fibre was also classified into the following three categories: 1) edible carbohydrate polymers naturally existing in food; 2) carbohydrate polymers extracted from raw food materials by physical, enzymatic, or chemical means that have been shown to have physiological benefit to health; 3) synthetic carbohydrate polymers proven by scientific evidence that they have physiological benefits to health (Cumming, Mann, Nishida, &Vorster, 2009).

With the advantages of being inexpensive and abundant, DF that naturally exists in plant material are extensively used in the food industry.

2.1.2 Physicochemical properties and health benefits

2.1.2.1 Solubility

DF can be classified as soluble and insoluble, according to its ability to dissolve in water. For soluble dietary fibre (SDF), it includes pectic polysaccharides, gums, mucilage and some hemicellulose; while insoluble dietary fibre (IDF) includes other types of hemicellulose, cellulose

and lignin (Davidson & Mc Donald, 1998; Roehrig, 1988; Schneeman, 1987).

Solubility is affected by the structure of the polysaccharides. Generally, the SDF contains a hydrophilic backbone and/or side chains, while the hydrophobic substitution group such as COOH and SO_4^{2-} decreases the solubility. The solubility of DF can also be influenced by temperature and ionic strength (Bertin, Rouau, & Thibault, 1988; Fleury & Lahaye, 1991).

The differences in solubility contribute to the varied utilization of dietary fibre in food processing. In terms of SDF, the capability of gel formation, emulsification, and viscosity development enable the broad application of SDF as a gelling agent, thickener and stabilizer in processed food and drinks.

Table 1. Classification of dietary fibre components based on water solubility/fermentability (Dhingra, Michael, Rajput, & Patil, 2012)

Characteristic	Fibre component	Description	Main food source
Water insoluble Less fermented	Cellulose	The main structural component of the plant cell wall. Insoluble in concentrated alkali, soluble in concentrated acid.	Plants (vegetables, sugar beet, various brans)
	Hemicellulose	Cell wall polysaccharides, which contain the backbone of β-1,4 glucosidic linkages. Soluble in dilute alkali.	Cereal grains
	lignin	Non-carbohydrate cell wall component. Complex cross-linked phenyl propane polymer. Resists bacterial degradation.	Woody plants
	Pectin	Components of primary cell wall with D-galacturonic acid as principal components. Generally water soluble and gel forming	Fruits, vegetables, legumes, sugar beet, potato
Water soluble Well fermented	Gums	Secreted at site of plant injury by specialized secretory cells. Food and pharmaceutical use.	Leguminous seed plants (guar, locust bean), seaweed extracts (carrageenan, alginates), microbial gums (xanthan, gellan)
	Mucilage	Synthesized by plant, prevent desiccation of seed endosperm. Food industry use, hydrophilic, stabilizer.	Plant extracts (gum acacia, gum karaya, gum tragacanth)

2.1.2.2 Hydration properties and oil-binding capacity

Polysaccharides are hydrophilic substances, forming hydrogen bonds with water in the presence of numerous free hydroxyl groups (Oakenfull, 2001). Hydration capacity is demonstrated in three aspects: water absorption (WA), water holding capacity (WHC) and swelling (SW). According to earlier studies, WA refers to the kinetic of water movement under certain conditions and determined by the Baumann apparatus; while WHC is demonstrated as the water amount retained by a known weight of DF under specific conditions, and is also measured with the Baumann apparatus (Fleury & Lahaye, 1991; Robertson, 1998). However, SW measures the volume occupied by a certain weight of DF under certain conditions in the volumetric cylinder (Kuniak & Marchessault, 1972; Robertson, 1998). The hydration properties are associated with the properties of DF, such as chemical structure, porosity, particle size and ionic form (Elleuch et al., 2011). External environment factors such as pH, temperature, ionic strength, the type of ion in solution and stresses upon the fibre, could also influence the hydration properties (Elleuch et al., 2011).

At the same time, DF has oil holding capacity (OHC), which is portrayed as the amount of oil retained by the DF under defined conditions (Lin, Humbert, & Sosulski, 1974; Thammakiti, Suphantharika, Phaesuwan, & Verduyn, 2004). The OHC is related to the chemical composition, as well as the porosity of the DF (Lin et al., 1974; Tanti, Barbut, & Marangoni, 2016). The mechanism of OHC mainly lies in the binding sites of oil provided by the non-polar sidechains and the physical entrapment of oil within the porous structure.

The excellent WHC, SW and OHC give fibres the possibility of being used as ingredients in food processing. DF with good WHC is usually regarded as a functional ingredient to avoid syneresis, alter the viscosity, and change the texture in certain formulated foods (Grigelmo-Miguel

& Martín-Belloso, 1998). DF with great OHC often serves as an emulsifier and stabilizer in high-fat foods and emulsions.

2.1.2.3 Viscosity

Almost all SDF have the capacity to develop viscous solutions. Viscosity (η), also known as resistance to flow, is calculated as the ratio of shear stress (Γ) to shear rate (γ). Viscosity originates from the physical interactions of the polysaccharide molecules in solution. The majority of polysaccharide solutions belong to the non-Newtonian fluid category, which exhibits increased or decreased viscosity with an increase in shear rate (Sanderson, 1981). In particular, most non-Newtonian fluids are called pseudoplastic fluids, which exhibit shear-thinning with viscosity decreasing as the shear rate increases (Liu & Deng, 2017). The behaviour of plasticity was due to the orientation of particles in the direction of flow under shearing (Colin-Henrion, Cuvelier, & Renard, 2007). According to an earlier study, SDF was the major component that caused the increase of viscosity in solution (Abdul-Hamid & Luan, 2000). Viscosity is related to the intrinsic characteristics of the polysaccharides, DF concentration, temperature, and the solvent (Guillon & Champ, 2000). Moreover, viscosity is positively correlated to the fibre concentration but negatively correlated to the temperature. The viscosity displayed by the fibre solution makes it an ideal food additive as a gelling agent, thickener, and stabilizer in food processing.

2.1.2.4 Health benefits

The interest in the possible health benefit of DF was stimulated by the prevalence of chronic disorders in western and developed countries. In those countries, people consume substantial amounts of refined foods and animal-based foods while rather low DF-containing foods. Dietary fibre as food ingredients or component offers various health benefits, including 1) laxative; 2) regulation of blood cholesterol and blood glucose; 3) reduction of the risk of chronic disorders,

e.g., coronary heart disease, diabetes, obesity and certain forms of cancer. According to a previous study of the effects of DF, SDF led to an decrease in constipation, while conflicting results were observed with IDF (Suares & Ford, 2011). In the previous study, various SDF shared similar ability in reducing total and low-density lipoprotein (LDL) cholesterol (e.g., 3 g SDF from oat reduced total and LDL cholesterol by <0.13mmol/L), and increasing SDF intake made a limited contribution in cholesterol reduction (Brown, Rosner, Willett, & Sacks, 1999). DF was reported to attenuate blood sugar level and contribute to the risk reduction of type 2 Diabetes by delaying gastric emptying, amylolysis and sugar diffusion and absorption (Goff et al., 2018). In a previous study, the intake of dietary fibre was inversely related to the risk of large bowel cancer and observed to have a largest protective effect on the left side of the colon (Bingham et al., 2003). Therefore, it is necessary for us to understand the functional role of DF in health.

Table 2. Functions and benefits of dietary fibre on human health

Functions	Benefits
Adds bulk to the diet, making feel full faster	May reduce appetite
Attracts water and turns to gel during digestion, trapping carbohydrates and slowing absorption of glucose	Lowers variance in blood sugar levels
Lowers total and LDL cholesterol	Reduces risk of heart disease
Regulates blood pressure	May reduce onset risk or symptoms of metabolic syndrome and diabetes
Speeds the passage of foods through the digestive system	Facilitates regularity
Adds bulk to stool	Alleviates constipation
Balances intestinal pH and stimulates intestinal fermentation production of short-chain fatty acids	May reduce risk of colorectal cancers

2.1.3 Influence of dietary fibre on digestion and absorption of other nutrients

2.1.3.1 Gastrointestinal absorptive and digestive processes

Food digestion plays an important role in sustaining life. Using the studies conducted in the

past 300 years, we have a better understanding of the basic principles of digestion. Gastric digestion includes both physical and chemical food breakdown. The key organs involved in the process are the mouth, stomach, small intestine and large intestine (Mun, Kim, McClements, Kim, & Choi, 2017).

The process of food digestion begins with mastication in the mouth. Food is sufficiently chewed and mixed with saliva to form a bolus and hydrolyzed by human salivary α-amylase into smaller particles, such as maltose, maltotriose and dextrins. With the proximal stomach relaxing, bolus moves through the esophagus into the stomach, where the food is hydrolyzed by HCl and enzyme. During digestion, HCl and pepsinogen are secreted from parietal cells and chief cells distributed on the fundus, which are mixed to convert into the proteolytic enzyme pepsin. The digested food particles are stored in the proximal stomach and then, after size reduction in the antrum, pass through the pyloric sphincter into small intestine, under the peristaltic contractions of the stomach wall. During digestion in the small intestine, segmentation and peristaltic muscle contractions aid in the mixing of chyme and the forward movement of the chyme in the small intestine, respectively. Duodenum, jejunum and ileum comprise the three sections of the small intestine. In the duodenum, the chyme is neutralized and adjusted to proper pH by bicarbonate for further enzymatic digestion. Afterward, the chyme will be mixed with the bile and digested using enzymes secreted from the liver and pancreas. Lipase, phospholipase A, amylase, amyloglucosidase, trypsin, chymotrypsin, carboxypeptidase and elastase take part in this phase of digestion to break down the lipid, starch and protein into small molecules. The jejunum is the major absorption spot of micronutrients, such as vitamins and minerals; as well as subunits of micronutrients, such as amino acids, di/tripeptides, monosaccharides and fatty acids/glycerol. The ileum is the third section where absorption completed. With the segmentation and peristaltic

muscle contraction, the chyme is transported to the large intestine. In the large intestine is located more than 10^{10} microorganisms/g of the intestinal content. The partially disintegrated but non-absorbable food particles left from the small intestine are partially fermented by those anaerobic bacteria and the by-products of the fermentation can then be absorbed in the form of short-chain fatty acids and water.

2.1.3.2 Influence on digestion and absorption

DF has been shown to have the effect of delaying gastric emptying and lowering the uptake of a range of nutrients in previous studies (Prasad et al., 2018). This effect was attributed to the increased viscosity of digesta generated by the dietary fibre, thereby impacting the interaction among dietary fibre, nutrients and enzymes, as well as the susceptibility to microbial fermentation (Grundy et al., 2016). Thus, soluble fibre may hinder nutrient digestion and mass transfer, as well as decreasing the GI value after a meal. Furthermore, the reduced nutrients digestion and absorption by water-soluble fibre can be explained from following aspects.

Bile salt metabolism has been reported to be modified by water-soluble fibre, which decreased the fasting plasma cholesterol concentration and slowed down lipid digestion (Gunness & Gidley, 2010). In previous studies, the oat β-glucan and guar gum were observed to reduce the plasma cholesterol concentration and increase the excretion of bile acid (Ellegård & Andersson, 2007; Lia et al., 1995; Moriceau et al., 2000). Although there is limited work done on the interaction and binding type between bile salts and dietary fibre (Gunness, Flanagan, Mata, Gilbert, & Gidley, 2016), one possible explanation demonstrated the mechanism as the entrapment of bile salts in the polysaccharides network (Bowles, Morgan, Furneaux, & Coles, 1996; Ebihara & Schneeman, 1989; Gunness et al., 2016; Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2011). Thus, insufficient mixing of bile salt may result in the weakening of emulsification and mass transfer of

lipolytic products along the way from the gut lumen and mucosal surface. After that, the emulsified bile salt will then either be fermented in the colon and/or excreted as faeces. In this case, the loss of native bile salt by faecal excretion facilitates the hepatic synthesis of cholesterol, although the total bile salt and cholesterol level are decreased (Dawson & Karpen, 2015; Gunness & Gidley, 2010).

The interaction among digesta, digestive enzymes and dietary fibre was reported to be another factor in reduced digestion and absorption. On the one hand, the increased viscosity of digesta could reduce the susceptibility of the digesta to the digestive enzymes; On the other hand, the dietary fibre present in digesta could also directly interact with the digestive enzymes, thus reducing the hydrolysis of macronutrients (Brennan, Blake, Ellis, & Schofield, 1996; Grundy et al., 2016; Slaughter, Ellis, Jackson, & Butterworth, 2002). Similar results were found in the study of starch digestion. In an *in-vitro* study, the polysaccharides extracted from *Plantago asiatica* L were reported to slow down the diffusion of glucose and inhibit the activity of α-amylase, pancreatic lipase and protease (Hu, Nie, Li, & Xie, 2013). In an earlier study of Gourgue et al. (1992), mango fibre interfered with enzyme absorption due to a large quantity of free carboxylic groups (Gourgue, Champ, Lozano, & Delort-Laval, 1992).

The structure of cell wall contributes to reduced digestion and absorption as well. Due to the indigestibility of cell wall components in the upper gastrointestinal tract by mastication and endogenous enzyme hydrolysis, the nutrients encapsulated in the cell wall are not absorbed as much (Bhattarai, Dhital, Mense, Gidley, & Shi, 2018; Tydeman et al., 2010; Waldron, Parker, & Smith, 2003). For foods that are rich in starch, the digestion and absorption were limited by the cell wall matrix due to the physical barrier for α-amylase, limited gelatinization during cooking and the binding between α-amylase and cell wall components (Dhital, Gidley, & Warren, 2015).

There is also evidence from recent work on almonds that showed that the intact cell wall in almond tissue reduced the lipaemic response (Grassby et al., 2014; Grundy et al., 2014; Mandalari et al., 2014).

Compared to other substrates, dietary fibre is also less digested in the colon. For the SDF, it is rapidly fermented by the microbiota to generate short chain fatty acids and gases; while less soluble dietary fibre, such as most of the cellulose, hemicelluloses and cross-linked pectin, are slowly fermented and maintain more intact (McDougall, Morrison, Stewart, & Hillman, 1996). According to the previous study, the digestibility of dietary fibre was related to not only its composition, but also the inter-polymer cross-links between polymers (Fry, 1986).

2.2 Sunflower fibre

Nowadays, sunflower (*Helianthus annuus* L) is one of the most important oilseed crops grown in 72 countries around the world, which yields the fifth highest total oil production behind soybean, rapeseed, cottonseed and peanut (Seiler, Qi, & Marek, 2017). After removal of sunflower seeds, the by-products from sunflower include sunflower head, sunflower hulls and sunflower stalk. So far, limited research has been done on the sunflower by-products, the majority of which were focused on the lignocelluclosic part.

Previous studies on sunflower head are focused on the extraction and the characterization of the pectin. The chelators-assisted acidic extraction method of pectin from sunflower head has been comprehensively investigated from 1970s to 1990s (Chang, Dhurandhar, You, & Miyamoto, 1994a; Kim, Sosulski, & Campbell, 1978; Kim, Sosulski, & Lee, 1978). The structure of the pectin extracted from sunflower head has been widely studied. Generally, it has the MW of 30,000-50,000 g/mol, galactose content (Gal content) of 70-90%, degree of methylation (DM) of 10-40% and acetylation content of 2-4% (Iglesias & Lozano, 2004; Miyamoto & Chang, 1992; Sahari,

Akbarian, & Hamedi, 2003). The rheological behavior of the sunflower head pectin was studied by Hua et al. (2015). In the study, the extracted pectin exhibited a stiff rod-like conformation in the intrinsic viscosity analysis. The viscosity of the solution was significantly increased when the concentration of pectin was in excess of 1.0% (w/w). The aggregation of the pectin was promoted by decreasing the pH of the solution to below 3.0. The extracted pectin also showed the property of being heat sensitive, the solution of which turned to a low-viscous solution (Hua, Wang, Yang, Kang, & Zhang, 2015).

The chemical composition of sunflower seed hull has been investigated in prior studies. The major components of sunflower seed hull were lipid (5.17% of the total hull weight), proteins (4% of the entire hull weight) and carbohydrates (around 50% of the total hull weight) (Cancalon, 1971). Moreover, the major carbohydrates of the hull are cellulose and lignin. Several degradation methods for sunflower hull were investigated, including mesophilic and thermophilic treatment with different nitrogen co-substrates (Conghos, Aguirre, & Santamaria, 2006).

The stalks contain fibre (external part, 90%), and the pith (internal part, 10%) (Marechal & Rigal, 1999). The previous study on sunflower focused on the degradation of hemicellulosic material and the potential application of the cellulosic part in the environmental protection industry. By hydrothermally pre-treating the sunflower stalk, 90% glucose conversion and degradation of hemicellulosic sugar were achieved (Díaz, Cara, Ruiz, Pérez-Bonilla, & Castro, 2011). The possibilities of sunflower hull utilization, such as ethanol production, sugar production, absorbents for hazardous elements, manufacture of particleboard and thermoplastic, as well as biogas production, were widely investigated in previous studies (Hesami et al., 2015; Kaymakci et al., 2013; Ruiz et al., 2013; Sharma et al., 2004; Sun & Shi, 1998).

2.3 Application in bread

2.3.1 Introduction

Bread is one of the oldest foods in history. The earliest evidence of bread prepared from barley or barley mixed with wheat or millet can be dated back to Neolithic in Europe (Popova, 2017). Later, grains and yeast spores were widely used in bread making around 10,000 BC. In 1961, the Chorleywood bread process was developed by the British Baking Industries Research Association. It enabled the usage of lower-protein wheat and shorter production time.

Bread is made from a dough of flour and water and is baked in an oven. Compared to other kinds of foods, bread has the following advantages: 1) easy-to-produce: the complete set of production equipment and the mature processing method in industrial production largely saved energy, human resources and time; 2) easy-to-store: baking in high temperature enables thorough sterilization and extends the shelf life; 3) rich-in-nutrients: bread contains variety of nutrients including carbohydrates, protein, dietary fibre, vitamins, micronutrients, and antioxidants. Bread is consumed by people all around the world and is regarded as a staple food in the Middle East, Central Asia, Africa, Europe, Australia as well as the Americas.

The diversity in bread depends on the culture, flour type and formulation. Based on different formulation, the main bread type can be classified into pan bread, hearth bread or sour bread, flatbread or roti/chappati, as well as rolls and other small fermented bread. According to the bread flour, bread can be classified into wheat bread and non-wheat bread, which contains a high level of gluten and low level of gluten, respectively. Gluten-free bread was created due to the dramatic rise in celiac disease patients. It is usually made with mixed flours from rice, sorghum, corn, legumes or almonds.

2.3.3 Bread making process

In bread production, the ingredients in the formula are precisely weighed. Salt, sugar and

oxidizing agents are pre-mixed with yeast in a solution form. The activated yeast, other ingredients and flour are then mixed to develop the dough. Mixing is a process of gluten network formation. The gluten formation is affected by many factors, including applied mechanical energy in stretching and shearing stage, mixing time, temperature, shear rate and flour-to-water ratio (Avramenko, Tyler, Scanlon, Hucl, & Nickerson, 2018). Gluten is formed by various amino acids, in which glutamine (around 35%), glycine (20%) and proline (10%) account for the major amino acids in gluten development (Fermin and others 2003; Pommet and others 2005; Wellner and others 2005). For all the proteins involved in gluten development, they are classified into gliadin (alcohol-water soluble) and glutenins (insoluble) based on the solubility. During dough development, gliadins provide the dough with viscosity and extensibility, while glutenins create the required cohesiveness and elasticity for dough (Delcour et al., 2012; Goesaert, Brijs, Veraverbeke, Courtin, Gebruers, & Delcour, 2005; He, Roach, & Hoseney, 1992; Joye, Lagrain, & Delcour, 2009b).

Afterward, the optimally mixed dough is transferred to the fermentation cabinet for a specific time to develop the porous structure. In fermentation, sugars are broken down to carbon dioxide and ethanol by yeast. The carbon dioxide produced during fermentation is responsible for the foam structure in the dough, which converts into a sponge structure of breadcrumb after baking (Campbell, Rielly, Fryer, & Sadd, 1998; Scanlon & Zghal, 2001). The flavor and aerated structure of breadcrumb are closely related to the yeast activity and affected by the fermentation condition, including the temperature and relative humidity.

During fermentation, the dough is punched at certain intervals. Punching also plays a crucial role in dough development. On the one hand, the dough temperature throughout the mass is equalized. On the other hand, the excessive carbon dioxide accumulated in the dough is renewed

with atmospheric oxygen.

Then the dough is sent to dough make-up step, including scaling, rounding, intermediary proof and molding, to give a uniform shape and weight. Subsequently, the molded dough pieces are proofed in the baking pans and then subjected to baking in the pre-heated baking oven at 220-250℃.

2.3.4 Influence of food additives on dough properties

The major components in dough and bread are carbohydrates, protein, water, lipids and air (Autio & Laurikainen, 1997). The dough ingredients combined with processing condition largely affect the microstructure of the dough, thereby determine the appearance, texture, taste and stability of the final product. To improve the handling properties of the dough, increase quality and extend the shelf-life of the bread, the application of additives has become a common practice in the bakery industry (Rosell, Rojas, & De Barber, 2001). Generally, the additives includes the oxidants, for example, potassium bromate, azodicabonamide, calcium iodate, and ascorbic acid; emulsifiers/surfactants, for example, glycerol monostearte (GMS), sodium stearoyl lactylate (SSL) and diacetyl tartaric ester of monoglycerides (DATEM); and enzyme, for example, α-amylase, lipoxygenase and protease (Ravi, Manohar, & Rao, 2000; Tebben, Shen, & Li, 2018a).

The application of oxidants contributes to the flour and subsequent bread crumb whitening, and the formation of the disulfide bond between glutenin protein (Bloksma, 1972; Kaya & Topaktaş, 2007; Yamada & Preston, 1992). As the promotion of the disulfide bond, the dough is strengthened and has better gas retention properties (Dong & Hoseney, 1995; Sandhu, Manthey, Simsek, & Ohm, 2011). The new emerging technique such as ozone treatment is considered as a possible safe alternative to those commercial oxidants, as ozone gas is safer and easier to decompose (Sandhu et al., 2011).

Emulsifier are usually used as dough strengthener and crumb softener in the bakery industry (Stampfli & Nersten, 1995). Emulsifiers possesses both hydrophilic and lipophilic groups within their structures. Such special chemical structure provides emulsifiers with the ability to concentrate at the water/oil interphase and stabilize a thermodynamically unstable system (Kohajdová, Karovičová, & Schmidt, 2009). The effect of the emulsifiers on the dough are varied due to the different chemical structure of ionic emulsifiers (such as sodium stearoyl-2-lactylate and diacetyl tartaric acid) and nonionic emulsifiers (such as sucrose esters of fatty acids, monolycerides, epoxylated monoglycerides, and distilled monoglycerides). Ionic emulsifiers could be anionic or cationic (not used in foods), in particular, amphoteric emulsifiers (lecithin) have both anionic and cationic groups (Stampfli & Nersten, 1995). Although the mechanism of the dough strengthening effect by emulsifier has not been fully understood, there are two possible theories. The first theory illustrated the stronger dough as the result of the promoted complex formation between gluten protein and emulsifiers by hydrophobic interaction (Gómez et al., 2004; Miyamoto, Sakamoto, Maeda, & Morita, 2005). Another theory suggested that the gliadin may associate with the liquid-crystalline phase formed by polar emulsifiers (Ribotta, Pérez, Añón, & León, 2010).

Later, compared to those chemical improving agents, the enzymes became more prevalent in the baking market due to the natural and clean label. The impacts on the dough are different from the type of the enzyme and affected by the temperature, water activity, pH, and enzyme concentration (Tebben et al., 2018a). The specific effects and related mechanisms of major enzymes used in whole wheat dough are summarized in Table 3. Overall, α-amylase, G4-amylase, and xylanase significantly improved the whole wheat dough handling, and other enzymes including glucose oxidase, and phytase improved the characteristics of whole wheat dough in varied manners.

Table 3. Improving effects of typical ingredients in whole wheat dough and bread (Tebben, Shen, & Li, 2018b)

Ingredient	Overall impact level	Observed effects
α-amylase	Major	Increased loaf volume, decreased crumb hardness and staling
G4-amylase	Major	Increased loaf volume, decreased crumb hardness and staling. Needs further study
Xylanase	Major	Decreased WA, increased loaf volume, decreased crumb hardness and staling, improved sensory characteristics
Glucose oxidase	Moderate	Increased dough strengthen, decreased dough resistence to extension, increased loaf volume
Phytase	Moderate	Dependent on enzyme strain and flour composition. Activation of endogenous α-amylase can lead to increased loaf volume, decreased crumb hardness
Amyloglucosidase	Minor	Various effects on dough strength, increased loaf volume
Lipase	Minor	Dough hardening, decreased loaf volume
Cellulose	Minor	Decreased crumb hardness
Transglutaminase	Minor	Decreased loaf volume, increased crumb hardness, improved sensory characteristics
Vital wheat gluten	major	Increased dough strength, increased loaf volume, improved sensory characteristics
DATEM	Moderate	Increased loaf volume, decreased crumb hardness, improved sensory characteristics
SSL	Moderate	Increased loaf volume, decreased crumb hardness, improved sensory characteristics
Ascorbic acid	Minor	Increased dough strength, increased loaf volume
HPMC	Minor	Increased loaf volume, decreased crumb hardness, improved sensory charaterisitics

However, within the groups of additives used in the food industry, the applications of DF in the bakery industry are limited. Field pea hull, sugar beet fibre, wheat bran, rice bran, whole grain rye, apple fibre, psyllium husk, pseudo-cereals, corn bran, oat bran, carob fibre, flaxseed fibre, peanut hull, and hydrocolloids are commonly enriched in baked products (Anil, 2002; Kupper, 2005). DF could affect dough properties from both chemical and microstructural aspects. As shown in recent studies, the particle size of the fibre and the number of the hydroxyl group present in fibre were associated to WA of the dough (Chartrand, Russo, Duhaime, & Seidman, 1997; Lohiniemi, Mäki, Kaukinen, Laippala, & Collin, 2000). Consequently, the WA influenced the rheological properties of the dough. In the study of Rosell et al. (2001), the rheological characteristic of dough were modified in different manners by four hydrocolloids (sodium alginate, κ-carrageenan, xanthan gum and hydroxyl propyl methyl cellulose), and the greatest effect at dough level was promoted by xanthan and alginate (Rosell et al., 2001). Hydrocolloids are also used in the gluten-free formulations to increase the water retention and thickness, as well as control the dough rheology (Arslan, Rakha, Xiaobo, & Mahmood, 2018). The research conducted on the cereal fibre source including wheat bran, rice bran, oat bran, and barley bran suggested that the resistance to extension and extensibility of the dough were decreased with the increase in the bran level (Sudha, Vetrimani, & Leelavathi, 2007). Considering the detrimental effect of the DF on dough, some proposals have been made. One is the incorporation of other additives such as emulsifiers, exogenous enzymes, SSL, vital gluten, and bromate (Haseborg & Himmelstein, 1988; Krishnan, Chang, & Brown, 1987; Laurikanen, Härkönen, Autio, & Poutanen, 1998b; Shogern, Pomeranz, & Finney, 1982; Sidhu, Al-Hooti, & Al-Saqer, 1999). Another proposal is the use of bran with different particle size (Glitsø & Knudsen, 1999).

2.3.4 Nutritional value

The fundamental ingredients for bread making are cereal flour, water, salt and yeast (or another leavening agent) (Martin, 2004; Sluimer, 2005). As the major ingredient, cereal flour offers a considerable amount of most nutrients in a balanced diet form (Truswell, 2002). Generally, bread provides human with nutrients, including starch, dietary fibre, protein, lipids and vitamins (Dewettinck et al., 2008). However, over thousands of years, bread has been developed widely around the world, which results in the different formulas and production techniques used in bread production. In this case, the nutritional value of bread can be affected by the ingredients and bread making methods.

Other ingredients added for better processing outcome and specialty bread could change the nutritional value of bread (Jackel, 1994; Sluimer, 2005). Bread with the incorporation of rye, barley and oats often have increased the nutritive value. Addition of SDF may impair digestion through reducing the contact of enzyme and starch with increased viscosity. Bread made with flour substituted by barley or wheat had higher resistant starch content (Åkerberg, Liljeberg, & Björck, 1998; Liljeberg, Åkerberg, & Björck, 1996; Van Hung, Yamamori, & Morita, 2005). Through addition or sourdough fermentation, the digestibility of starch was reduced with the presentation of lactic acid in the interaction of starch and gluten (Östman, Nilsson, Elmståhl, Molin, & Björck, 2002). The reduced bioavailability was attributed to the barrier created by the gluten.

As shown in the Table 4, bread making method may alter the nutritional value of bread by increasing or decreasing the level and bioavailability of bioactive compounds in grains. According to the earlier study, the resistant starch content in pumpernickel, wholemeal barley and white wheat bread was improved by increasing both baking temperature and time (Kale, Kotecha, Chavan, & Kadam, 2002). However, increasing the water level decreased the resistant starch content in bread. Vitamin content was also influenced by the physical factors in bread making, such as the mixing

process, pH, temperature and baking time (Batifoulier, Verny, Chanliaud, Remesy, & Demigne, 2005; Khetarpaul & Chauhan, 1989; Pedersen, Bach Knudsen, & Eggum, 1989). However, conflicting results were observed in the influence of bread making on phenolic content. The phenolic content in the crust of white bread slightly increased after baking, which is possibly generated from the Maillard reaction (Gélinas & McKinnon, 2006). In contrast, similar results were not found in wholemeal bread (Gélinas & McKinnon, 2006; Hansen et al., 2002).

Table 4. Potential strategies to increase bioavailability and bioaccessibility of phenolic compounds in bread (Angelino et al., 2017)

Strategy			Reason/mechanism	References
Raw materials	Type of grain/ cereal	Whole grains	Keeping all the anatomic parts of the kernel, where phenolic compounds are located Raw material naturally rich in phenolic compounds	(Hemery, Rouau, Lullien-Pellerin, Barron, & Abecassis, 2007) (Dykes & Rooney, 2007) (Taylor & Duodu, 2015) (Yu, 2015) (Abdel-Aal, Choo, Dhillon, & Rabalski, 2012) (Abdel-Aal & Rabalski, 2008) (Abdel-Aal, Hucl, Shipp, & Rabalski, 2016) (Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2010)
		Rye, barley Minor cereals Pseudocereals		
		Ancient grains		
		Pigmented grains		
Pre-processing	Selected fractions	Bran Aleurone layer	Anatomic parts of the kernel, rich in phenolic compounds	(Rosa-Sibakov, Poutanen, & Micard, 2015)
	Fractionation	De-branning	Selection of phenolic-rich fractions	(Martini, D'Egidio, Nicoletti, Corradini, & Taddei, 2015) (Zanoletti et al., 2017) (Blandino et al., 2013)
	Physical treatment	Air classification	Selection of phenolic-rich layers	(Verardo, Gómez-Caravaca, Marconi, & Caboni, 2011)
	Mechanical treatment	Micronization	Ultrafine grinding which damages the fiber matrix and increases the phenolic compounds available for extraction	(Zhu, Huang, Peng, Qian, & Zhou, 2010)
	Bio-technological process	Germination	Metabolic changes and/or increase in extractability by the activation of endogenous enzymes which break the bonds of bound phenolic compounds	(Hübner & Arendt, 2013) (Alvarez-Jubete et al., 2010)
		Fermentation/leavening	Release of insoluble bound phenolic compounds by activity of exogenous enzymes	(Katina et al., 2007) (Poutanen, Flander, & Katina, 2009) (Zhang, Gao, Chen, & Wang, 2014)
		Enzymatic treatment	Addition of enzymes which act to increase free phenolic compounds available for extraction	(Sørensen, Meyer, & Pedersen, 2003) (Moore, Cheng, Su, & Yu, 2006)

Bread-making process	Mixing and kneading		Release of bound phenolic compounds into free forms by mechanical action and/or activation of oxygenase and peroxidase	(Abdel-Aal & Rabalski, 2013) (Hilhorst et al., 1999)
	Fermentation/ leavening	Length of fermentation	Prolonged fermentation time increase the phenolic compounds available for extraction	(Yu, 2015)
		Type of fermentation (sourdough *vs.* dry yeast)	Increase in the release of insoluble bound phenolic compounds during sourdough fermentation favoured by the lowering of pH	(Hansen et al., 2002) (Konopka, Tańska, Faron, & Czaplicki, 2014)
	Baking		Possible decrease in phenolic content due to degradation (thermal labile)	(Alvarez-Jubete et al., 2010) (Vogrincic, Timoracka, Melichacova, Vollmannova, & Kreft, 2010)
		Temperature	Possible increase in phenolic bioaccessibility due to the release resulting from intense heat	(Lu et al., 2014)
			e.g., The upper crust, exposed to the greatest heat, generally has the highest level of phenolic compounds	(Yu, 2015) (Gélinas & McKinnon, 2006)
		Maillard reactions	May result in newly generated phenolic compounds	(Gélinas & McKinnon, 2006) (Michalska, Amigo-Benavent, Zielinski, & del Castillo, 2008)
		Time	No known effect	(Gélinas & McKinnon, 2006)

2.3.5 Previous studies on functional bread

Nowadays, there is a trend among customers to adopt a healthy lifestyle and proper nutritional habits. To meet the needs of the increasing population, researchers are focusing on the exploitation of substitution crops or underutilized materials to improve the nutritional value, flavor and mouthfeel.

Due to excellent physiological functions and physicochemical properties, DF has attracted the attention of the food industry. Table 5 presents partial DF sources investigated in previous studies. DF-rich by-products from primary or secondary food production streams are considered to be used as a partial replacement of flour, fat or sugar for enhancing the ability of WHC, OHC and emulsification or oxidation. Generally, DF is added to prolong the freshness and shelf life of the bread, thereby reducing economic losses. Up until now, cereal brans are the most common source of DF in the baking industry, followed by fruit or vegetables. Compared to fibre-free products, the fibre-containing products share some adverse effects, such as reduced loaf volume, dark color, unpleasant flavor and hard crumb. The effects differ by fibre source and dosage. With those known disadvantages, various methods were explored to improve the quality of the fibre-enriched bread. In a study, by reducing the particle size of fibre from sugarcane, the softness, elasticity, color, odor and taste of bread were improved (Sangnark & Noomhorm, 2004). Incorporation of the fibre after enzymatic treatment was also shown to have a positive effect on bread quality (Laurikanen, Härkönen, Autio, & Poutanen, 1998a).

Table 5. Dietary fibre sources used in baked products

Type of flour	Dietary fibre	Influence on bread quality	References
Wheat flour	Fenugreek fibre	Increased water absorption, and dough strength; prevented the starch retrogradation	(Huang, Guo, Wang, Ding, & Cui, 2016)
Wheat flour	Potato fibre	Softer bread, higher water retention, and reduced amylopectin	(Curti, Carini, Diantom, & Vittadini, 2016)
Wheat flour	Guar and xanthan gums	Guar increased the volume, porosity, and color; reduced the firmness compared with control and xanthan gum	(Hejrani, Sheikholeslami, Mortazavi, & Davoodi, 2017)
Rice flour	Rice bran	Increased sensory acceptance, extended shelf life	(Phimolsiripol, Mukprasirt, & Schoenlechner, 2012)
Wheat flour	Lemon pomace fibre	The hardness of bread was increased; high substitution of lemon pomace fibre resulted in an adverse effect on the formation of the gluten network, and a led to lower cohesiveness, elasticity, and specific volume	(Chang, Li, & Shiau, 2015)
Wheat flour	Pineapple pomace fibre	The water- and oil-holding capacity was increased with pineapple pomace fibre addition level (0, 5%, and 10%). Bread with 5% pineapple pomace fibre addition had the highest acceptance. Little differences were found in the specific volume and texture.	(Chareonthaikij, Uan-On, & Prinyawiwatkul, 2016)
Wheat flour	Chicory inulin	The extent of loaf volume reduction and crust color was increased with the increasing addition of inulin.	(Sirbu & Arghire, 2017)
Wheat flour	Oat fibre	Increasing the proportion of oat fibre powder resulted in increased firmness. The addition of oat fibre powder with smaller particles resulted in a product with the rheological and colour parameters that more closely resembled control sample.	(Kurek, Wyrwisz, Piwińska, & Wierzbicka, 2016)
Wheat flour	Mesquite flour	final specific volumes of composite bread were lower than that of control wheat bread. Hardness and chewiness increased, and cohesiveness and resilience decreased when higher contents of mesquite flour were added (25–35%).	(Bigne, Puppo, & Ferrero, 2016)

Polyphenol extracts from various plants are reported to have health benefits of reducing postprandial glycemia through inhibition and/or prolonging intestinal digestion (Bryans, Judd, & Ellis, 2007; Chai, Wang, & Zhang, 2013; Hanhineva et al., 2010). This has aroused interests in the application of certain polyphenols as functional food ingredients for health promotion. In recent literature, antioxidants have been added either in the form of extracts from natural sources, or as plant material rich in phenolic compounds (Korus et al., 2012; Świeca, Sęczyk, Gawlik-Dziki, & Dziki, 2014; Wang, Zhou, & Isabelle, 2007). The antioxidant potential in end-products was reported to be strongly dependent on the processing condition and product formula. In a previous study, sour dough wheat bread (durum and kamut) exhibited better protection effect than wheat bread on antioxidation (Gianotti et al., 2011). According to (Hye-Min Han & Bong-Kyung Koh, 2011), the antioxidant activity and free phenolic acid level were decreased during mixing, while partially recovered in the finished products. The mechanism was explained as the hydrolysis and releasing of antioxidants during fermentation. The effects of baking on the antioxidant level were widely studied in recent years, but conflicting results were observed. This may be explained by the Maillard reaction, in which the destruction-formation of natural- labile and thermally-introduced antioxidant compounds happen at the same time (Delgado-Andrade, Conde-Aguilera, Haro, De La Cueva, & Rufián-Henares, 2010). Nevertheless, strategies have been explored to improve the bioavailability and bioaccessibility of phenolic compounds, such as the utilization of raw material with higher phenolic content and various pre-processing technologies (Angelino et al., 2017). The properties of extended shelf life, reduced loaf volume and increased hardness were found in the polyphenol-added bread (Culetu, Duta, & Andlauer, 2018; Xu, Wang, & Li, 2019). According to previous study, the bread quality was affected by both covalent and non-covalent interactions between polyphenol and bread components such as wheat proteins (Sivam, Sun-

Waterhouse, Waterhouse, Quek, & Perera, 2011; Xu et al., 2019).

Chapter 3 Materials and methods

3.1 Materials

The dry sunflower stalks were obtained from Morden Research and Development Center located in Manitoba. The pith of sunflower stalks was peeled manually and then ground into powder. Wheat flour, yeast, sugar, salts, and shortening were purchased from local grocery stores (Bulk barn and Walmart, Guelph, ON). The green tea polyphenols were received from a Chinese company (Hongda Tea Co., Ltd, Wuyuan, Jiangxi province). Glacial acetic acid (1.05 g/cm^3, certified ACS Grade), sodium hexametaphosphate (powder, Laboratory Grade), α-amylase (from human saliva, 102.6 units/mg solid), sodium potassium tartrate (99%, certified ACS Grade), invertase (from baker's yeast, ≥ 300 units/ mg solid), guar gum (powder, Laboratory Grade), and pepsin (from porcine pancreas, 8 x USP specifications) were purchased from Sigma Aldrich. Commercial pectin standards were purchased from Fisher Scientific. Amyloglucosidase (3300 units/mL on soluble starch), regular maize starch (93% starch (dw), 8.3% moisture) were from Megazyme total starch kit. Disodium hydrogen phosphate (99%, certified ACS Grade), sodium dihydrogen phosphate (99%, certified ACS Grade), sulfuric acid (18 M, certified ACS Grade), and hydrochloric acid (36.5% to 38%, certified ACS Grade) were purchased from Caledon Laboratories.

3.2 Sample preparation

The centre of sunflower stalk was peeled manually and ground into powder using a grinder (M20, IKA, U.S.A). The sunflower stalk pith powder (SSP) was then sieved through 30 mesh (1.19 mm). After sieving, the sunflower stalk pith powder was stored in a desiccator before analyzing.

3.3 Extraction of sodium hexametaphosphate-extractable pectin (SEP)

The extraction method was adapted from Xu (Xu, 2016). The complete extraction process is

demonstrated in Figure 1. The sunflower stalk pith powder was suspended in 0.75% sodium hexametaphosphate (SHMP) solution in a ratio of 1:25 (weight to volume, g/mL). The pH of the mixture was then adjusted to 3.5 with HCl. The extraction was performed in a 75 ℃ water bath under stirring for 60 min. Subsequently, the mixture was centrifuged at 10,000 rpm under room temperature for 20 min and filtered through the cheesecloth to get the supernatant and pellet. The supernatant was concentrated using a rotary evaporator. The concentrated extracts were precipitated with 3 times-volume of anhydrous ethanol. The precipitated pectin and pellet were freeze dried. The dried two fractions were dialyzed against distilled water for 48 hours. Following with the freeze drying, the purified SEP and residue fraction (RF) were obtained.

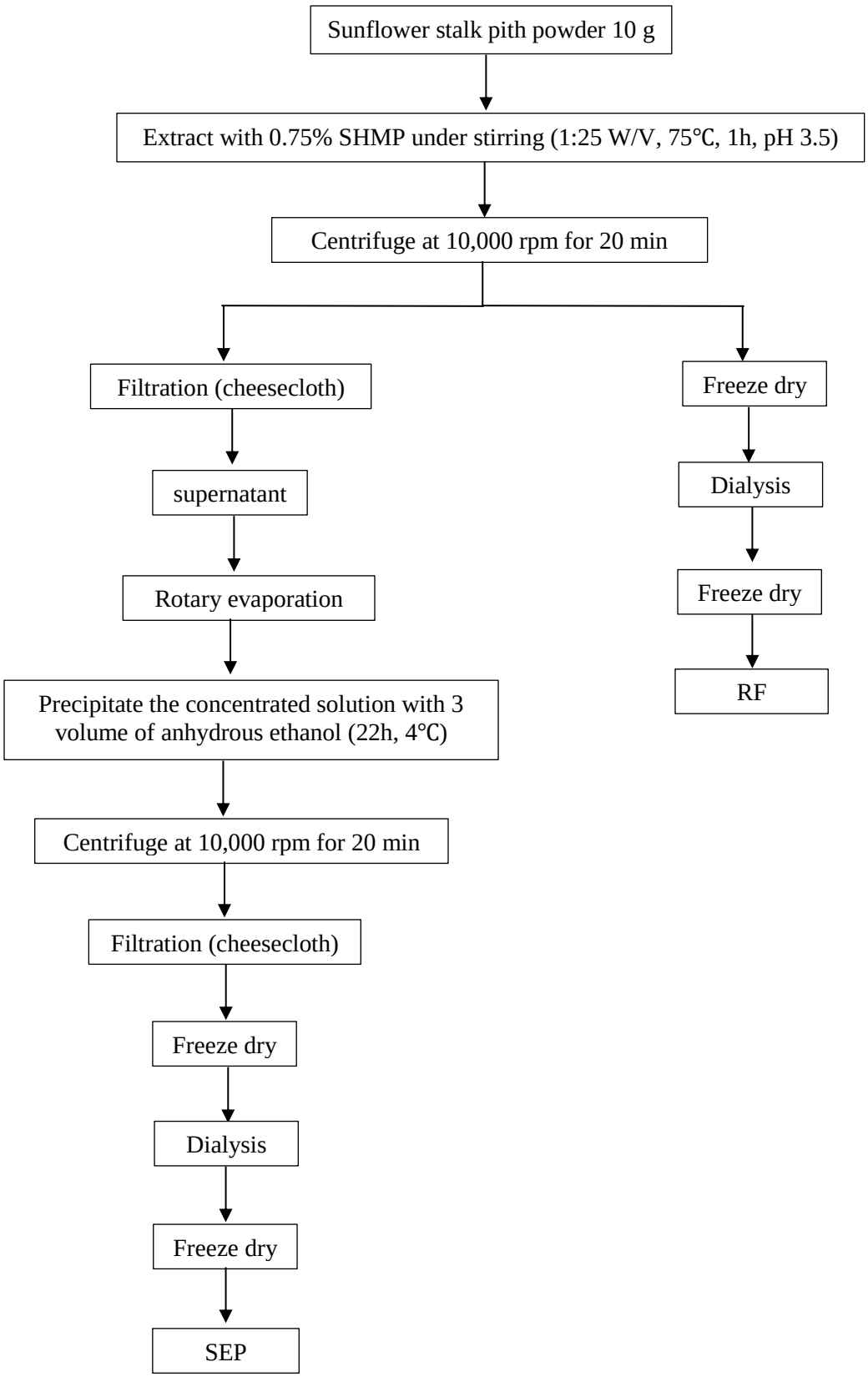

Figure 1. Extraction of SEP and RF from SSP

3.4 Proximate analysis

The ash and moisture content were determined according to the official method (AOAC., 1990). The content of total galacturonic acid was determined according to the m-hydroxydiphenol method (Blumenkrantz & Asboe-Hansen, 1973). The protein content was calculated based on the content of nitrogen determined by the protein analyzer (FLASH 2000 Organic Elemental Analyzer, Thermo Scientific, CA), using 6.25 as the nitrogen conversion factor. The quantitative determination of mineral elements (Ca, Cu, Fe, Mg, Mn, P, K, Na, S, Zn) in SSP was carried out by another independent lab (Laboratory Services, University of Guelph, Canada).

3.5 Monosaccharides composition analysis

Samples (50 mg) were weight into a glass screw cap tube, followed by adding of 0.5 mL 12 M sulfuric acid to hydrolyze at room temperature for 30 min. The mixture was then hydrolyzed for 120 min at 100 °C water bath after addition of 4.5 mL Milli-Q water, and then diluted and filtered through the 0.45 filter. The analysis was carried out by the high-pressure anion exchange chromatography combined with pulsed amperometric detection (HPAEC-PAD) (DX600, Thermo Scientific Dionex, Sunnyvale, CA) using a CarboPac PA1 column (4 × 250 mm) and guard (3 × 25 mm). Samples were eluted in 10 mM and then 100 mM NaOH. Standard solutions between 10 and 100 μg/mL were prepared with sugar standards (rhamnose, arabinose, galactose, glucose, mannose, and xylose) (Wood, Weisz, & Blackwell, 1994). The standards at gradient concentration were applied in the quantification of sugar residues from the hydrolysate.

3.6 Fourier transform infrared spectroscopy (FT-IR) analysis

SSP, SEP, RF, and pectin standards were kept in a desiccator before FT-IR analysis. The FT-IR spectra were recorded with the FT-IR spectrometer (PerkinElmer, CA) equipped with a universal attenuated total reflectance (ATR) sampling accessory. The FT-IR spectra of samples

were recorded under absorbance mode from 4000 to 650 cm-1 interval (Chylińska, Szymańska-Chargot, & Zdunek, 2016; Karadag et al., 2018). The resolution of 4 cm^{-1} with 4 co-added scans was applied to obtain an optimal signal-to-noise ratio. The degree of esterification (DE) of pectin is proportional to the ratio of the number of total esterified carboxylic groups to the number of the total carboxylic group. The original form of the esterified carboxylic group and the carboxylic group were reported to responsible for the absorbance around 1740 cm^{-1} and 1600 cm^{-1}, respectively (Filippov, 1992). The esterification degree of SEP was determined by calculating the ratio of $\frac{A_{1740}}{A_{1740}+A_{1600}} \times 100\%$. The standard pectin with known DE of 26%, 59%, and 94% were purchased from Sigma-Aldrich.

3.7 α-amylase assay

The method was an adaption from Tan et al. (Tan & Gan, 2016). The components of inhibitor solutions are depicted in Table 7. The human salivary enzyme solution, the inhibitor solutions, and the 1% starch solution were pre-incubated in a 25℃ water bath. 100 µL of digestive solution and inhibitor solution were pre-mixed for another 10 min, followed by adding the 1% starch solution. After 10 min, the reaction was then stopped by adding 20 µL 3,5-dinitrosalicylic color reagent, and the color was developed over 5 min boiling. The digested solutions were diluted with distilled water and cooled down to room temperature. Subsequently, the absorbance of the digested solution was measured at 540 nm. The inhibitory rate was calculated according to the following equation:

$$\%\ Inhibition = \frac{(A_{control} - A_{control\ blank}) - (A_{sample} - A_{sample\ blank})}{A_{control} - A_{control\ blank}} * 100\%$$

where $A_{control}$, $A_{control\ blank}$, A_{sample}, and $A_{sample\ blank}$ refer to the absorbance of reaction vials containing live enzyme and PBS buffer, dead enzyme and PBS buffer, live enzyme and inhibitor, as well as dead enzyme and inhibitor, respectively.

Table 6. Chemical composition of TP

Composition	Content [a] (%)
EGC	15.10
C	2.69
EC	7.21
EGCG	51.86
GCG	2.15
ECG	12.24
6 Catechin in total	91.25
Caffeine	0.41

[a] based on the total weight of raw material

Table 7. Composition of inhibitor solutions

Concentration (SEP, mg/mL)	Composition (SEP +TP)				
	10:2 (SEP:TP)	10:1(SEP:TP)	10:0.5(SEP:TP)	SEP	TP
10.00	10.00+2.00	10.00+1.00	10.00+0.50	10.00	1
5.00	5.00+1.00	5.00+0.50	5.00+0.25	5.00	0.5
2.50	2.50+0.50	2.50+0.25	2.50+0.125	2.50	0.25
1.25	1.25+0.25	1.25+0.125	1.25+0.0625	1.25	0.125
0.625	0.625+0.125	0.625+0.0625	0.625+0.03125	0.625	0.0625

3.8 Application in bread

3.8.1 Farinograph test

The farinography analysis was performed with Farinograph-E (Brabender® GmbH & Co. KG, Duisburg, NJ07606 Germany, Serial #071150). According to the AACC Approved Method 54-21.02, the Farinograph tests were conducted on the wheat flour blended with SSP, RF, and TP at three substitution levels (substitution level: 6%, 9%, and 12%), and 6% yellow mustard gum

(YMG) substitution combined with 0.1% TP addition, respectively. The water absorption was recorded as the water amount added to maintain the Farinograph curve at 500 ± BU.

3.8.2 Baking procedure

Flour used in bread consisted of: 6% SSP substitution, 6% RF substitution, 6% YMG substitution, 0.1% TP addition, and 6% YMG substitution combined with 0.1 % TP addition. The bread recipes of each flour blending are listed in Table 8. Bread baking procedure was modified according to AACC international method 10-10.03. The water absorptions for each flour blending were obtained in the farinograph analysis. Hard wheat flour was premixed with fibres (SSP, RF, YMG) or/and TP. Initially, dough mixing was performed with a 100 g micromixer (National Manufacturing, Lincoln, NE 68508 U.S.A, Serial #44942B) for 2 min at room temperature. After mixing, the dough was placed in a lightly greased bowl and fermented in a fermentation cabinet (Duke Manufacturing), at 86 F and high humidity. The dough was punched at 52, 77, and 90 min intervals of fermentation with a dough sheeter (National Manufacturing, Lincoln, NE 68508 U.S.A, Serial #49520). The fermented dough was rounded and transferred into a three-sided greased metal baking tin and proofed in the fermentation cabinet for 36 min. Finally, the proofed dough was baked in a rotary oven (National Manufacturing, Lincoln, NE 68508, U.S.A, Serial #44942A) at 215 °C for 24 min.

Table 8. Bread recipe with fibres and TP fortification

Ingredients	Water g/100g	Salt /g	Sugar /g	Yeast /g	Shortening /g	Flour (g)/ fibre or (and) TP (g)
Control	66.2	1.5	6.0	5.3	3.0	100.0/0
6% SSP substituted	70.7	1.5	6.0	5.3	3.0	94.0/6.0
6% RF substituted	77.4	1.5	6.0	5.3	3.0	94.0/6.0
6% YMG substituted	70.5	1.5	6.0	5.3	3.0	94.0/6.0
0.1% TP enriched	65.3	1.5	6.0	5.3	3.0	100.0/0.1
YMG-TP fortified	70.1	1.5	6.0	5.3	3.0	94.0/6.0 + 0.1

[a] flour was premixed with fibre or/and TP

3.8.3 Specific volume

The resulted bread was cooled down at room temperature for 2 h. The weight of each bread was recorded. According to AACC international method 10-05.01, the loaf volume (cm^3) was determined by rapeseed displacement method with a volumeter (National Manufacturing, Lincoln, NE 68508, U.S.A). The specific volume was defined as the loaf volume divided by the bread weight. Data are reported as the mean value of measurement from three loaves.

3.8.4 Porosity of bread

The images of loaf were taken by Nikon DSLR D700 equipped with 24-70 mm/f 2.8 mm lens (Nikon Corporation, Japan) at 80 cm from loaf surface. Specific area images were cut from the center of the loaf.

3.8.5 Color analysis

The color value of the bread crumb from bread samples was determined by the Hunter Lab color measuring system (Labscan XE, Serial# LX17334, Hunter Lab, U.S.A). The results were expressed as L* (luminosity), a* (red versus green), b* (yellow versus blue), and ΔE (total color difference). The ΔE was calculated with the following equation: $\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$.

3.8.6 Texture profile analysis

Two slices of bread, each 1.25 mm thick were cut from the center of the bread loaf with a

slicer (Cuisinart, LB-9/014, Canada). Two sliced bread were stacked up to 2.5 mm thick. The texture profile of bread, including hardness, cohesiveness, gumminess, resilience, chewiness, and springiness were measured using the texturometer (EZ-LXHS, SHIMADZU Scientific, CA). The instrument was set at 40% compression with a 36 mm cylindrical jade at a test speed of 10 mm/sec (AACC international method 74-10.02). The results were analyzed by TRAPEZIUM X (SHIMADZU Autograph Software, CA).

3.9 Statistical analysis

The results (except Table 6) were expressed as means ± standard deviation (SD). The statistical analysis for farinograph analysis, color analysis, and texture profile analysis was performed with one-way analysis of variance (ANOVA) by using SPSS (Version 13.0, Chicago, USA). Results were regards as statistical significance if $p < 0.05$.

Chapter 4 Results and discussion

4.1 Extraction and purification

SEP was isolated from sunflower stalk pith with 0.75% sodium hexametaphosphate solution in a 75℃ water bath. The SEP and RF were further dialyzed against distilled water for 48 h. The yields of SEP and RF before dialysis were 20.93±1.36% and 71.10±2.21%, respectively, relative to the total weight of sunflower stalk pith powder. The yield of SEP was higher than the reported yield from sunflower head, although the extraction temperature used by them was 65℃ (Sahari et al., 2003). The difference in extraction yield was affected by the sunflower cultivar, degree of maturity, storage condition, raw materials, size of the particle, and extraction conditions (Chang, Dhurandhar, You, & Miyamoto, 1994b; Sahari et al., 2003).

Table 9. Chemical compositions of SSP, SEP, and RF

Composition [b]	Neutral sugar (%)	Uronic acid (%)	Protein (%)	Ash (%)	Moisture (%)
SSP	53.46±2.11	- [a]	4.89±0.02	19.56±0.17	11.05±0.06
SEP	7.75±0.14	67.07±0.19	3.19±0.05	18.67±0.32	9.22±0.19
RF	68.49±3.01	- [a]	5.10±0.07	13.50±0.72	3.14±0.36

[a] not measured; [b] data are given as "mean ± SD", n=3; based on the total weight of raw material

Table 10. Mineral contents of SSP

Contents	SSP
Calcium (μg/g)	17000
Copper (μg/g)	9.1
Iron (μg/g)	31
Magnesium (μg/g)	3900
Manganese (μg/g)	10
Phosphorus (μg/g)	2400
Potassium (μg/g)	63000
Sodium (μg/g)	2800
Sulfur (μg/g)	2000
Zinc (μg/g)	35

The chemical compositions of three fractions and the mineral contents of SSP are shown in Table 9 and Table 10, respectively. The results of the proximate analysis revealed the high content of neutral sugar in SSP, which was higher than the reported 42.20% in our previous study (Xu, 2016). The ash content in SEP was approximately 18%, which was lower than that reported in an earlier study (Iglesias & Lozano, 2004). The dissimilar ash content resulted from the extraction and dialysis process (Iglesias & Lozano, 2004; Singthong, Ningsanond, Cui, & Goff, 2005). During the extraction, the application of chelating agent of SHMP, low pH, and precipitation methods could affect the ash content (Iglesias & Lozano, 2004; Lin, Sosulski, & Humbert, 1978; Xu, 2016). The dialysis process was effective in lowering the concentration of potassium. However, the concentration of calcium and sodium was increased after dialysis due to the stronger binding between these ions and pectin (Singthong et al., 2005). The rich mineral elements present in the raw SSP, such as calcium, potassium, and manganese, were consistent with the high ash content. The increased neutral sugar content in RF suggested the separation of uronic acids from SSP. The galacturonic acid content in SEP was 67.07%, relative to the total weight of SEP,

reflecting the pectic nature of the extracted substance.

4.2 HPAEC analysis

Table 11. Monosaccharides compositions of SSP, SEP, and RF

Mono[b]/ fraction	Rha (%)	Ara (%)	Gla (%)	Glu (%)	Xyl (%)	Man (%)
SSP	2.17±0.10	2.88±0.13	3.40±0.23	43.67±1.84	4.41±0.33	2.85±0.13
SEP	1.13±0.03	1.45±0.02	3.42±0.09	1.74±0.04	ND[a]	ND[a]
RF	2.26±0.17	2.80±0.15	2.90±0.13	54.82±2.23	4.56±0.31	3.31±0.17

[a] not detected; [b] data are given as "mean ± SD", n=3; based on the dry weight of raw material

The monosaccharides composition of three fractions is demonstrated in Table 11. The primary neutral sugars in SEP were rhamnose, arabinose, and galactose, which were the building units of rhamnogalacturonans type I (RGI) and rhamnogalacturonans type II (RGII) (Cui, 2005). Xylose and mannose were not found in SEP, suggesting the high content of RGI structure. It is noted that the pectin in the form of RGII was found to be lower than 5% (Yapo, 2007). Moreover, the low content of neutral sugar in SEP (7.75%) indicated the relatively smaller proportion of rhamnogalacturonan structure. The main component in SSP and RF was glucose, reflecting their cellulose nature (Habibi, Mahrouz, & Vignon, 2009). The proportion of galactose, xylose and arabinose in SSP and RF further confirmed the presence of xylans and xyloglucan, the most common neutral hemicellulose (Mateos-Aparicio, Redondo-Cuenca, & Villanueva-Suárez, 2010). In the study of Hua et al. (2015), xylose and mannose were reported to be the products after hydrolysis from cellulosic and semi-cellulosic part (Hua et al., 2015).

4.3 FT-IR analysis

The spectra of raw SSP and RF are shown in Figure 2. SSP and RF displayed intense absorbances in the range of 4000 to 650 cm^{-1}. In particularly, SSP and RF shared a similar shape

of spectrum. The broad absorption peaks in the range of 3600 to 3000 cm^{-1} were correlated to the stretching vibration of free, inter, and intramolecular –OH (Yuen, Choi, Phillips, & Ma, 2009). The FT-IR bands around 1640 from RF, SSP and TP were assigned to the stretching vibrations of C=C (Cai, Xie, Chen, & Zhang, 2013). The weak band around 2900 cm^{-1} from SSP and RF indicated the stretching vibration of C-H of the methyl group from the sugar ring (Li, Wang, Zheng, & Li, 2017). The absorption peak at 1430 cm^{-1} was correlated to the CH_2 bending vibration of cellulose and recognized as "crystallinity band" (Wang, Shankar, & Rhim, 2017). The absorption peaks in SSP and RF around 890 cm^{-1} corresponding to the C-O-C stretching vibration was assigned to the β-(1→4)-glycosidic linkage from cellulose (amorphous band) (Wang et al., 2017).

Figure 3 shows the spectrum of SEP and the pectin standards with known DE of 26%, 59%, and 64%. The weak absorption peaks around 1430 cm^{-1} indicated the plane deformation vibration of aliphatic or aromatic C-H from methyl, methylene, and methoxy groups (Grassino et al., 2016). Generally, the peaks between 800 and 1200 cm^{-1} are recognized as the "fingerprint region", which is unique for individual polysaccharides and hard to interpret the overlap (Cui, Phillips, Blackwell, & Nikiforuk, 2007; Kozarski et al., 2011). The absorption bands between 1100 and 1200 cm^{-1} were attributed to the ether (R-O-R) and cyclic C-C bonds from the pectic ring structure (Grassino et al., 2016). The peaks around 1100 and 1005 cm^{-1} were assigned to the glycosidic linkage of sugar units (Gnanasambandam & Proctor, 2000). The absorption bands around 920 and 830 cm^{-1} were attributed to the D-glucopyranosyl and α-D-mannopyranose, respectively (Zhang et al., 2013). Other typical characteristic peaks of pectic material found in our study included the absorbance at 880 (pyranose ring) and 1270 cm^{-1} (C-O dilatation vibration). According to the evidence, it can be concluded that the obtained substance was rich in polygalacturonic acids.

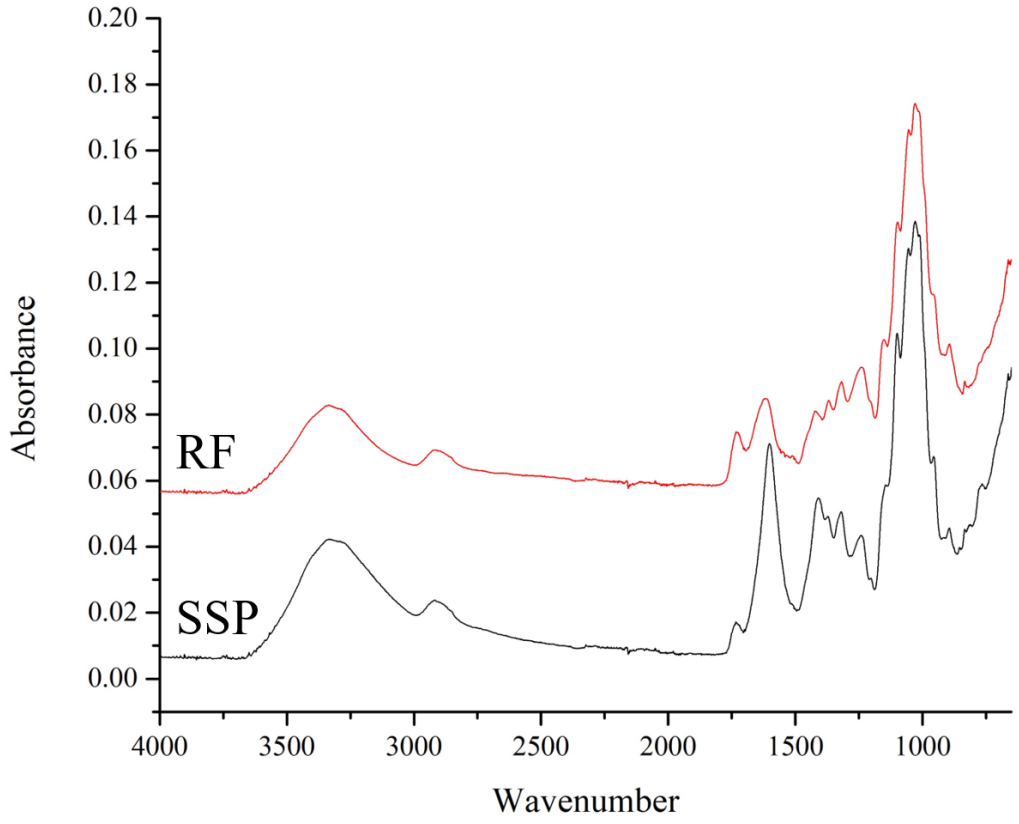

Figure 2. FT-IR spectra of SSP and RF at 4000-650 cm^{-1} wavenumber

According to the previous study, the bands in the range of 1630-1650 and 1740-1760 cm^{-1} were assigned to the free and esterified carboxyl group, respectively (Grassino et al., 2016; Naji-Tabasi, Razavi, Mohebbi, & Malaekeh-Nikouei, 2016). With the increasing DE values, the intensities and band area of the esterified carboxyl groups were increased (Grassino et al., 2016). Consequently, the DE of pectic material could be calculated from the corresponding band area. In Figure 4, the calibration curve was built as the DE versus the ratio of $A_{1740}/(A_{1740}+A_{1600})$. The square linear correlation coefficient of 0.9981 indicated the strong linear relationship between DE and the ratio of $A_{1740}/(A_{1740}+A_{1600})$. According to the regression equation, the DE of the SEP was determined to be 34.5%, indicating the characteristic of low-methoxyl pectin (LMP). This fact was

consistent with the previous results from studies conducted on the pectin isolated from sunflower head residue and sunflower stalk (Hua et al., 2015; Iglesias & Lozano, 2004; Lin, Humbert, Sosulski, & Downey, 1975). The LMP was also found in melon peel and sour orange peel, which have the DE of 1.33-29.33% and 6.77-28.07% respectively (Hosseini, Khodaiyan, Kazemi, & Najari, 2019; Raji, Khodaiyan, Rezaei, Kiani, & Hosseini, 2017).

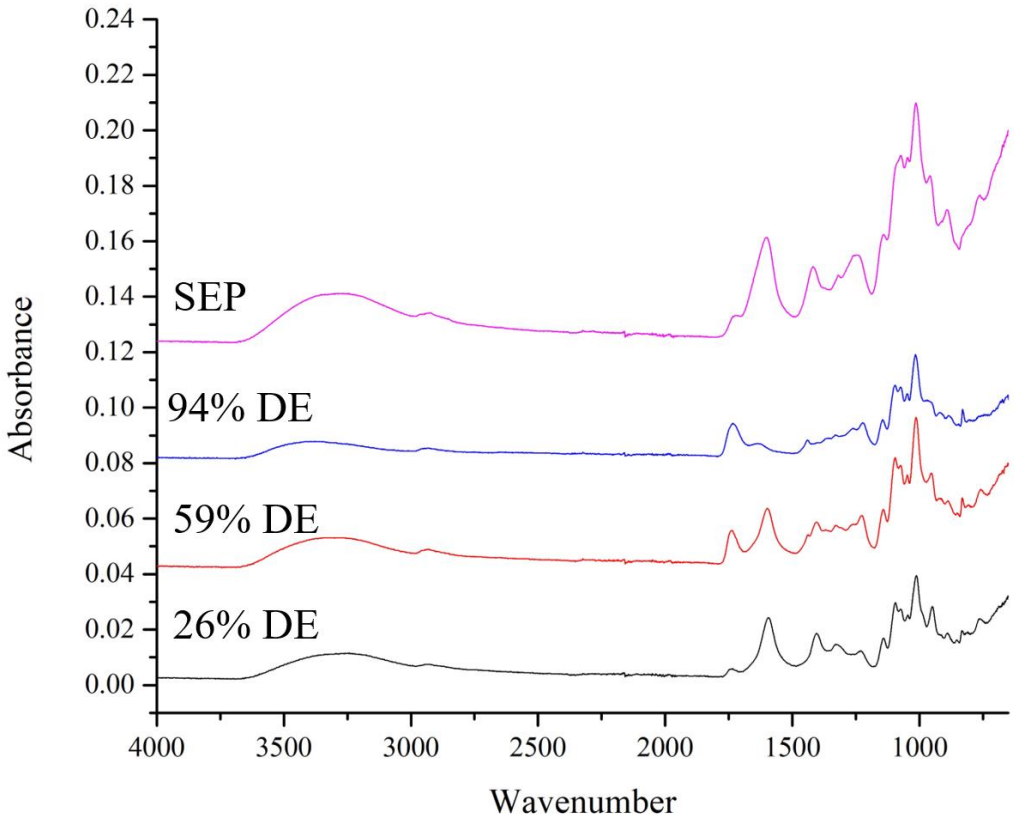

Figure 3. FT-IR spectra pf SEP, and citrus pectin standards of known DE at 4000-650 cm^{-1} wavenumber

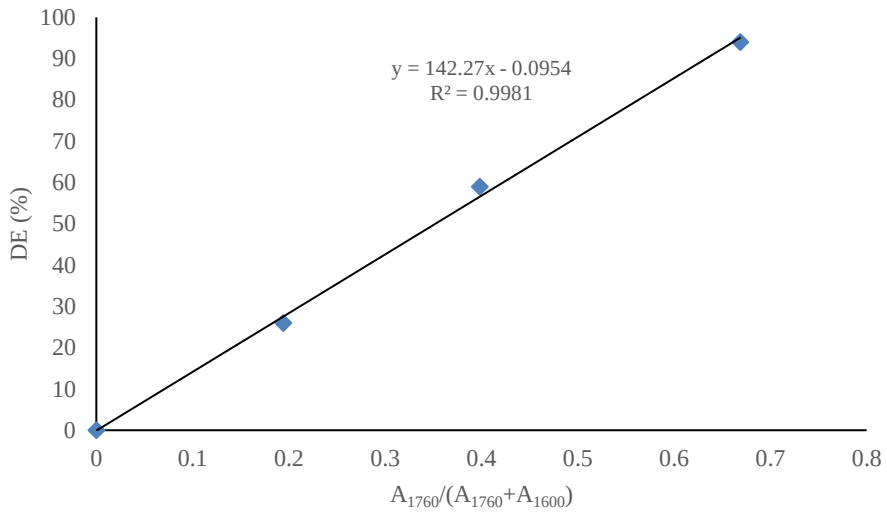

Figure 4. Calibration curve of esterification degree (DE) determined by FT-IR spectra of pectin standards

Figure 5 presents the spectra of TP and three SEP/TP complexes. In the spectra of TP, the broad band between 3500-3000 cm^{-1} was attributed to the –OH stretch (Robb, Geldart, Seelenbinder, & Brown, 2002). Absorption due to C=O was observed around 1687 cm^{-1} (Wu et al., 2019). The peak around 1610 cm^{-1} was assigned to the C=C stretching of the aromatic ring, indicating the functional group from phenolic compounds (Siripatrawan & Harte, 2010). The peak near 1190 cm^{-1} was due to the CO stretch of the aromatic alcohol. Peaks between 900 and 700 cm^{-1} were correlated to the Ar-H out-of-plane bond (Grzesik et al., 2018). The spectra of SEP/TP complexes at three mixing levels (SEP:TP at 10:0.5, 10:1, and 10:2) are shown in Figure 5. In comparison to TP, the peaks between 3600 and 3000 cm^{-1}, corresponding to the stretching vibration of free –OH, are slightly increased in SEP/TP complexes. The absorption peak at 1640 and 1700 cm^{-1} became more noticeable with increasing TP concentration, indicating the increasing amount of C=O and C=C in functional group of phenolic compounds. The increased intensities in those peaks were consistent with the increasing proportion of TP. In the previous study, the intensity of peak correlated to –OH was decreased with increasing green tea extract concentration

present in the chitosan solution (Siripatrawan & Harte, 2010). In their study, the coincidence of decreased peak at 3600-3000cm^{-1} and the increased peak at 1640 and 1700cm^{-1} confirmed the assumption of specific arrangement in the complexes due to the interaction by hydrogen bonding. As no decrease in the peak assigned to –OH was found, the interaction between SEP and TP by hydrogen bonding remained unclear. According to previous studies on the polyphenol-polysaccharides interaction, the formation of hydrogen bonding is mainly driven by enthalpy but also might be induced by conformational changes of compounds in the aqueous environment (Watrelot, Le Bourvellec, Imberty, & Renard, 2014; Zhu, 2018). Furthermore, the association between polyphenol and polysaccharides could also result from the hydrophobic interaction, which occurs between dihydropyran heterocycle from polyphenol and methyl group from pectic fraction (Renard, Watrelot, & Le Bourvellec, 2017).

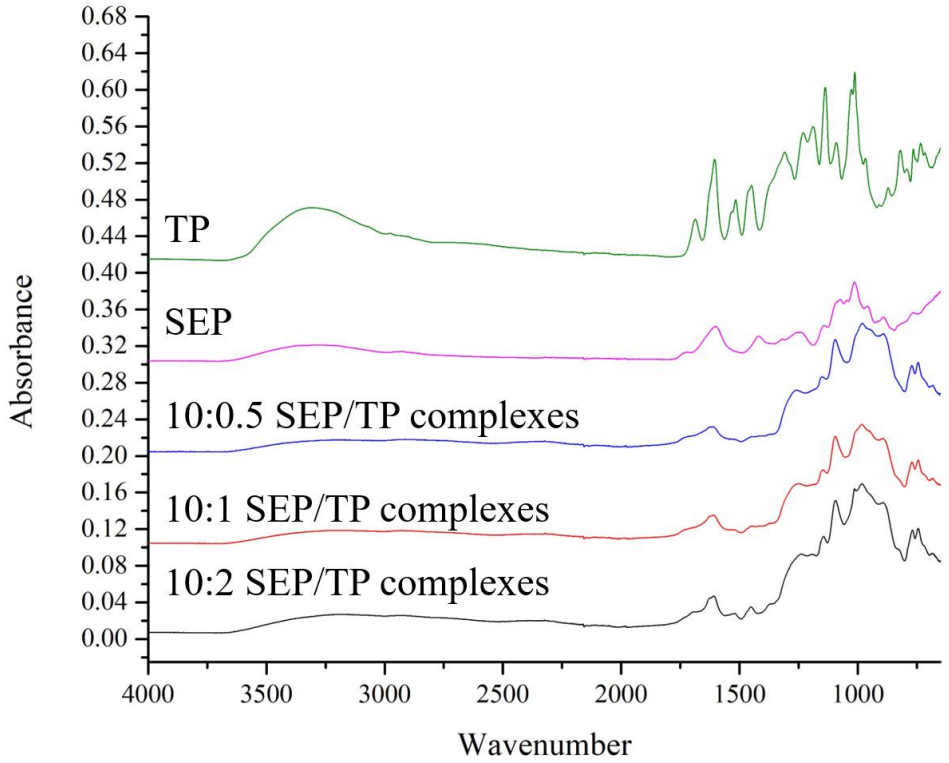

Figure 5. FT-IR spectra pf SEP/TP complexes in ratios (SEP:TP) of 10:0.5, 10:1 and 10:2

4.4 α-amylase analysis

The inhibition rate of each inhibitor is shown in Table 12. Evidently, the type and the concentration of inhibitor significantly affected α-amylase activity. The recognizable inhibitory effect was found in SEP/TP complexes and TP. However, the inhibitory effects of SEP seem random and non-dose dependent. Among three inhibitors, TP showed the strongest inhibitory effect on α-amylase at each concentration (except at TP concentration of 0.125 mg/mL), which was followed by SEP/TP complexes and SEP. The highest α-amylase inhibitory effect of TP was obtained at concentration 1 mg/mL as 85.15% reduction. The inhibition rate at TP concentration of 0.5 mg/mL was comparable with previous study from He et al. (2007), although the concentration of the green tea extract they used was 10-times lower with different composition (C and EGC, 5.1%; EGCG, 40.9%; ECG, 30.4%; GCG, 10.9%; EC, 6.3%) (He, Lv, & Yao, 2007). The inhibitory effect of SEP/TP complexes in three compositions was dose-dependent in the concentration range of SEP from 0.625 to 10.00 mg/mL. The SEP/TP complexes had the highest α-amylase inhibitory rate around 72% at the ratio of 10:2 (SEP:TP), when the concentrations of SEP within the complexes was 10 mg/mL and 5 mg/mL. It is worth mentioning that the inhibition rate of SEP/TP complexes in three compositions were not seen to increase after the concentration of SEP within the complexes reached 5 mg/mL.

As shown in the results, TP was the mainstream in the inhibition of α-amylase activity. According to the previous study on the inhibitory effect of TP against α-amylase, the mechanism may lay in the binding of TP to the active site residues in protein (Miao, Jiang, Jiang, Zhang, & Li, 2015). In comparison to pure TP, the relative lower inhibitory effects of SEP/TP complexes and pure SEP were related to the limited solubility of the SEP. Besides the rheological effects, SEP mainly acted as a physical barrier in the interaction between starch and α-amylase. Another

possible mechanism pointed out by Chau et al. (2003) was that the insoluble fibre could inhibit the activity of α-amylase by adsorbing glucose and retarding the glucose diffusion (Chau, Huang, & Lee, 2003). During the formation of the SEP/TP complexes, the TP could interact with SEP through hydrogen bonding and hydrophobic interaction (Zhu, 2018). In this case, fewer active sites were available for TP to bind with α-amylase, thus resulting in lower inhibition capacity of SEP/TP complexes. In terms of the inhibitory capacity of polysaccharides against α-amylase, more pronounceable results were found in the water-soluble fibre. In the study of polysaccharides from *Momordica charantia*, the α-amylase inhibition rate was 89.1% at the polysaccharides concentration of 10 mg/mL (Tan & Gan, 2016). A study on the polysaccharides from mulberry fruit fractioned by 0.2 M NaCl also reported the highest inhibitory effects in the tested concentrations as approximately 60% reduction on amylase activity, when the concentration was 5 mg/mL (Chen et al., 2016).

Table 12. Inhibition rate of inhibitors in different compositions and concentrations

Concentrations (SEP, mg/ml)	Inhibition rates (%)				
	10:2 (SEP:TP)	10:1(SEP:TP)	10:0.5(SEP:TP)	10 SEP	1 TP
10.00	72.05±3.65	54.97±3.00	27.11±1.55	10.59±5.81	85.15±2.11
5.00	72.74±1.37	54.48±2.05	27.02±3.34	1.68±8.60	67.19±3.66
2.50	41.19±2.58	38.75±2.94	14.82±8.88	0.39±7.96	48.84±2.02
1.25	31.97±1.31	28.10±4.35	11.84±1.27	-1.87±4.99	23.69±1.79
0.625	8.31±3.86	5.44±4.98	0.00±6.03	2.57±5.12	10.33±2.94

[a] data is given as "mean ± SD", n=3; concentration of each monomer was equal to that in the complexes.

4.5 Bread characteristic

4.5.1 Farinograph: the effects of fibres and TP on dough properties during mixing

SEP was not investigated in latter research due to the limited yield. Alternatively, yellow mustard gum (YMG), the physical and structural characteristics of which were well studied in our previous studies, was included in the latter part of the study. In this phase of research, the effects of three fibres (SSP, RF, and YMG), as well as TP, were determined on farinograph. In addition,

the impact of the combination of YMG and TP were investigated and compared with that of YMG substituted bread and TP fortified bread. Farinograph evaluates the dough properties by determining the resistance against mixing action of blades as a function of time (TömöSközi, GyeNGe, PeLCéder, AboNyi, & Lásztity, 2011). Generally, it is applied in the determination of the effects of ingredients on dough mixing properties, the water absorption and blending requirements during the dough development. Additionally, the farinograph also can be used in the prediction of the product texture characteristics. The dough parameters determined by farinograph include water absorption (WA, the water amount required to maintain the mixing curve around 500 BU), dough development time (DDT, the time interval from the first addition of water to reach maximum consistency), stability (the time interval of the arriving time and departure time), mixing tolerance index (MTI, the difference of BU value at peak time and 5 min after the peak), and time to break down (TTB, the time between the start of the mixing and there has been a decrease of 30 BU from the peak point).

The farinograph and registered parameters of wheat flour with SSP replacement in three substitution level (6, 9, 12g/100g) are presented in Table 13. The characteristics of bread dough, including WA, rheological behavior, and baking qualities, were significantly modified by the substitution of SSP. In comparison to control, the WA value was increased by 4.5, 10.3, and 14.6 g/100 g. This fact was correlated to the high water holding capacity of SSP. Similar results were reported in previous studies (Huang et al., 2016; Roberts, Cui, Chang, Ng, & Graham, 2012). With the increasing substitution of SSP, the DDT was increased, indicating a longer hydration process in the dough formation. Due to the large number of hydroxyl groups in fibre, the hydration process involves the balancing of the water among the flour components, mainly including gluten, starch, and SSP (Messia et al., 2016; Miś, Nawrocka, & Dziki, 2017). The stability of the dough was

decreased with increasing SSP substitution, suggesting reduced dough strength and bread-making ability. This fact was possibly correlated to the dilution effect of SSP in gluten crosslinking formation. The minor difference in MTI value was found in the dough with the control and SSP replaced dough at the substitution levels of 6 g/100 g and 9 g/100 g. The highest MTI value was found at the substitution level of 12 g/100 g, showing a softer texture after dough mixing. Time to breakdown (TTB) was slightly reduced with increasing SSP substitution, which reflected the overall minor influence of SSP on the dough stability. Although the dilution effect of SSP replacement on gluten protein weakened the interaction between gluten proteins, it was counteracted by the strong interaction between the gluten matrix and SSP (Miś, Grundas, Dziki, & Laskowski, 2012). In a study on the effect of Moldavian dragonhead leaves on the wheat flour properties and bread quality, where the WA, DDT, and MTI value were increased, the stability was reduced (Dziki et al., 2019).

In Figure 6, the farinograph of SSP substituted dough at the substitution level of 9, and 12 g/ 100 g had a noisy initial stage. This was attributed to the inconsistency of the hydration process and instability of the dough. With the increasing percentage of SSP, two peaks could be identified in the graph. The double peak was more obvious at the substitution levels of 9 and 12 g/100 g, where the first peak was relative comprised. Moreover, the identification of the second peak was reported in previous research, and regarded as the result of the dehydration-induced increase in dough consistency (Huang et al., 2016; Roberts et al., 2012; Salinas, Carbas, Brites, & Puppo, 2015; Teng, Liu, Bai, & Liang, 2015). Accordingly, the first peak was assigned to the formation of the gluten network by disulfide bonding and the second peak was assigned to the fibre (Huang et al., 2016). However, the explanation of the two peaks was controversial. An adverse report was found in the study of rice bran treated dough (Teng et al., 2015).

Table 13. Farinograph parameters of the dough with different SSP substitution level. Significant difference within column at confidence level of $P<0.05$

SSP substitution	WA (g/100g flour) (corrected for 500 BU)	DDT (min)	Stability (min)	MTI (BU)	Time to break down (min)
Control	66.2 ± 0.3^{a}	6.9 ± 0.9^{a}	14.8 ± 0.9^{c}	15.0 ± 3.0^{a}	17.8 ± 1.2^{b}
6 g/100 g	70.7 ± 0.3^{b}	8.1 ± 0.2^{b}	15.7 ± 0.2^{c}	11.3 ± 1.2^{a}	17.7 ± 0.2^{b}
9 g/100 g	76.5 ± 0.1^{c}	8.4 ± 0.2^{b}	12.7 ± 0.9^{b}	16.3 ± 3.5^{a}	16.6 ± 0.5^{ab}
12 g/100 g	80.8 ± 0.1^{d}	11.0 ± 0.2^{c}	9.8 ± 0.7^{a}	31.7 ± 1.5^{b}	15.8 ± 0.5^{a}

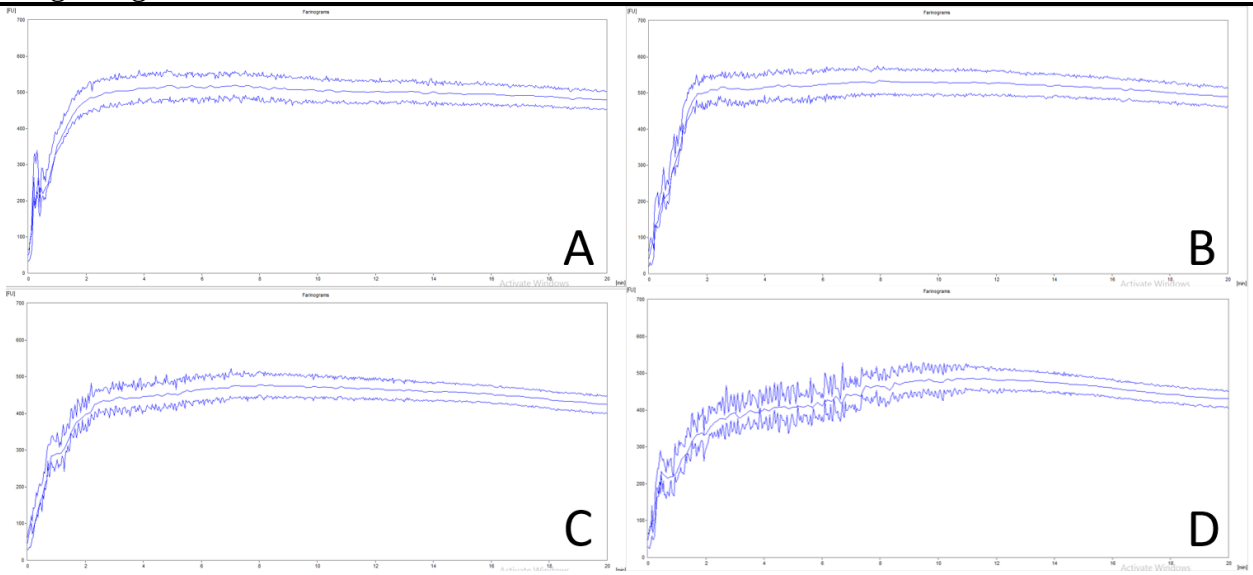

Figure 6. Farinograph of the dough with flour replaced by of SSP in different levels (A: control; B: 6 g/100 g; C: 9 g/100 g; D:12 g/100 g)

Table 14 and Figure 7 present the farinograph and related parameters of the RF replaced dough in various substitution levels (0, 6, 9, 12 g/100 g). With the increased RF replacement, the WA went up from 66.2 to 90.4 g/100 g. At the same time, the DDT significantly increased from 6.9 to 23.3 min. In comparison to SSP, the greater DDT value in RF substituted dough reflected the better water holding capacity of RF, as the hydration process became tougher. The stability showed a declining trend with the increasing RF substitution, except 9 g/100 g demonstrated higher stability. On the contrary, the TTB showed an overall growing trend, except a significantly lower value at 6 g/100 g. The MTI were comparable in three RF substituted levels, revealing the similar degree of softening dough after mixing.

In figure 7, RF substituted doughs had similar noisy initial stage as SSP exhibited due to the alike cellulous nature. The period of the initial noisy stage and the intensity of the second peak were increased with increasing fibre substitution.

Table 14. Farinograph parameters of the dough with different RF substitution level. Significant difference within column at confidence level of $P<0.05$

RF substitution	WA (g/100g flour) (corrected for 500 BU)	DDT (min)	Stability (min)	MTI (BU)	Time to break down (min)
Control	66.2 ± 0.3^{a}	6.9 ± 0.9^{a}	14.8 ± 0.9^{c}	15.0 ± 3.0^{a}	17.8 ± 1.2^{b}
6 g/100 g	77.4 ± 0.0^{b}	5.4 ± 0.1^{a}	6.5 ± 0.3^{a}	50.3 ± 2.1^{b}	8.9 ± 0.2^{a}
9 g/100 g	84.5 ± 0.2^{c}	13.7 ± 0.8^{b}	11.4 ± 0.4^{b}	42.3 ± 5.7^{b}	17.8 ± 0.5^{b}
12 g/100 g	90.4 ± 0.1^{d}	23.3 ± 0.5^{c}	5.3 ± 0.7^{a}	49.7 ± 5.0^{b}	26.1 ± 0.4^{c}

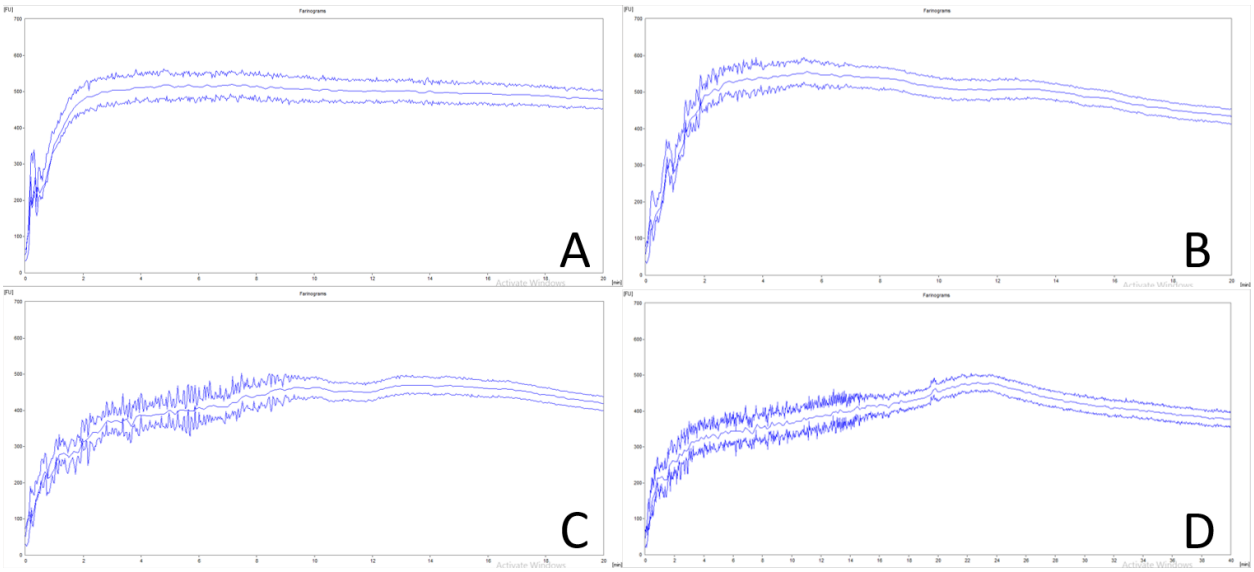

Figure 7. Farinograph of the dough with flour replaced by of RF in different levels (A: control; B: 6 g/100 g; C: 9 g/100 g; D:12 g/100 g)

The farinograph and related parameters are demonstrated in Table 15 and Figure 8. As the concentration of YMG increased, the WA was gradually increased. The highest WA value was found at the substitution level of 12 g/100 g. Such enhanced farinographic WA by hydrocolloids was reported by several authors in a wide range of concentration from 0.1% to 5% (flour basis) (Correa, Añón, Pérez, & Ferrero, 2010; Linlaud, Puppo, & Ferrero, 2009; Maleki & MilaNi,

2013a; Tavakolipour & Kalbasi-Ashtari, 2007; Zannini, Waters, & Arendt, 2014). However, the DDT, stability, and TTB shared an alike declining tendency, indicating the weakened dough structure. Compared to the control, the MTI value dramatically rose to around 49 BU at 9 and 12 g/100 g, except a significantly lower value observed at 6 g/ 100g. This fact suggested the higher degree of dough softening at high YMG substitution level. In a study of the effects of hydrocolloids on dough rheology and bread physical properties, a direct correlation between WA and degree of softening was reported (Maleki & MilaNi, 2013b). Guatda et al. (2004) explained this as the thickening effect of the hydrocolloids on the gas cell wall (Guarda, Rosell, Benedito, & Galotto, 2004). It is noted that the impact of the hydrocolloids on the dough was varied from different hydrocolloid types, as the nature of the hydrocolloids defines its interaction with other ingredients within the system (Bárcenas, De la O-Keller, & Rosell, 2009).

As shown in Figure 7, a smooth initial stage in the mixing process was observed, showing the little impact of YMG on the consistency of the hydration process and stability of the dough. However, the curves were witnessed to rapidly climbed to reach 500 BU and then decreased to below 500 BU at substitution level of 9 and 12 g/100 g. This fact indicated the shorter balancing stage of water in the dough system and dough weakening effect of YMG. Furthermore, the second peak was not found in all substitution levels, indicating the weak interaction between YMG and starch molecules.

Table 15. Farinograph parameters of the dough with different YMG substitution level. Significant difference within column at confidence level of $P<0.05$

YMG substitution	WA (g/100g flour) (corrected for 500 BU)	DDT (min)	Stability (min)	MTI (BU)	Time to break down (min)
Control	66.2 ± 0.3^{a}	6.9 ± 0.9^{b}	14.8 ± 0.9^{c}	15.0 ± 3.0^{b}	17.8 ± 1.2^{c}
6 g/100 g	70.5 ± 0.2^{b}	2.0 ± 0.2^{a}	9.9 ± 0.2^{b}	7.3 ± 2.3^{a}	11.8 ± 0.3^{b}
9 g/100 g	75.2 ± 0.1^{c}	1.9 ± 0.1^{a}	2.0 ± 0.1^{a}	49.7 ± 1.2^{c}	3.4 ± 0.1^{a}
12 g/100 g	79.7 ± 0.4^{d}	2.5 ± 0.2^{a}	2.5 ± 0.2^{a}	46.7 ± 2.3^{c}	3.9 ± 0.2^{a}

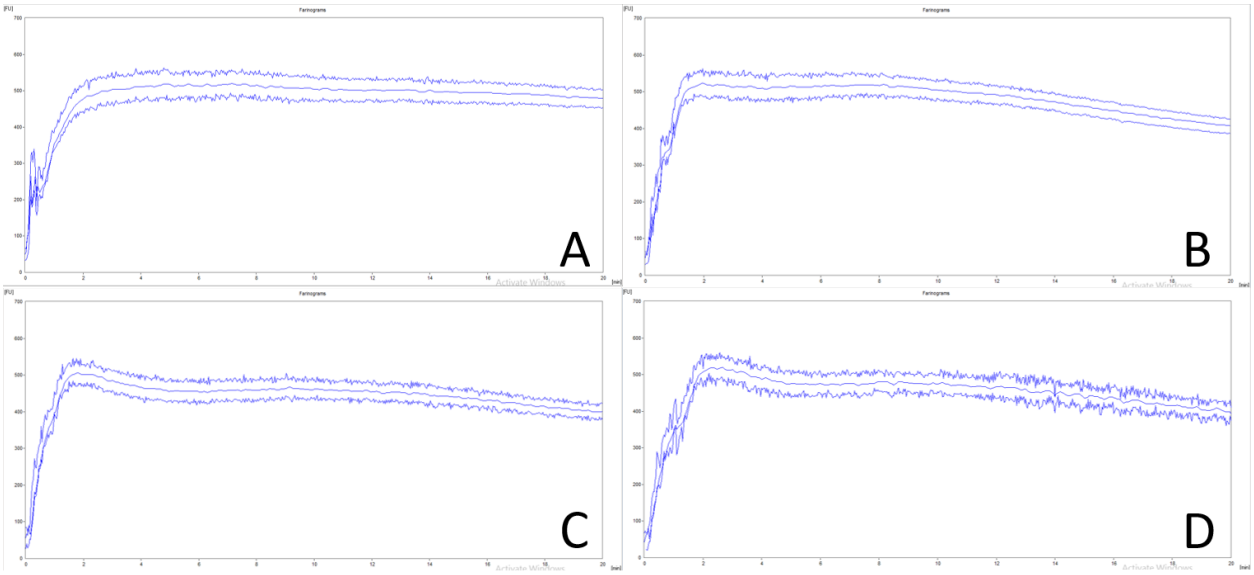

Figure 8. Farinograph of the dough with flour replaced by of YMG in different levels (A: control; B: 6 g/100 g; C: 9 g/100 g; D:12 g/100 g)

As the phenolic compounds added to the dough, the interaction among the starch, gluten, and polyphenols can impact the properties of the dough. During mixing, the phenolic compounds could interact with the free radicals and link with the gluten network, resulting in the reduced disulphide bond (SS) and increased thiol (SH) group (Hye-Min Han & Bong-Kyung Koh, 2011; Hye Min Han & Bong-Kyung Koh, 2011; Jackson & Hoseney, 1986; Schroeder, 1976). With the increasing SH group present in the dough, a weakened gluten network with lower viscosity and less resilient was observed (Sluimer, 2005).

The farinograph related parameters of the TP enriched dough are depicted in Table 16. With the increasing addition of TP, the WA value was gradually decreased to 63.6 g/100 g at addition level of 1.0 g/ 100 g. This result was not consistent with that reported by Ananingsih & Zhou. (2011) that the fortification of the green tea extract showed no impact on the WA (Ananingsih & Zhou, 2011). The DDT was significantly increased at the addition levels of 0.5 g/ 100 g and 1.0 g/ 100 g, indicating a longer interaction process between TP and the gluten. Compared to the control, the MTI values in tested addition levels were increased, but to different extents. This was

consistent with the previous report that adding of reducing agent promoted the SH/SS interchange reactions and improved the machinability of the dough (Joye, Lagrain, & Delcour, 2009a; Wang et al., 2015). However, the stability and TTB fluctuated over the test addition level with two relatively lower values found at 0.1 g/100 g and 0.5 g/100 g. Therefore, it was postulated that the covalent bonding formed between TP and thiol made the dough stable again at the fortification level of 1.0 g/100 g.

According to Figure 9, the presence of TP in dough had limited influence in the hydration process and dough stability, as the noisy stage was only observed at high addition levels of 0.5 g/100 g and 1.0 g/100 g. Compared to control, the low addition level of 0.1 g/ 100 g present in the dough resulted in the earlier departure from 500 BU. While at the addition level of 0.5 g/100 g and 1.0 g/100 g, the second peak related to the interaction between TP and gluten was witnessed, and the intensity was increased with the increasing TP concentration. However, similar results were not found in previous research.

Table 16. Farinograph parameters of the dough with different TP addition level. Significant difference within column at confidence level of $P<0.05$

TP addition	WA (g/100g flour) (corrected for 500 BU)	DDT (min)	Stability (min)	MTI (BU)	Time to break down (min)
Control	66.2 ± 0.3^{c}	6.9 ± 0.9^{a}	14.8 ± 0.9^{b}	15.0 ± 3.0^{a}	17.8 ± 1.2^{b}
0.1 g/100 g	65.3 ± 0.1^{b}	7.1 ± 0.1^{a}	9.7 ± 0.1^{a}	24.3 ± 1.5^{b}	12.6 ± 0.3^{a}
0.5 g/100 g	64.0 ± 0.2^{a}	8.1 ± 0.1^{b}	9.6 ± 0.3^{a}	45.0 ± 1.0^{c}	11.7 ± 0.1^{a}
1 g/100 g	63.6 ± 0.4^{a}	12.5 ± 0.1^{c}	15.1 ± 0.8^{b}	25.3 ± 2.1^{b}	18.3 ± 0.4^{b}

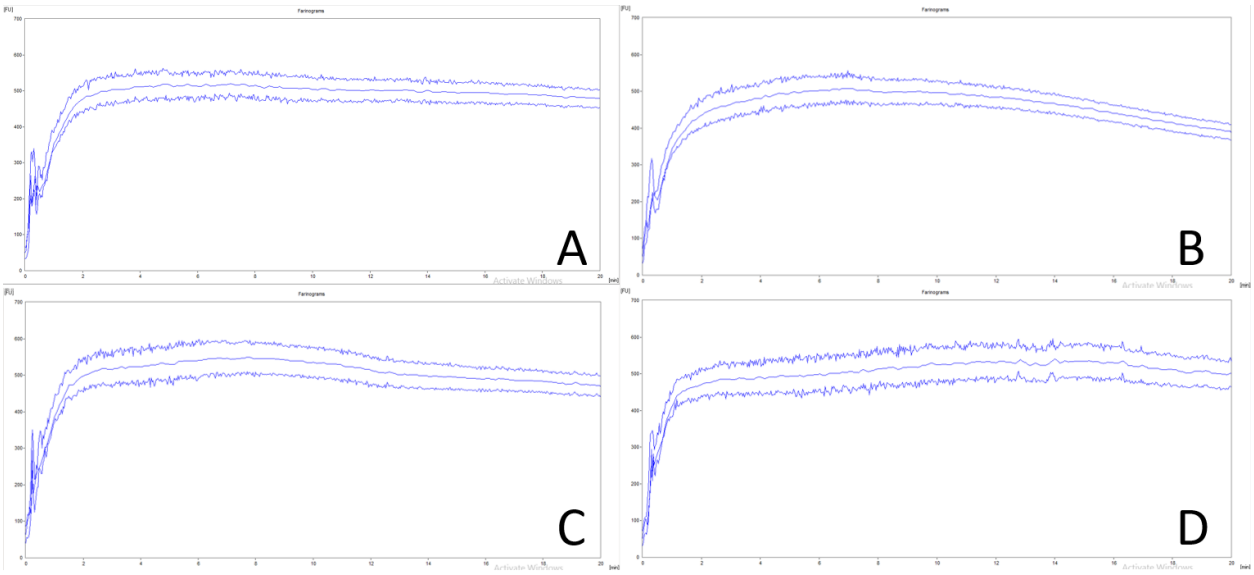

Figure 9. Farinograph of the dough with flour enriched by of TP in different levels (A: control; B: 0.1 g/100 g; C: 0.5 g/100 g; D:1.0 g/100 g)

In the dough containing both YMG and TP, other than the aggregation of YMG-protein and TP-protein, the formation of the YMG-TP complexes of higher molecular weight may also impact the dough properties. In case of the interaction among polysaccharides, phenolics, and gluten protein, the interaction was reported to possibly be mediated by the hydrogen bonding and hydrophobic interactions (Stathopoulos, Tsiami, Schofield, & Dobraszczyk, 2008). The difference between polyphenol-polysaccharides complexation and polyphenol-assisted aggregation was reported in previous studies. The interaction between polyphenols and protein had a relatively longer process than that between polyphenol and polysaccharides. The polyphenol-protein aggregation was reported to start with the binding of polyphenol to protein by hydrogen bonds and hydrophobic interaction and followed by the polyphenol-protein complexes aggregation through self-association and colloid formation (Jöbstl, O'Connell, Fairclough, & Williamson, 2004; McRae, Falconer, & Kennedy, 2010). However, polysaccharides-polyphenol aggregates were in the form of loose oligomers and (or) less compacted microgels, which do not collapse nor form tightly packed aggregates. Furthermore, the polyphenol-polysaccharides complexes exhibited

higher stability than that of polyphenol-protein micelles upon ultracentrifugation (Carn et al., 2012). After the formation of polyphenol-polysaccharides aggregates, the complexes of TP and YMG became more mobile and more vulnerable to the charged protein, thereby promoted the incorporation of the complexes into the gluten network (Sivam et al., 2011). According to the previous study, the hydroxyl group from polyphenols and polysaccharides could bond to the CO and N-H group from gluten repetitive domains by H bonds due to the steric hindrance. Such bonding in return changed the structure of the protein to a β-spiral structure, which does not rely on the H bonding foe stabilization (Secundo & Guerrieri, 2005).

The farinograph parameters of dough treated by YMG and TP are presented in Table 17. Compared to the results of YMG substituted dough and TP fortified dough, the combined dough had the features of both two doughs. Due to the double peaks, it is difficult to determine the farinograph parameters, as the resistance value may rise again at the second peak. The YMG-TP dough had comparable WA values with that of YMG substituted dough at the substitution level of 6.0 g/100 g. With the increasing amount of TP, the WA value slightly decreased at TP addition levels of 0.5 g/100 g and 1 g/100 g. This could explain by the interaction between YMG and TP. The DDT was gradually decreased with increasing TP proportion compared to YMG treated dough. At the same time, the stability and MTI value demonstrated an increasing trend in the tested TP concentration arrange. Compared to YMG, The TTB showed a decreasing trend with increasing TP addition, except the highest value at the TP level of 0.5 g/100 g.

Figure 10 presents the curves of three tested doughs. Initially, a quick rise to 500 BU was observed in three curves of the dough with various TP fortification, which was followed by a decrease at around 3 min. The intensity of the decline became more pronounceable with the increasing TP concentration. As TP was added in the dough, the second peak assigned to the

interaction between the gluten and TP, YMG and YMG-TP complexes was witnessed. The intensity of the second peak was lower than the TP fortified dough at each corresponding level, reflecting the interaction between YMG and TP. Although the interaction between YMG and TP reduced the damage on dough structure by YMG, the overall weakened structure was revealed by the decreased dough resistance.

Table 17. Farinograph parameters of the dough with 6% YMG substitution and different TP addition level. Significant difference within column at confidence level of $P<0.05$

Bread formula	WA (g/100g flour) (corrected for 500 BU)	DDT (min)	Stability (min)	MTI (BU)	Time to break down (min)
6% YMG	70.5 ± 0.2^{b}	2.0 ± 0.2^{ab}	9.9 ± 0.2^{a}	7.3 ± 2.3^{a}	11.8 ± 0.3^{c}
6% YMG+ 0.1% TP	70.6 ± 0.1^{b}	2.1 ± 0.1^{b}	9.5 ± 0.3^{a}	8.0 ± 4.4^{a}	11.2 ± 0.3^{b}
6%YMG+ 0.5% TP	70.1 ± 0.1^{a}	1.8 ± 0.1^{a}	12.4 ± 0.3^{b}	39.7 ± 2.5^{c}	13.9 ± 0.5^{d}
6% YMG+ 1% TP	70.1 ± 0.1^{a}	1.8 ± 0.1^{a}	16.6 ± 1.5^{c}	75.7 ± 3.8^{b}	3.1 ± 0.3^{a}

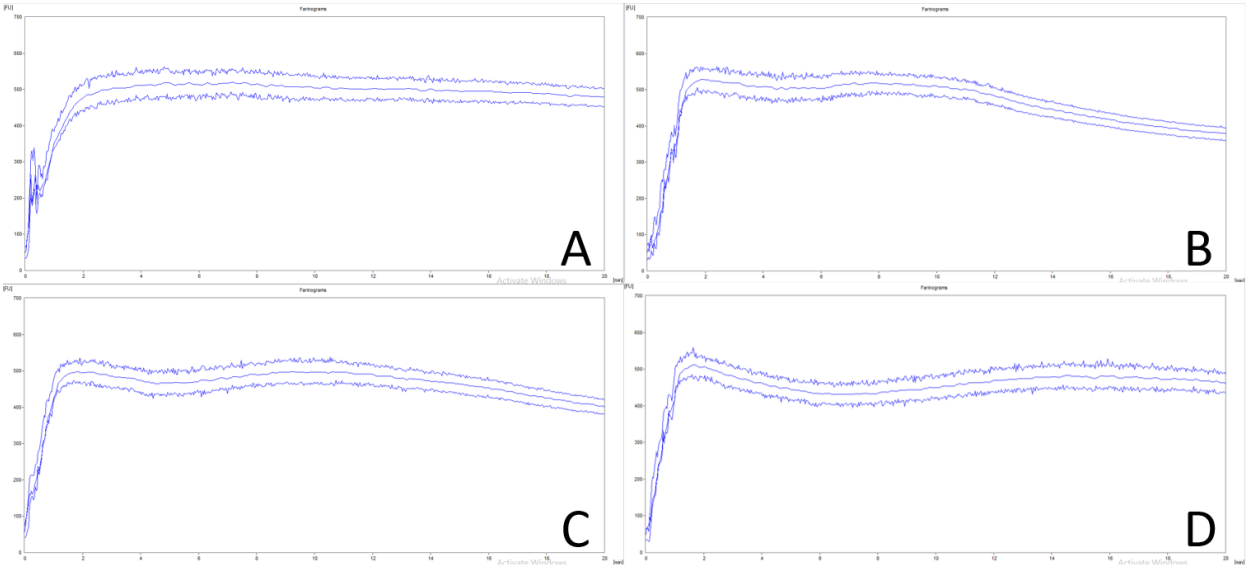

Figure 10. Farinograph of the 6% YMG substituted dough enriched by of TP in different levels (A: control; B: 0.1 g/100 g; C: 0.5 g/100 g; D:1 g/100 g)

From this part of the study, it can be seen that each fibre has the least effect on the dough properties at the substitution level of 6 g/100 g. This result was in good agreement with our

previous studies. The concentration of fibre in excess of 10 g/100 g not only damaged the dough structure but also developed an unpleasant flavor and color (Huang et al., 2016; Roberts et al., 2012). According to previous research on the sensory of TP enriched bread, the most of the astringency was established at the application level of 5 g/kg (Wang et al., 2007). Therefore, the fibre substitution level of 6 g/100 g and TP addition level of 0.1 g/100 g were selected to apply in subsequent experiments.

4.5.2 Specific volume

The effect of three types of fibres, TP, as well as the combination of YMG and TP on the weight, volume, and specific volume are summarized in Table 16. In three fibre treated breads, the lowest specific volume was observed in 6% RF-replaced bread, which was followed by 6% YMG replaced bread and 6% SSP replaced bread. Similar results on specific volume reduction have been reported extensively in previous studies (Huang et al., 2016; Noort, Mattila, Katina, & Van der Kamp, 2017). The TP at the addition level of 0.1 g/100 g was observed to have little impact on the specific volume, which may be correlated to the small dosage used in our study. In the study of Wang et al. (2007), the TP enriched bread had a decreased volume and increased density with the weakened dough structure (Wang et al., 2007). Considering the different solubility of SSP, RF, and YMG, different mechanisms were employed to explain the reduced volume. In terms of IDF, Gallilard et al.(1986) and Gan et al. (1989) assumed that the presence of particulate components in dough, in particular bran and epicarp fibres, may act as a physical disruption in the gluten matrix and the weakness point during the expanding of the dough cell wall (Galliard, Blanshard, & Frazier, 1986; Gan, Ellis, Vaughan, & Galliard, 1989). Later, in studies on the effects of both SDF and IDF on gluten network, both physical and chemical mechanisms were employed to explain this fact. The physical mechanism was associated with the water-binding capacity and size depletion phenomena of fibre. The chemical mechanism was associated with the interaction among

fibre, gluten, and ferulic acid (Noort, Van Haaster, Hemery, Schols, & Hamer, 2010; M Wang et al., 2003; Wang, Oudgenoeg, Van Vliet, & Hamer, 2003; Wang, Van Vliet, & Hamer, 2004a, 2004b). For SDF, one commonly accepted hypothesis was illustrated as the dilution effect on gluten by introducing the fibre fractions (Gan, Galliard, Ellis, Angold, & Vaughan, 1992). In terms of TP, the precise mechanism of the interaction between individual tea catechin remains unclear. However, a commonly accepted hypothesis claimed that tea catechins might interact with free radicals (GS*) and take part in the SH-SS interchange reaction (Dong & Hoseney, 1995; Hoseney, 1992). Excessive SH groups and insufficient SS bonds formed in the gluten crosslinking resulted in a weakened dough and smaller volume of the final product (Wang, Zhou, Yu, & Chow, 2006). The opposite results on the loaf volume were found in some modified polysaccharide derivatives, for example: carboxymethylcellulose (CMC), hydroxylpropylmethylcellulose (HPMC), and methyl cellulose (MC) (Gallagher, Gormley, & Arendt, 2004; Haque & Morris, 1994). The mechanism was attributed to the additional properties introduced by the hydrophilic and hydrophobic groups present in their molecule, such as the interfacial activity during proofing and gel forming network during heating. Thereby the viscosity of the dough was increased and gas cell boundaries was strengthened to give a higher gas retention capacity and better loaf volume (Bell, 1990).

Compared to YMG treated bread, the bread treated by both YMG and TP had a higher specific volume. In a study of biopolymers in bread with fibre and polyphenols fortification, the secondary structure of gluten protein was changed due to the H bonds formed in polyphenol-protein interaction and pectin-protein interaction (Sivam, Sun-Waterhouse, Perera, & Waterhouse, 2013). Taking account of the interaction between YMG and TP, the protein structure could be less modified, leading to a higher specific volume.

4.5.3 Porosity of bread

The porosity is an important physicochemical parameter in bread quality, and the crumb images of the control, fibre or (and) TP blended bread are presented in Fig 11. The gas cell in TP enriched bread was comparable with that of control. The increased porosity and decreased gas cell size were observed in the fibre replaced bread crumb (Fig 11. A-C, E, F). The relatively more compressed gas cell was a direct result of the weakened dough structure and the nucleating effect of added fibre, which was attributed to the interaction between fibre and dough. The increased porosity was also found in the research of gluten-free bread by Lazaridou et al. (2007), and such effect was observed at pectin addition level of 2%, as well as CMC and β-glucan addition level of 1% (Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007). Moreover, the uniformity of the gas cell is another essential parameter that influences the quality of bread. As shown in the figure 11, the gas cell was more evenly distributed from center to the boundary in those fibre substituted breads. Similar phenomenon was reported by Zettel et al. (2015). In their study, the gas bubbles was stabilized by adding the gels from chia seed (Zettel, Krämer, Hecker, & Hitzmann, 2015). Despite the stabilization effect, hydrocolloid could also act as an inhibiting factor in gas bubble growth with increased dough viscosity (Upadhyay, Ghosal, & Mehra, 2012).

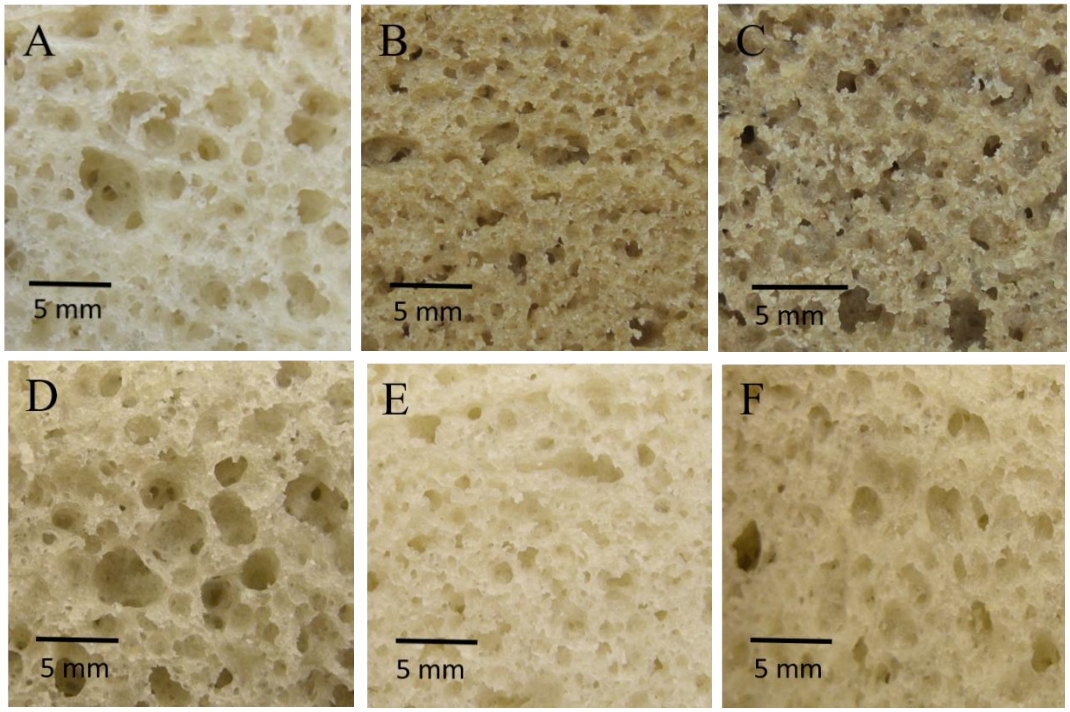

Figure 11. Crumb images of bread slices (A: control; B: 6% RF substitutued; C: 6% SSP substituted; D: 0.1% TP enriched; E: 6% YMG substituted; F: 6% YMG substituted + 0.1% TP enriched).

4.5.4 Color analysis

The color values of bread crumb, including L*, a*, b*, and ΔE, are depicted in Table 18. The L * scale extends from 0 dark to 100 light; the a* scale ranges from positive red to negative green; the b* scale extends from a positive value (yellow) to a negative value (blue). The color of bread crumb is mainly attributed to the physicochemical characteristics of the dough. As the temperature in bread crumb is limited for Maillard and caramelization reactions during baking, the differences in color of bread crumbs are mainly associated to ingredients applied in dough preparation (Kurek & Wyrwisz, 2015). A significant decrease on the L* was obtained in the breads with SSP, and RF replacement, respectively. Adding of TP also moderately reduced the L* value. On the contrary, bread with YMG substitution showed a higher value in L*. The darkening effect was also reported in the study on incorporation of green tea polyphenols, pea and broad bean pods fibres (Fendri et al., 2016; Pasrija, Ezhilarasi, Indrani, & Anandharamakrishnan, 2015). All the incorporated breads

exhibited significantly higher a* values, which indicated more redness in the bread crumb. Presence of SSP and RF increased the b*, while TP and YMG decreased the b*. This fact was correlated to the original brown color of TP, SSP and RF, as well as the white color of YMG.

Compared to YMG substituted bread and TP enriched bread, the bread treated with both YMG and TP exhibited a medium L* value, and very close to that of control. Similarly, the medium value also obtained in a*, b* and ΔE. This result could be attributed to the dilution of the white YMG on the natural browning color of TP.

4.5.5 Bread texture profile

The modification of crumb texture was observed with the presence of fibres. Related texture parameters registered in the texture profile analysis are summarized in Table 19. The firmness of the bread crumb with SSP and RF replacement were comparable and 2 times higher than that in control, which was followed by the bread with YMG substitution. While little difference in hardness was found in control bread and TP enriched bread. The different texture was the major problem of fibre modified bread reported in previous research. Hartikainen et al. (2014) added the bioprocessed bran into bread and observed a harder texture in the treated bread (Hartikainen, Poutanen, & Katina, 2014). Saccotelli et al. (2017) used durum in bread and observed that the tested sample showed an increased hardness (Saccotelli et al., 2017). Reduced softness was also found associated with both fibre level and the particle size (Majzoobi, Farahnaky, Nematolahi, Hashemi, & Ardakani, 2013; Zhang & Moore, 1999). The detrimental effect of bran on bread was attributed to several factors. In addition to the physical hindrance and disruption effect, other factors were related to the reactive components and endogenous enzyme of bran. The gluten protein binding and oxidative cross-linking hence influenced the water distribution (Noort et al., 2010; Piber & Koehler, 2005; Wang, Hamer, van Vliet, & Oudgenoeg, 2002; Mingwei Wang et al., 2003; Wang et al., 2004b; Zhou, Su, & Yu, 2004). Furthermore, selected enzymes, such as

amylase, xylanases, peptidase, and lipase, mainly displayed negative impact on the bread texture at high concentration (Chamberlain, Collins, & McDermott, 1981; Courtin & Delcour, 2002; Goesaert, Brijs, Veraverbeke, Courtin, Gebruers, Delcour, et al., 2005; Linko, Javanainen, & Linko, 1997). On the contrary, some oxidoreductases, namely lipoxygenase and peroxidases, were observed to improve the gluten network (Bahal, Sudha, & Ramasarma, 2013; Jerkovic et al., 2010; Manu & Prasada Rao, 2011). In terms of hydrocolloids, there was two commonly accepted hypotheses on the hydrocolloids modified crumb texture. Some studies proposed that the weakened starch structure may have better water distribution and water retention capacity, which resulted in a reduced crumb resistance (Collar, Andreu, Martınez, & Armero, 1999; Eidam, Kulicke, Kuhn, & Stute, 1995). However, a later study explained this fact from two opposite phenomena. On the one hand, the presence of the hydrocolloids could inhibit the association of amylose chains; on the other hand, the increased rigidity in starch structure may reduce the swelling of starch and intermolecular associations among amylopectin molecules (Biliaderis, Arvanitoyannis, Izydorczyk, & Prokopowich, 1997). The reduced swelling power of starch granules and amylose in the presence of YMG was also reported in our previous study (Liu, Eskin, & Cui, 2003). The dissimilar impact on bread texture was also associated with the type and the treatment of the fibre.

The springiness and cohesiveness in all bread samples were comparable, which reflected the equivalent elasticity. The gumminess, which is proportional to the hardness and cohesiveness, shared a similar trend with hardness. Likewise, similar results were also found in the chewiness, which is proportional to the hardness, cohesiveness, and springiness.

Table 18.The influence of different fibre and TP on the volume, weight, specific volume, and crumb color of bread. Significant difference within column at confidence level of $P<0.05$

Bread type	Volume/(ml)	Weight/(g)	Specific volume/(ml/g)	Color analysis			
				L*	a*	b*	ΔE
Control	613.89 ± 11.10^{a}	143.89 ± 2.05^{e}	4.27 ± 0.03^{a}	71.32 ± 0.29^{b}	-0.27 ± 0.18^{e}	20.71 ± 0.27^{c}	74.27 ± 0.35^{b}
6% SSP subsitutued	413.33 ± 6.01^{d}	149.85 ± 0.12^{c}	2.76 ± 0.04^{c}	54.20 ± 0.59^{d}	4.62 ± 0.10^{b}	21.13 ± 0.25^{b}	58.36 ± 0.50^{e}
6% RF subsitutued	377.22 ± 2.55^{e}	159.47 ± 0.25^{a}	2.37 ± 0.02^{e}	55.30 ± 0.69^{d}	5.30 ± 0.06^{a}	22.07 ± 0.19^{a}	59.78 ± 0.60^{d}
6% YMG subsituted	416.11 ± 3.85^{c}	154.64 ± 0.58^{b}	2.69 ± 0.02^{d}	75.68 ± 0.31^{a}	0.57 ± 0.07^{c}	20.29 ± 0.26^{d}	78.36 ± 0.35^{a}
0.1% TP enriched	618.89 ± 16.44^{a}	146.22 ± 1.08^{d}	4.23 ± 0.09^{a}	61.50 ± 0.42^{c}	0.09 ± 0.03^{d}	12.09 ± 0.15^{f}	62.68 ± 0.40^{c}
YMG -TP fortified	438.33 ± 10.14^{b}	155.44 ± 0.30^{b}	2.82 ± 0.07^{b}	71.95 ± 0.78^{b}	0.20 ± 0.12^{d}	17.11 ± 0.11^{e}	73.96 ± 0.73^{b}

Table 19. The influence of different fibre and TP on bread crumb texture. Significant difference within column at confidence level of $P<0.05$

Bread type	Hardness	Cohesiveness	Gumminess	Springness	Chewiness
Control	11.78 ± 1.98^{c}	0.67 ± 0.03^{a}	7.88 ± 1.13^{d}	0.90 ± 0.05^{a}	7.09 ± 0.79^{d}
6% SSP subsitutued	24.58 ± 1.02^{a}	0.66 ± 0.02^{a}	16.30 ± 0.28^{a}	0.90 ± 0.08^{a}	14.63 ± 1.12^{a}
6% RF subsitutued	23.94 ± 2.48^{a}	0.70 ± 0.01^{a}	16.64 ± 1.81^{a}	0.88 ± 0.06^{a}	14.65 ± 1.40^{a}
6% YMG subsituted	20.22 ± 0.88^{b}	0.69 ± 0.01^{a}	13.85 ± 0.29^{b}	0.84 ± 0.00^{a}	11.60 ± 0.18^{b}
0.1% TP enriched	11.44 ± 0.77^{c}	0.68 ± 0.01^{a}	7.78 ± 0.62^{d}	0.89 ± 0.01^{a}	6.89 ± 0.55^{d}
YMG-TP fortified	17.20 ± 2.55^{b}	0.67 ± 0.02^{a}	11.48 ± 1.48^{c}	0.86 ± 0.00^{a}	9.83 ± 1.21^{c}

Chapter 5 Conclusion and future work

In this study, extraction of SEP from sunflower stalk pith and determination of the chemical structure of the three fractions were achieved. The inhibitory effects of SEP, TP, and SEP/TP complexes in three compositions (10:0.5, 10:1, 10:2) against human salivary α-amylase were investigated in different concentrations. The influence of SSP, RF, YMG, TP, and YMG-TP on dough properties and bread quality were also determined.

SEP contained high level of galacturonic acid, as well as low levels of neutral sugar and protein. HPAEC analysis suggested that the primary neutral sugars in SEP were rhamnose, arabinose, and galactose. The characteristic bands at 880, 1270, and between 1100 and 1200 cm^{-1} in FT-IR analysis further proved SEP as pectin. The DE of SEP was 34.5%, indicating that it was an LMP. In the spectrum of the SEP/TP complexes, the decreased peak at 3600-3000 cm^{-1} was not observed, the hypothesis of specific arrangement in SEP/TP complexes by hydrogen bonding remained unconfirmed. In α-amylase assay, the inhibitory effects were associated with both the composition and concentration of the inhibitor. Among the tested inhibitors, TP exhibited strongest inhibitory effects, followed by SEP/TP complexes and SEP. The inhibition rate of SEP/TP complexes were dose-dependent at low concentration and increased with increasing TP proportion. The highest inhibitory rate of SEP/TP complexes was around 72% at the ratio of 10:2 (SEP:TP), when the concentrations of SEP within the complexes was 10 mg/mL and 5 mg/mL. With the increasing concentration, SSP, RF, YMG, TP, and YMG-TP affected the properties of dough in different manners. At substitution level of 6%, SSP had the least influence on the dough, which was followed by YMG and RF. YMG-TP dough displayed the features of both YMG substituted dough and TP enriched dough. Compared to YMG substituted dough, the weakened second peak in the farinograph of YMG-TP blended dough indicated the interaction between YMG and TP.

Reduced specific volume and harder crumb were observed in all the fibre-substituted samples. Among the fibre treated bread, the greatest specific volume and softest crumb were found in YMG-TP fortified bread.

For future study, a few directions could be followed. With the unknow acceptability of the bread quality of fibre-fortified bread, the sensory of tested breads should be complete. Besides, the anti-hyperglycemic effect of each fibre-substituted bread should be investigated. Taking account of the interaction between YMG and TP, the protective effect of YMG on TP during the breading making and digestion should be determined. Finally, work should focus on counteracting the detrimental effects of fibres on bread quality, which could consider the application of food additives (such as enzymes, emulsifiers, oxidants).

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