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**The effects of cereals bran, pseudocereal and enzymes on the
Chinese steamed bread dough and bread quality**

A thesis
submitted in partial fulfilment
of the requirements for the Degree of
Philosophy in Food Science

at
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by
Wenjun Liu

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Declaration

Some parts of this thesis have been published, submitted and/or presented at Conferences in advance of submission of the thesis:

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- Liu, W., Brennan, M., Serventi, L., & Brennan, C. (2017). Buckwheat flour inclusion in Chinese steamed bread: Potential reduction in glycaemic response and effects on dough quality. *European Food Research and Technology*, 243(5), 727-734.
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The effects of cereals bran, pseudocereal and enzymes on the bread dough and bread quality

by

Wenjun Liu

Wheat and oat bran are by-product derived from milling industry and are widely used as a good source of dietary fibre for incorporation into processed foods, particularly cereal products. Buckwheat (*Fagopyrum*) belongs to the family of *Polygonaceae*, which is usually classified as a pseudocereal and widely used in food products such as breads, noodles, cakes and extruded snack products. The major dietary fibre component of wheat bran, oat bran and buckwheat are arabinoxylan, β -glucan and resistant starch, respectively. Chinese steamed bread (CSB), also known as mantou, is a Chinese traditional fermented food, which has been widely consumed as a staple food and represents about 40 % of the wheat consumption in China.

The effect of incorporation of wheat bran, oat bran and buckwheat into Chinese steamed bread on dough rheology and quality of steamed bread were investigated. The addition of bran and buckwheat flour into wheat flour can influence the dough rheology and final quality of Chinese steamed bread due to the excellent hydration properties of dietary fibre and the disruption of the starch-gluten network by dietary fibre. For instance, the addition of bran significantly increased water absorption, development time and stickiness, whereas decreased extensibility. In consequence, the specific volume of CSB reduced significantly and hardness, gumminess and chewiness increased significantly as the substitution increased from 0 to 15 %. Additionally,

the results also show that the substitution level of bran and buckwheat flour into wheat flour can be increased up to 15 % resulting in a reduced predicted glycaemic response which may confer significant health benefits to the consumers.

In order to overcome the negative effects of bran inclusion on bread quality, three types of commercial enzymes were added into the Chinese steamed bread incorporated with 15 % bran and buckwheat flour. Two experimental designs were included: the first one to evaluate the effect of a single enzyme on the dough rheology and breadmaking properties, and the second one to investigate the effect of combined enzymes on the dough rheology and breadmaking properties. The results showed that the dough handling and bread making properties could be improved with use of enzymes, whereas no significant difference in fibre content, starch content and AUC value. In comparison with a single enzyme, the enzymes combinations were more effective in the rheological properties of dough and quality of Chinese steamed bread incorporated with 15 % bran and buckwheat flour due to the synergistic effect of α -amylase, xylanase and cellulase. Additionally, the combined enzymes reduced the fibre and starch content, resulting in varied AUC value. The results obtained from full factorial design suggested that the combination of α -amylase, xylanase and cellulase induced interactions among enzymes and their coupled reactions.

The combination of α -amylase, xylanase and cellulase showed potential bread improvers in baking industry. This research found the optimum concentrations of enzymes resulted in higher AUC value than the high fibre CSB without enzymes. This study is a first step towards understanding the effect of cereal brans and enzymes on the dough rheology and bread quality and will contribute to the optimisation of industrial processes.

Keywords: cereal bran, buckwheat, dietary fibre, α -amylase, xylanase, cellulase, dough rheology, physicochemical parameters, glycaemic response, DoughLab.

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Chapter 1

Introduction

1.1 Background

In recent years, consumers have developed a growing awareness of the link between diet and nutrition, thus there has been an increased demand for healthier products with a consequent rise in interest in functional and nutritional items by the food industry (Brennan, Merts, Monro, Woolnough, & Brennan, 2008; Campbell, Ross, & Motoi, 2008; Chareonthaikij, Uan - On, & Prinyawiwatkul, 2016; Grigor, Brennan, Hutchings, & Rowlands, 2016; McGill & Devareddy, 2015). Dietary fibre is the edible part of plants or analogous carbohydrate that resists digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine (Codex, 2009). Accordingly, the chemical nature of dietary fibre is composed of non-digestible carbohydrates, including oligosaccharides, polysaccharides and lignin, e.g., cellulose, β -glucan, hemicelluloses, arabinoxylan, gums, mucilage, pectin, inulin, resistant starch (Lunn & Buttriss, 2007). A previous study by Brennan (2005) illustrated that dietary fibre has many beneficial effects on human health, for example decreased intestinal transit time, reduction of blood cholesterol levels (total and LDL), and a reduction in glycaemic response and insulin levels. Additionally, dietary fibre has a potential role for disease prevention and control, such as diabetes, obesity, heart disease, diseases of the large bowel, and colon cancer (Birkett & Cho, 2013; Grigor et al., 2016; Slavin, Tucker, Harriman, & Jonnalagadda, 2016).

The majority of dietary fibre is concentrated in outer layers of the cereal grain (Coda, Katina, & Rizzello, 2015). Cereal bran is the outer layer of the cereal grain, comprising the pericarp, testa, aleurone, germ, and part of the starchy endosperm (Brownlee, 2011; Kiszonas, Fuerst, Luthria, & Morris, 2015; Serna-Saldivar, 2016; Uraipong & Zhao, 2016). Thus, cereal bran can be a good source of dietary fibre, including arabinoxylan, β -glucan, cellulose and lignin as major components (Elleuch et al., 2011; Kiszonas et al., 2015). Apart from dietary fibre, cereal

bran is rich in protein, lipids, minerals, and B-vitamins (Begum, Goswami, & Chowdhury, 2015; Nordlund, Katina, Aura, & Poutanen, 2013; Onipe, Jideani, & Beswa, 2015; Sobota, Rzedzicki, Zarzycki, & Kuzawińska, 2015). In the past, the main utilization of cereal bran was in the feed industry as livestock feed and only a small part was used as additive in foods. Currently, with an expanding market for health food, cereal bran is considered as a nutritionally valuable ingredient in food processing due to the potential health benefits (Chinma, Ilowefah, Shammugasamy, Mohammed, & Muhammad, 2015; Coda et al., 2015; Thamnarathip, Jangchud, Jangchud, & Vardhanabhuti, 2016). As a low-cost by-product, cereal bran is widely used as a source of dietary fibre for incorporation into processed foods, particularly bread. However, the addition of cereal bran into bread generally results in poor dough rheology properties and poor textural properties of final products, for example reducing loaf volume, darkening the crumb, and increasing the firmness (Heiniö et al., 2016; Hemdane et al., 2016; Mastromatteo et al., 2015; Oliveira, Rosell, & Steel, 2015; Rashid, Rakha, Anjum, Ahmed, & Sohail, 2015).

Chinese steamed bread (CSB), also known as mantou, is a Chinese traditional fermented food, which has been widely consumed as a staple food and represents about 40 % of the wheat consumption in China (Liu et al., 2014; Wu et al., 2012; Zhu, 2014). The main ingredients of CSB include wheat flour, yeast and water with processing by steam cooking. Due to the differences in processing, the properties of CSB and western style baked bread are significantly different. For instance, the flavours of CSB are formed during the fermentation, while the flavours of western style breads are derived during baking by Maillard reaction (Lin, Chen, Lu, & Wang, 2012). In addition, the lower processing temperature (100 °C) may result in the greater retention of nutrients in CSB (Zhu, Sakulnak, & Wang, 2016). A few previous studies have illustrated the addition of cereal bran to CSB also leads to many negative effects as the effects on western bread.

In order to improve the quality of bread enriched in cereal bran, various types of improvers have been used in the baking industry, such as surfactants, emulsifiers, reducing agents, non-volatile acids and enzymes (Al-Hadi, Periasamy, Athinarayanan, Al-Khalifa, & Alshatwi, 2017; Purhagen, Sjö, & Eliasson, 2011; Reed, 2012). As natural and safe additives, enzymes have completely or partially replaced other chemical synthetic additives to improve dough handling, flexibility, machinability, stability of fresh bread quality, loaf volume and shelf life of bread in breadmaking industry (Baines & Seal, 2012; Monica Haros, Rosell, & Benedito, 2002). Different commercial enzymes preparations have been used in baking industry, such as amylases, xylanases, lipases, oxidases and other enzymes. Fungal α -amylase is an enzyme derived from fungi, with widespread application in food industry. The action of amylase is to catalyze the hydrolysis of α -1, 4-glycosidic linkages into starch molecules (amylose and amylopectin), at a lower rate, maltodextrins and oligosaccharides (Martínez-Anaya, 1996). Xylanase is a hydrolase, which can randomly attack the AX backbone and break the glycosidic linkages in AX, result in changing the functional and physicochemical properties of AX (Ahmad et al., 2014; Ghoshal, Shivhare, & Banerjee, 2013). Xylanases have been used in various industries, such as animal feeds, food, textiles and pulp and paper (Polizeli et al., 2005). The use of xylanases in bread-making facilitates an increase in bread volume and water absorption. Cellulase belongs to the glycoside hydrolase family, which can catalyze the hydrolysis of (1,4)-beta-D-glucosidic linkages in cellulose and other beta-D-glucans. The main utilization of cellulase has been in the textiles industry, pulp and paper industry, animal feeds and food industry (Sukumaran, Singhanian, & Pandey, 2005). Recent studies have reported that cellulases were used to increase the yield and process performance of fruit and vegetable juices in conjunction with xylanase and pectinase (Kuhad, Gupta, & Singh, 2011; Reed, 2012). There is a paucity of information regarding the applications of cellulase in bread-making. Due to their particular action mechanism, these enzymes may produce positive effects during breadmaking, such as rheological behaviour of dough and the quality of final products (Caballero, Gómez, &

Rosell, 2007b). However, the reports on the effects of enzymes combination, especially, the combination of cellulase, xylanase and α -amylase on the dough rheology and nutritional quality of final products (glycaemic response) are limited.

1.2 Knowledge gaps

In the last decade, most of research has focused on the effect of various cereal brans on the dough rheology and final bread and CSB quality (Gómez, Jiménez, Ruiz, & Oliete, 2011; Sudha, Vetrmani, & Leelavathi, 2007; J. Wang, Rosell, & de Barber, 2002; Zhao et al., 2009). Some studies fully discussed the mechanisms between the fibre and gluten-starch network. For instance, Rosell, Santos, and Collar (2006) and Bonnard-Ducasse, Della Valle, Lefebvre, and Saulnier (2010) suggested that the addition of bran fibre into wheat flour can disrupt the starch-gluten network structure, thus affecting dough viscoelastic behavior and bread quality. With the full understanding of fibre impact, some studies proposed to use the specific enzymes to improve the dough rheology and baking performance.

Therefore, enzymes have been widely used in the current baking industry with clean and natural label. Many studies just focused on the effect of single enzyme on the rheological properties of the bread dough and bread with high bran fibre (Ghoshal et al., 2013; J. H. Kim, Maeda, & Morita, 2006; Stojceska & Ainsworth, 2008). Furthermore, a few current studies have reported that the enzymes combination were more efficient than individual enzyme in improving bread quality (Alaunyte, Stojceska, Plunkett, Ainsworth, & Derbyshire, 2012; Altuna, Ribotta, & Tadini, 2015; Caballero et al., 2007b). However, few studies have pointed out the reaction mechanisms of enzymes mixture, especially, the combination of xylanase, amylase and cellulase.

Unfortunately, there has been no published article about how the enzymes combination affects the glycaemic response of bread and CSB.

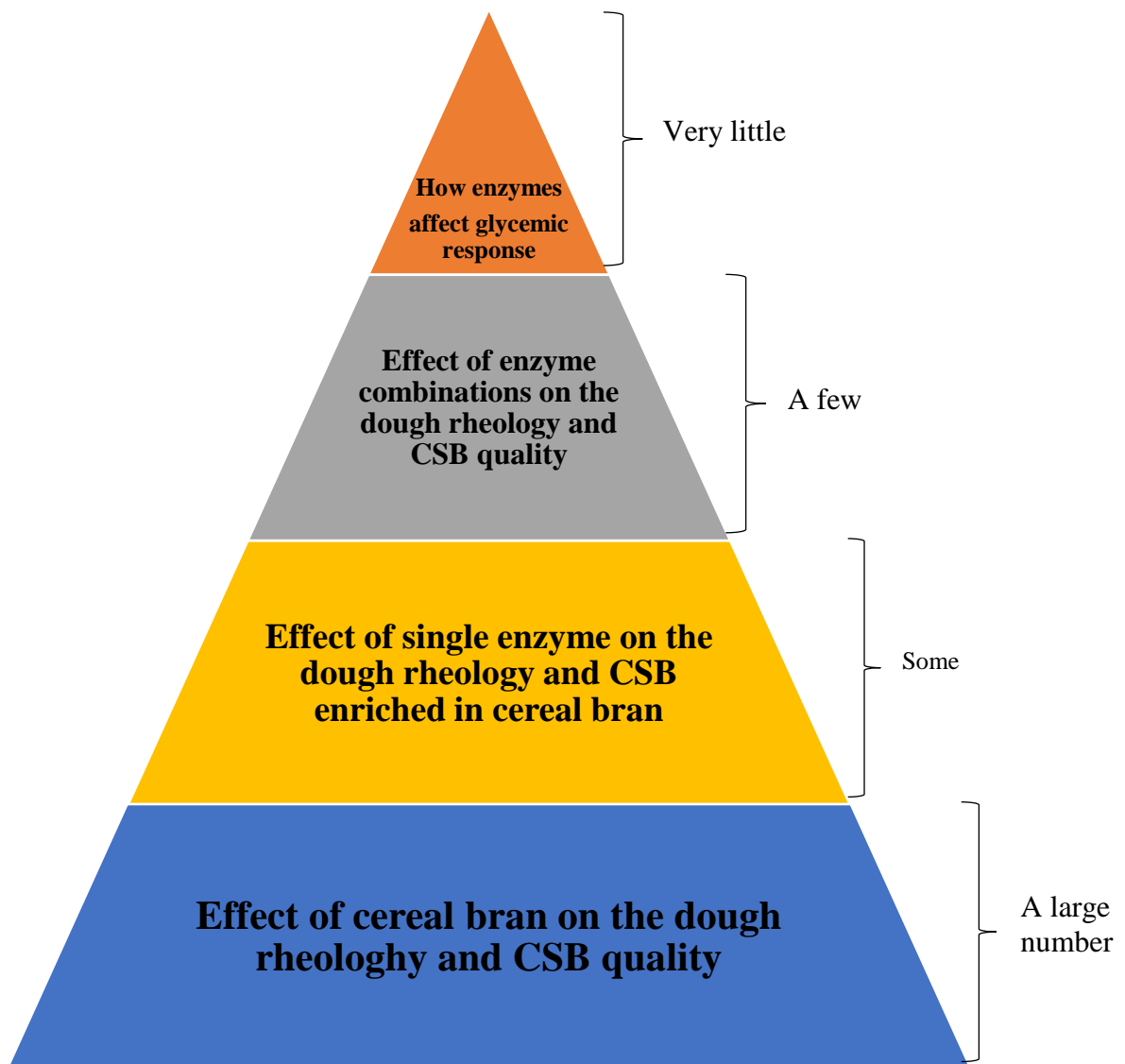


Figure 1.1 Areas covered in current research

1.3 Aim and objectives of this research

1.3.1 Aim of this research

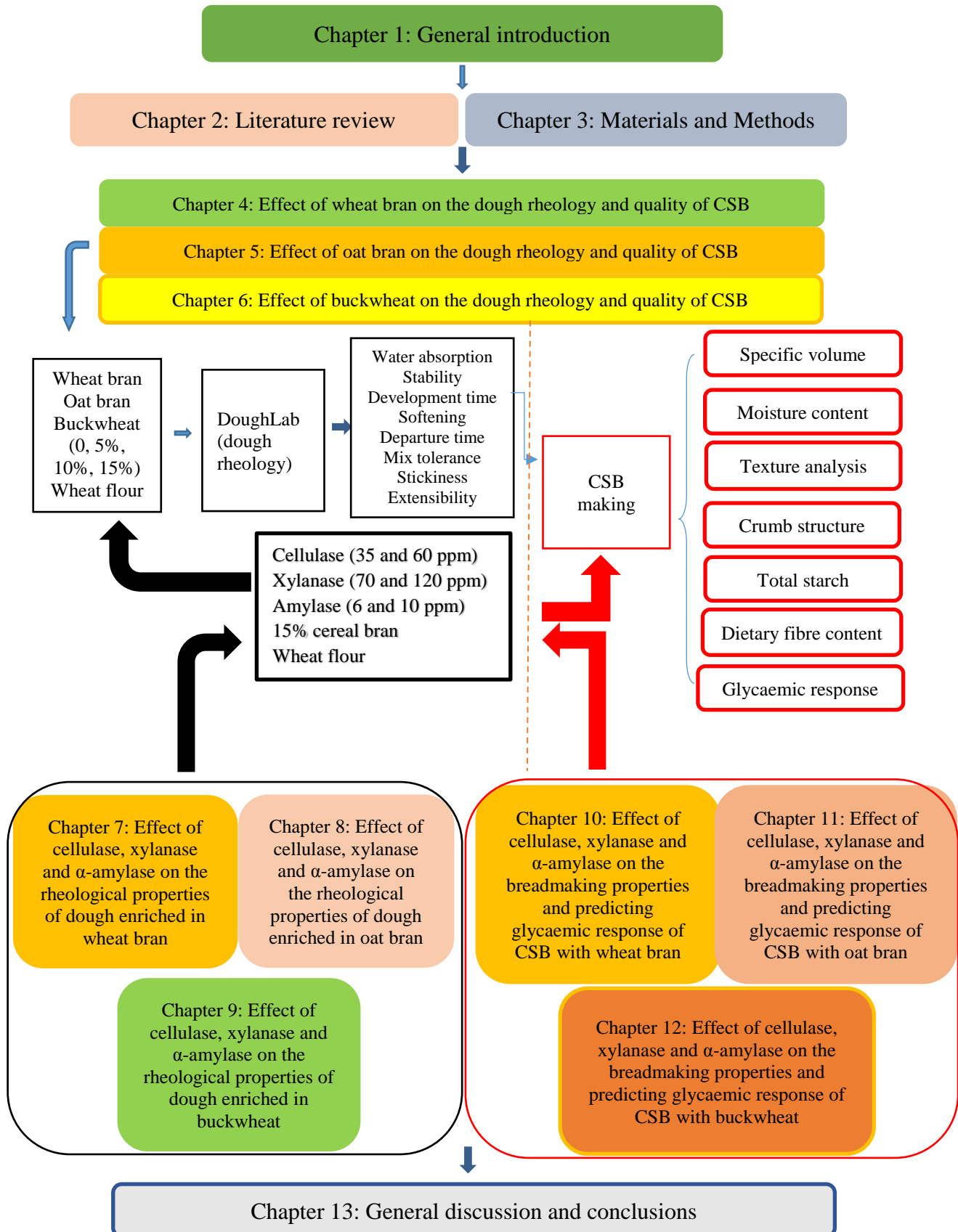
The aim of this project was to investigate the effects of the addition of different cereal brans (oat, wheat and buckwheat) and enzymes on dough rheology and CSB quality, and the optimum enzymes (xylanase, α -amylase and cellulase) combination to improve bread quality and dough rheology.

Hence, this research aimed to illustrate that the addition of different cereal brans may decrease the glycaemic response of products. However, the addition of cereal brans will decrease the quality of products. Enzymes can improve quality but there are few studies focused on the effects of enzymes combination on the CSB quality. Therefore, the optimum enzyme combination will be used in this research, and further research will illustrate how the enzymes combination affect the glycaemic response.

1.3.2 Objectives of this research

- 1) To investigate how cereal brans inclusion in CSB affects dough rheology and CSB quality from a physical - chemical perspective.
- 2) To investigate how single enzyme inclusion in high fibre CSB affects dough rheology and CSB quality.
- 3) To investigate how enzymes combination affects dough rheology compared with single enzyme.
- 4) To investigate how enzymes combination affects quality and glycaemic response of CSB enriched cereal bran.
- 5) To optimize concentration of enzymes combination in order to improve quality of the CSB enriched cereal bran.

1.4 Thesis structure



Chapter 2

Literature Review

2.1 Dietary fibre

2.2 Definition of dietary fibre

Dietary fibre is a complex compound, which is difficult to define by only chemistry or nutrition. The evolution of the definition of dietary fibre began in 130 A.D. when the physician Galen first reported that some unknown foods excite the bowels to evacuate and those that prevent them. Until the 1940s, the term of dietary fibre was used in human nutrition. Currently, there are numerous various definitions of dietary fibre in different countries as demonstrated in (Table 2.1). According to the report of dietary fibre definition committee to the board of directors of the American Association of Cereal Chemists (AACC, 2001), the definition of dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine, including polysaccharides, oligosaccharides, lignin, and associated plant substances. The beneficial physiological effects of dietary fibre to human health including laxation, blood cholesterol attenuation, and blood glucose attenuation.

In June 2009, CODEX Alimentarius Commission defined the dietary fibre as the carbohydrate polymers with 10 or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and belong to the following categories (Codex, 2009):

- Edible carbohydrate polymers naturally occurring in the food as consumed

- Carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.

- Synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.

Table 2.1 Current definitions for dietary fibre.

Definitions	Institution
<p>Fibre means carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine to the following categories:</p> <ul style="list-style-type: none"> -Edible carbohydrate polymers naturally occurring in the food as consumed -Edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence. -Synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence. <p>Dietary fibre is the edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre and other Carbohydrates continues to support the definition includes polysaccharides, oligosaccharides, lignin and associated plant substances.</p> <p>Dietary fibre means that fraction of the edible part of plants or their extracts, or synthetic analogues that are resistant to digestion and absorption in the small intestine, usually with complete or partial fermentation in the large intestine; and promotes one or more of these beneficial physiological effects: laxation, reduction in blood cholesterol, and/or modulation of blood glucose and includes polysaccharides, oligosaccharides (DP>2), and lignins.</p> <p>Dietary fibre means carbohydrate (CHO) polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine (SI) of humans and belong to the following categories:</p> <ul style="list-style-type: none"> •Edible CHO polymers naturally occurring in the food as consumed •CHO polymers, obtained from food raw material by physical, enzymatic, or chemical means² •Synthetic CHO polymers² <p>¹The footnote allows international authorities to decide whether those compounds with DP of 3–9 would be allowed.</p> <p>²For the isolated or synthetic fibres in category ‘2’ or ‘3’, they must show a proven physiological benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.</p>	<p>European Union (EU) The current EU definition of dietary fibre (Commission of European Communities)</p> <p>American Association of Cereal Chemists (AACC) The AACC International Technical Committee on Dietary adopted by the AACC (2001)</p> <p>Australia New Zealand Food Authority (ANZFA) Standard 1.2.8</p> <p>CODEX Alimentarius Commission 2009 (international standards for food and food imports).</p>

Adapted from (Jones, 2014)

Although the method for measurement of dietary fibre is recommended by the Codex Committee on Nutrition and Foods for Special Dietary Uses – CCNFSDU (Codex, 2009), there is some debate about the methods for dietary fibre analysis. Currently there are three categories of determination, non-enzymatic-gravimetric, enzymatic-gravimetric, and enzymatic-chemical (Elleuch et al., 2011; Knudsen, 2001). Both enzymatic-gravimetric Association of Official Analytical Chemists (AOAC) and enzymatic-chemical methods are the major common methods for dietary fibre analysis (Barry V McCleary, 2003; Schweizer & Würsch, 1979). According to chemical nature of dietary fibre, it is composed of non-digestible carbohydrates,

included oligosaccharides, polysaccharides and lignin, e.g., cellulose, glucans, hemicelluloses, AX, gums, mucilage, pectin, inulin, resistant starch (Table 2.2) (Elleuch et al., 2011).

Table 2.2 Chemical composition of dietary fibres.

Fibres	Main chain	Branch units
Cellulose	β -(1,4) glucose	
β -glucans	β -(1,4) glucose and β -(1,3) glucose	
Hemicelluloses		
Xylans	β -D-(1,4) xylose	
Arabinoxylans	β -D-(1,4) xylose	Arabinose
Mannans	β -D-(1,4) mannose	
Glucmannans	β -D-(1,4) mannose and β -D-(1,4) glucose	
Galactoglucmannans	β -D-(1,4) mannose and β -D-(1,4) glucose	Galactose
Galactomannans	β -(1,4) mannose	α -D-galactose
Xyloglucans	β -D-(1,4) glucose	α -D-xylose
<i>Pectin</i>		
Homogalacturonan	α -(1,4)-D-galacturonic acid (some of the carboxyl groups are methyl esterified)	
Rhamnogalacturonan-I	(1,4) galacturonic acid, (1,2) rhamnose and 1-, 2-, 4- rhamnose	Galactose, arabinose, xylose rhamnose, galacturonic acid
Rhamnogalacturonan-II	α -(1-4) galacturonic acid	Unusual sugar such as: apiose, aceric acid, fucose
Arabinanes	α -(1-5)-L-arabinofuranose	α -arabinose
Galactanes	β -(1-4)-D-galactopyranose	
Arabinogalactanes-I	β -(1-4)-D-galactopyranose	α -arabinose
Arabinogalactanes-II	β -(1-3)- and β -(1-6)-D-galactopyranose	α -arabinose
Xylogalacturonan	α -(1-4) galacturonic acid	xylose
Inulin	β -(2-1)-D-fructosyl-fructose	
Oligofructose	β -(2-1)-D-fructosyl-fructose	
Polydextrose	D-Glucose	
Resistant maltodextrins	α -(1-4)-D-Glucose	α -(1-6)-D-Glucose
Lignin	Polyphenols	

Adapted from (Elleuch et al., 2011).

Based on the solubility in water, dietary fibre is categorized as soluble or insoluble (Dhingra, Michael, Rajput, & Patil, 2012; Lahaye, 1991). Soluble dietary fibre is characterized by the ability cause gel formation, reduction of the glycaemic response and plasma cholesterol. These include gums, mucilage, pectin substances, and some hemicelluloses. Insoluble fibres include cellulose, lignin and other types of hemicellulose, and are believed to be responsible for an increase in faecal bulk and a reduction in the intestinal transit time because of their porosity and

low density (Chawla & Patil, 2010; Hollmann, Themeier, Neese, & Lindhauer, 2013; Mudgil & Barak, 2013; Thebaudin, Lefebvre, Harrington, & Bourgeois, 1997) (Figure 2.1).

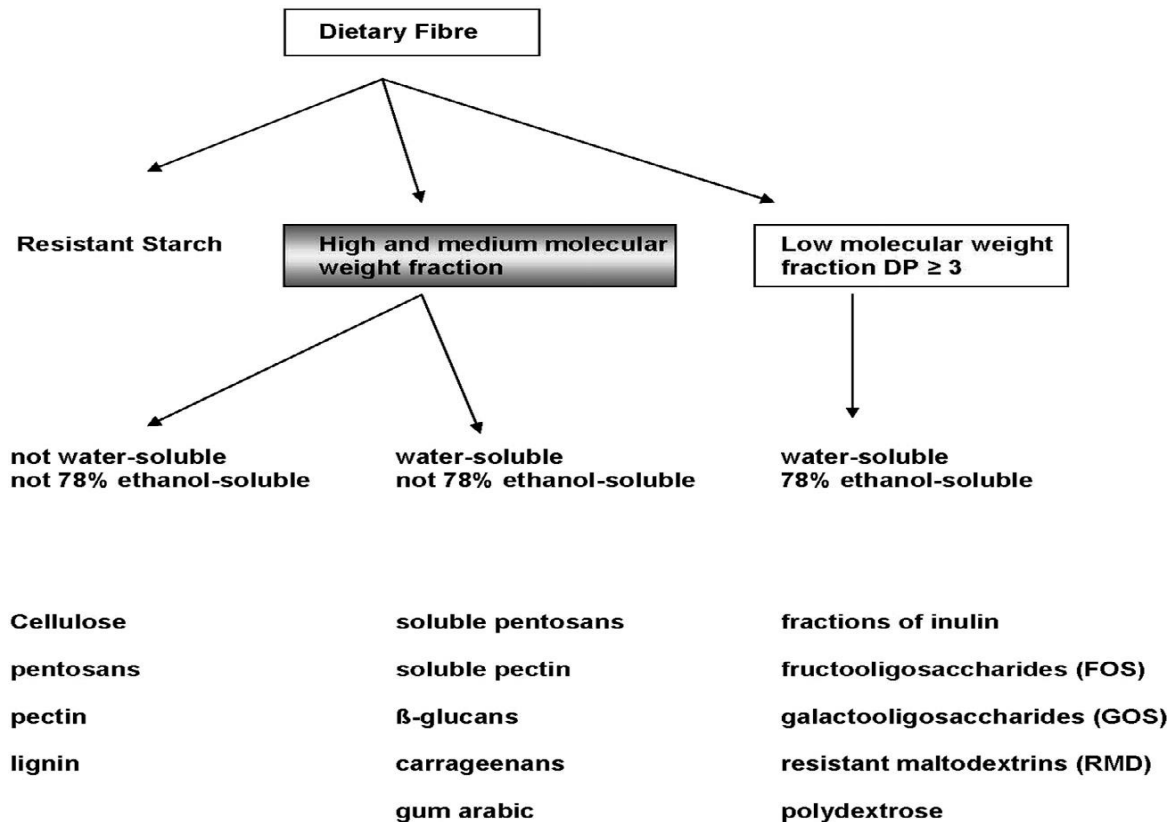


Figure 2.1 Dietary fibres classified according to their solubility
Adapted from (Hollmann et al., 2013)

2.2.1 Physicochemical properties of dietary fibre

The physicochemical properties of dietary fibre are the major factors of their physiological effects, which include solubility, water-holding capacity, viscosity, bulking, binding, and fermentability (Mudgil & Barak, 2013).

Solubility of dietary fibre depends on the conformation of the individual polysaccharides chains, which may be set regularly or irregularly on the backbone or as side chains, and the way that different polysaccharides interact with each other (Guillon & Champ, 2000). Additionally, both temperature and ionic strength also affect the solubility of dietary fibre (Chawla & Patil, 2010).

Compared to the insoluble dietary fibre, soluble dietary fibre is more versatile in food processing as it has capacity to provide viscosity along with an ability to form gels and/or act as emulsifiers and making it easier to incorporate in food products (Mudgil & Barak, 2013).

The hydration properties of dietary fibre are characterized by several different aspects, such as water absorption, water holding capacity (retention) and swelling capacity (Raghavendra et al., 2006). Water absorption is defined as the kinetic of water movement under defined conditions (Robertson et al., 2000). Water holding capacity (WHC) is defined as the amount of water retained by 1 g of dry fibres under specified conditions of temperature, time soaked, and duration and speed of centrifugation (Elleuch et al., 2011; Raghavendra et al., 2006). Swelling means the volume occupied by a defined weight of fibre under the condition used, which can be assessed by the bed volume technique, determined by swelling the fibres in water overnight, in a volumetric cylinder (Table 2.3) (Guillon & Champ, 2000). The hydration properties of dietary fibre depends on the chemical composition of the component polysaccharides and other factors, such as porosity, particle size, ionic form, ionic strength, pH, temperature, type of ions in solution and stresses upon fibres (Elleuch et al., 2011; Fleury & Lahaye, 1991). The amount of water measured by centrifugation is generally higher than the amount of water absorbed (Weightman, Renard, Gallant, & Thibault, 1995). In food processing, dietary fibres with high WHC can be used as ingredients in food products to avoid syneresis and modify the viscosity and texture of some formulated foods (Elleuch et al., 2011).

Viscosity is related to a fibre's water absorption and the formation of a gelatinous mass. The definition of viscosity (η) is the ratio of shear stress (T) to shear rate (γ). Water soluble fibres, such as gums, pectins, psyllium, and β -glucans, are the major component that would increase the viscosity of a solution (Elleuch et al., 2011; Guillon & Champ, 2000). For example, soluble fibres form gels which increase the viscosity of the contents of the gastrointestinal tract, whereas, defatted rice bran has low viscosity, because it contains only 9 % soluble fibre (Smits, 1996).

Dietary fibre is capable of absorbing metal ions, bile acids, and some organic components, because of the charged polysaccharides. The ability to bind or exchange mineral depends on the pH, the type of fibre, and the nature of bile acids. Binding ability is measured often by pHmetry and sometimes by conductimetry in controlled conditions (Elleuch et al., 2011; Guillon & Champ, 2000).

Table 2.3 Hydration properties of fibres

Source of fibre	Particle size (μm)	Swelling (mL/g)	Water retention g water g ⁻¹ dry pellet	Water absorption (ml water g ⁻¹ dry fibre)
Sugar beet fibre	500-200	11.5	26.5	
		19.3	32.9	
	390	14.7	19.7	
	385	21.4	22.6	8.8
	205	15.9	19.2	7.3
	540	11.0	26.6	
Citrus fibre	600	13.5	7.2	
		15.7	11.2	5.2
	540	15.7	10.4	7.0
	235	13.3	8.6	7.0
	420	14.7	10.4	
Apple fibre	139	10.4	10.7	4.6
	540	9.6	6.9	3.8
	250	8.6	5.5	4.6
	133	7.4	5.4	
	500	6.0	7.1	2.4
	80	5.6	7.1	2.7
	950	9.9	4.3	1.9
	300	7.8	6.2	2.8
	560	6.2	4.2	2.7
	100	6.5	3.9	3.3
Wheat bran	67	6.6	3.8	3.7
	500-250		6.4	2.7
	900	11.9	6.8	1.0
	320	5.9	3.0	0.9
	1000-500	7.0	7.0	
	Coarse	7.4	5.6	
Maize bran	Ground	6.4	6.6	
		5.7	2.4	
Oat bran		5.53	3.5	
Resistant starch	40	5.6	3.5	3.0
	80	7.4	3.1	3.9

Adapted from (Guillon & Champ, 2000).

2.2.2 Health benefits

Intake of dietary fibre has many beneficial effects on human health, e.g. decreased intestinal transit time, increased stool bulk, reduction of blood cholesterol levels (total and LDL), and reducing glycaemic response and insulin levels (Brennan, 2005; Marlett, McBurney, & Slavin, 2002). According to Burkitt's research, dietary fibre plays a significant role in the prevention and management of the diseases, such as diabetes, obesity, heart disease, diseases of the large bowel, and colon cancer (Table 2.4) (Burkitt, Walker, & Painter, 1972; Mann & Cummings, 2009).

Table 2.4 Evidence for preventive or therapeutic role of dietary fibre in frequently occurring chronic diseases

	Comparisons between countries / population groups or over time	Case control or cohort studies	Experimental studies	Randomized controlled trials
Type 2 diabetes / prediabetes	++	+++	+++	++
Coronary heart disease	++	++	++	++
Obesity	++	++	++	++
Constipation	++	-	+++	+++
Diverticular disease	++	++	++	++
Colon cancer	++	++	++	-
Appendicitis	+	+	-	-
Gallstones / cholecystitis	+	+	-	-
Peptic ulceration	-	-	-	-
Ulcerative colitis / Crohn's disease	-	-	-	-
Varicose veins / haemorrhoids	+	-	-	-

+ = Protective or therapeutic effect; +++ = Strongly protective or beneficial; -= No or inadequate data (the grading of the evidence is based somewhat upon the authors' assessment of the published literature).

Adapted from (Burkitt et al., 1972; Mann & Cummings, 2009).

2.2.3 Sources of dietary fibre

The major sources of dietary fibre include grain, fruits, legumes, nuts, seeds, and vegetables. The total dietary fibre derived from grains varies depending on the native amount present and the degree of processing. Wholegrain foods contain a large amount of dietary fibre (Table 2.5). According to current research, cereals are the main source of dietary fibre in the western countries, contributing to around 50 % of fibre intake. Cereals mainly contain a large amount

of AX, β -glucan, cellulose and lignin. Compared to cereals, vegetables contribute to 30-40 % of dietary fibre intake while fruits contribute 16 % (Table 2.6) (Buttriss & Stokes, 2008). In addition, both vegetables and fruits are the primary source of gums, pectin and mucilage.

Table 2.5 Fibre content of some commonly consumed grains

Low (less than 3 g per 100 g)		Medium (3-6 g per 100g)		High (6 g or more per 100 g)	
Source	NSP value (g/100g)	Source	NSP value (g/100g)	Source	NSP value (g/100g)
White rice	0.1	Wheat flour, white	3.1	All bran	24.5
Brown rice	0.8	Granary bread	4.3	Crispbread	11.7
Porridge	0.8	Puffed wheat	5.6	Oat bran flakes	10
Rice Krispies	0.7	Rye bread	4.4	Shredded wheat	9.8
Spaghetti, white	1.2	Spaghetti, wholemeal	3.5	Weetabix	9.7
White bread	1.5	Brown bread	3.5	Wheat flour, wholemeal	9

NSP = non-starch polysaccharides. Adapted from (Buttriss & Stokes, 2008).

Table 2.6 Fibre content of fruits, vegetables and pulses

Low (less than 3 g per 100 g)		Medium (3 g per 100g)		High (6 g or more per 100 g)	
Source	NSP value (g/100g)	Source	NSP value (g/100g)	Source	NSP value (g/100g)
Broccoli	2.3	Baked beans	3.8	Red kidney beans	24.5
Carrots	2.5	Brussels sprouts	3.1	Figs	11.7
Apples (no skin)	1.6	Butter beans	5.2	Apricots	10
Apples (with skin)	1.8	Chickpeas	4.3		
Pears (no skin)	1.7	Lentils	3.8		
Pears (with skin)	2.2	Avocado	3.4		
Baked potato (no skin)	1.4	Passion fruit	3.3		
Baked potato (with skin)	2.7				

NSP = non-starch polysaccharides. Adapted from (Buttriss & Stokes, 2008).

2.2.4 Applications of dietary fibre in food industry

Currently the food industry uses dietary fibre in food product processing in order to improve the viscosity, texture, sensory characteristics and shelf-life of food products (Elleuch et al., 2011). Many fibre-rich by-products (such as cereal bran, fruit and vegetable peel) may be used as a source of fibre for incorporation into processed foods. These by-products may also be incorporated into food products as inexpensive, non-caloric bulking agents for partial replacement of flour, fat or sugar, and enhances the water and oil retention to improve emulsion and oxidative stabilities (Dhingra et al., 2012). However, the percentage of dietary fibre added in the products is limited, because it can cause undesirable changes to colour and texture of foods (Elleuch et al., 2011). According to recent reports, dietary fibre can be added into various food products such as baked goods, beverages, confectionery, dairy, meat, pasta and soups (Elleuch et al., 2011; Guillon & Champ, 2000). Commonly, the use of fibres in bakery products is widespread due to the water retention capacity of dietary fibre. Dietary fibre can modify bread loaf volume, springiness, the softness of bread crumb, and the firmness of bread loaf (Wang et al., 2002).

2.3 Cereal

2.3.1 Definition

Cereals are classified as grasses that yield edible seeds (grains) suitable for use as foods. The use of animal feed accounts for the second largest use of the world cereals. Cereal grains are major dietary staple, which may provide 80 % of calories in some populations (Evers, O'Brien, & Blakeney, 1999). The grains of wheat, rice, maize, barley, oats, and rye, and the lesser cereals triticale, sorghum, and the millets share many common attributes but there are few characteristics in any two species are identical. Therefore, in all species of cereal grains the

starchy endosperm accounts for the greatest dry weight of the whole, and the major component of the endosperms is starch (Chakraverty, Mujumdar, & Ramaswamy, 2003).

2.3.2 Structure

The fruit of a grass is a ‘caryopsis’, which contains a single seed accounting for the major part of the entire fruit when mature. The cereal seed is comprised of the embryonic axis, scutellum, endosperm, nucellar tissue, and seed coat (testa), and it is surrounded by the fruit coat or ‘pericarp’. In anatomy, the cereal structure is essentially similar with some minor differences. For example, wheat and maize are surrounded by a fruit coat or pericarp and seed coat or testa. However, an additional husk is found surrounding the kernel in the barley, oats, and rice. Because of the consistent structure of cereal caryopses, the extent can be described by generalized (Figure 2.2) (Chakraverty et al., 2003; Evers et al., 1999).

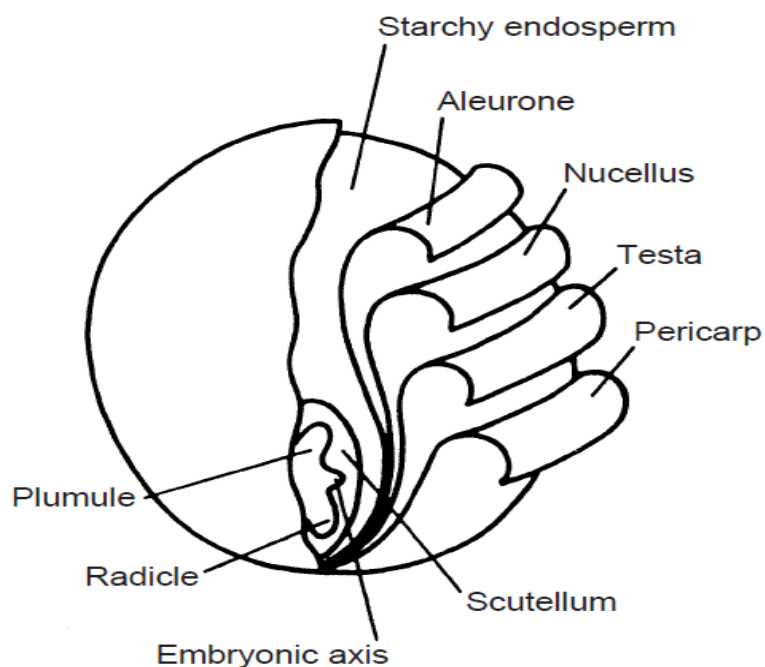


Figure 2.2 Generalized grain showing the main common characteristics
Adapted from (Evers et al., 1999)

Embryo (germ)

The embryo is comprised of embryonic axis and the scutellum. The embryonic axis is the plant of the next generation, which comprises primordial roots and shoot with leaf initials. It is connected to the shield-like scutellum lying between it and the endosperm. The term 'germ' is also used by cereal chemists to describe part or all the embryo. The scutellum serves the requirements of the embryonic axis as a secretory and absorptive organ (Evers et al., 1999; Evers & Millar, 2002).

Endosperm

The endosperm is the largest tissue of the cereal grains, which consists of two components. The majority of endosperm is a central mass described as starchy endosperm. It is comprised of cells packed with nutrients that can be mobilized to support growth of the embryonic axis at the onset of germination. There are two major nutrients stored in insoluble form, they are carbohydrate present as starch, and protein. These compounds account for 80 % and 20 % of the endosperm, respectively. The wall of the starchy endosperm of wheat consists mainly of AX, while in oat and barley (1 → 3, 1 → 4)-β-D-glucan (β-glucan) predominate. Cereal walls of the starchy endosperm contain little cellulose except in the case of rice (Evers et al., 1999; T. Evers & Millar, 2002).

The other endosperm tissue is the aleurone layer found surrounding the starchy endosperm. The aleurone layer comprises 1 to 3 layers of thick-walled cells with dense contents and prominent kernel, the number of layers depends on the cereal species, wheat, rye, oats, maize, rice, and sorghum have only 1 layer, while barley has 3 layers and can be up to 6 layers in rice. However, the number of layers may be greater in the region adjacent to the conducting tissue. Compared to the starchy endosperm, aleurone cells contain no starch but they have a high protein content and they are rich in lipid. Aleurone cells play an important role in both grain development, during which they divide to produce starchy endosperm cells, and germination. In addition,

they are also a site of synthesis of hydrolytic enzymes responsible for solubilizing the reserves in most species (Evers et al., 1999).

Seed coats

Surrounding the endosperm and embryo lie the remains of the nucellus, the body within the ovule in which the cavity known as the embryo sac develops. Following fertilization the embryo and endosperm expand at the expense of the nucellus, which is broken down except for a few remnants of tissue and a single layer of squashed empty cells from the nucellar epidermis. In many higher plants, epidermal cells secrete a cuticle and a cuticle is present on the outer surface of the nucellar epidermis of many cereals. The testa or seed coat is the outermost tissue of the seed (the nucellar epidermis is also regarded as a seed coat but its origin is different from that of the testa which develops from the integuments) (Evers et al., 1999; Evers & Millar, 2002).

Pericarp

The pericarp is a multi-layered structure that consists of several complete and incomplete layers. In all cereal grains, it is dry when the seed is mature, consisting of largely empty cells. It can serve to protect and support the growing endosperm and embryo during development. In addition, all starch has disappeared and the cells in which was present are largely squashed or broken down at maturity (Evers et al., 1999).

2.3.3 Chemical components of cereals

Cereal grains supply macronutrients for humans, the components of cereal grains include carbohydrates (approximately 75 % of grain), protein (about 10 %), fat (about 2 %), vitamins and minerals (1.5 %) (Table 2.7). The composition of cereals varies and depends greatly on the type of grain, growing conditions, and crop husbandry (Charalampopoulos, Wang, Pandiella, & Webb, 2002; Kent, 1984).

Table 2.7 Composition of cereal grain

Parameter	Malt	Rice	Corn	Wheat	Sorghum	Millet
Water (%)	8	12	13.8	12	11	11.8
Protein (g)	13.1	7.5	8.9	13.3	11	9.9
Fat (g)	1.9	1.9	3.9	2.0	3.3	2.9
Carbohydrates (g)	77.4	77.4	72.2	71.0	73	72.9
Fibre (g)	5.7	0.9	2.0	2.3	1.7	3.2
Ash (g)	2.4	1.2	1.2	1.7	1.7	2.5
Ca (mg)	40	32	22	41	28	20
P (mg)	330	221	268	372	287	311
Fe (mg)	4.0	1.6	2.1	3.3	4.4	68
K (mg)	400	214	284	370	350	430
Thiamin (mg)	0.49	0.34	0.37	0.55	0.38	0.73
Riboflavin (mg)	0.31	0.05	0.12	0.12	0.15	0.38
Niacin (mg)	900	1.7	2.2	4.3	3.9	2.3
Mg (mg)	140	88	147	113	n.d.	162

Composition of foods expressed as 100 g of edible portion.

Adapted from (Charalampopoulos, Wang, Pandiella, & Webb, 2002).

Carbohydrates

In the literature, the term carbohydrate is described as naturally occurring compounds of this class can be represented formally as the hydrates of carbon, i.e. $C_x(H_2O)_y$. It is common to classify carbohydrates according to their molecular size and degree of polymerization, with each group being subdivided according to the number and composition of saccharide units, such as monosaccharides (1 unit), disaccharides and oligosaccharides (2-20 units), and polysaccharides (> 20 units). Thus, this classification includes sugars (monosaccharides and disaccharides), oligosaccharides, starch (amylose and amylopectin) and non-starch polysaccharides (β -glucans) (Evers et al., 1999). Carbohydrates are the major components of cereal grains, accounting for almost 75 % of the mature grain (Henry, 1985; Lafiandra, Riccardi, & Shewry, 2014). According to research, the most abundant carbohydrate in all cereal grains is starch, accounting for about 64 % of the dry matter of the whole wheat grain and around 73 % of dry matter of the dent maize grain, about 1 % or less monosaccharides (glucose and fructose) and disaccharides (sucrose and maltose), about 1 % oligosaccharides (raffinose and fructo-

oligosaccharides), 1-2 % fructans, and about 10 % cell wall polysaccharides (mainly cellulose, AX, and β -glucan), which are major source of dietary fibre (Eskin & Shahidi, 2012; A. Evers et al., 1999; Haard, 1999; Lafiandra et al., 2014).

Starch consists of two different types of polymers: amylopectin and amylose. They are both composed of α -D-glucose and are different in their level of branching, with amylose being essentially linear and able to pack tightly while amylopectin is highly branched. Due to this difference in branching, the characteristics and functionality of both starches are different to each other with other differences arising due to chain length distribution and clustering (Evers et al., 1999; Evers & Millar, 2002; Lafiandra et al., 2014).

The main polysaccharides in the cell walls of cereal grains (wheat, barley, oats and rye) are AX and β -glucan. Arabinoxylan comprises a backbone of β -D-xylopyranosyl residues linked through (1 \rightarrow 4) glycosidic linkages, with residues being substituted with α -L-arabinopyranosyl residues at either position 3 or positions 2 and 3. Compared to AX, β -glucan has a simpler structure, consisting of only glucose residues which are linked by (1 \rightarrow 4) and (1 \rightarrow 3) bonds (Cui & Wang, 2009; Izydorczyk, Macri, & MacGregor, 1998).

Protein

Protein is the second most important component of cereal grains. The content of protein in cereal varies accounts for 7-22 % and highly depends on the cereal species and the growing environment. For instance, protein accounts for 5.8-7.7 % on a dry weight of rice, 8-15 % for barley, and 9-11 % for maize (Shewry, 2007). Protein is classified into four types, albumins, globulins, prolamins and glutelins (Fukushima, 1991; Shewry, Jenkins, Beaudoin, & Mills, 2004). Both globulins and prolamins are two major protein classes in cereal grains and varies depending on the cereal species. All types of protein are all found in the endosperm, except for

the 7S globulins which are found in the embryo and aleurone layer (Higgins, 1984; Shewry & Halford, 2002).

The nutritional quality of protein depends on the proportions of essential amino acids, essential amino acids are those that cannot be synthesized by the human body and hence they must be supplied in the diet for human health. When one of essential amino acids is limiting, the others will be broken down and excreted, this leads to poor growth of humans and loss of nitrogen in diet. There are ten essential amino acids: lysine, isoleucine, leucine, phenylalanine, tyrosine, threonine, tryptophan, valine, histidine and methionine (Friedman, 1996). Cysteine can also be considered an essential amino acid, because it can only be synthesized from methionine. According to current research, the ten essential amino acids are found in cereal grains (Table 2.8) (Shewry, 2007).

Table 2.8 Essential amino acid in cereal grain

	Wheat		Barley Grain	Oats Groat	Rye Grain	Rice Milled	Maize Cornflour	FAO recommendations	
	Grain	White flour						Children	Adults
Histidine	2.3	2.2	2.3	2.2	2.2	2.4	2.7	2.6	1.6
Isoleucine	3.7	3.6	3.7	3.9	3.5	3.8	3.6	4.6	1.3
Leucine	6.8	6.7	7.0	7.4	6.2	8.2	12.5	9.3	1.9
Lysine	2.8	2.2	3.5	4.2	3.4	3.7	2.7	6.6	1.6
Cysteine	2.3	2.5	2.3	1.6	1.9	1.6	1.6	4.2	1.7
Methionine	1.2	1.3	1.7	2.5	1.4	2.1	1.9		
Phenylalanine	4.7	4.8	5.2	5.3	4.5	4.8	5	7.2	1.9
Tyrosine	1.7	1.5	2.9	3.1	1.9	2.5	3.8		
Threonine	2.9	2.6	3.6	3.3	3.3	3.4	3.7	4.3	0.9
Tryptophan	(1.1)	(1.1)	1.9	ND	1.1	1.3	0.6	1.7	0.5
Valine	4.4	4.1	4.9	5.3	4.8	5.8	4.8	5.5	1.3

Values are g/100 g protein or g/16 g N. Adapted from (Shewry, 2007).

Fats (lipids)

Cereal grains contain generally low levels of lipids in the endosperm. The levels of lipids are much higher in the embryo, especially for rice and maize. Rice oil has a low content of linolenic acid, being a good source of tocopherols. In addition, both maize and rice oils are reliable sources of the essential fatty acid linoleic acid. Commercial production of oil from the two major grains is a significant industry worldwide, utilizing the 'bran' fraction, containing both the germ and the outer layers that are removed in the respective milling process (Buitimea - Cantúa et al., 2013; Evers et al., 1999; Evers & Millar, 2002).

2.4 Cereal bran

Cereal bran is outer layer of the cereal grain, comprising pericarp, testa, aleurone, germ, and part of the starchy endosperm (Brownlee, 2011). Cereal bran is a good source of dietary fibre, including AX, β -glucan, cellulose and lignin as major components. In addition, cereal bran is rich in protein, lipids, minerals, and B-vitamins (Nordlund, Katina, Aura, & Poutanen, 2013).

In the past, the main use of cereal bran was in feed industry as livestock feed and only a small part is used as additive in foods. Currently, with the increasing demand for health food in the market, cereal bran is considered as a nutritionally valuable ingredient in food processing due to the potential health benefits (Coda, Katina, & Rizzello, 2015).

However, the addition of cereal bran to the food products often leads to a poor consumption rates because of the dark colour, increased firmness, and taste. In the baking industry, for instance, the addition of cereal bran to bread will weaken the structure and baking quality of the dough decreasing bread volume and elasticity of the crumb, leading to an overall reduction in quality. Therefore, current research focuses on the processing techniques needed to improve the bread quality, such as hydrocolloids as an additive in rye bread, enzymes as improvers in bakery (Curti, Carini, Bonacini, Tribuzio, & Vittadini, 2013; Lebesi & Tzia, 2011).

Wheat bran

Wheat bran is produced worldwide in enormous quantities, as a by-product derived from wheat flour production. Wheat bran is a good source of dietary fibre, and consists of 36.5 – 52.4 % dietary fibre (Zhu, Huang, Peng, Qian, & Zhou, 2010). The main composition of wheat bran includes non-starch carbohydrates (58 %), starch (19 %) and protein (18%), with the non-starch polysaccharides being primarily arabinoxylans (70 %), cellulose (24 %) and β -(1,3) (1,4)-glucan (Sun, Liu, Qu, & Li, 2008). The major dietary fibre component of wheat bran is insoluble arabinoxylan (AX), which has many health benefits including immunomodulatory activity, cholesterol lowering activity, attenuation of type II diabetes, enhanced absorption of certain minerals, fecal bulking, and a prebiotic effect (Apprich et al., 2014; Gómez et al., 2011; Mendis & Simsek, 2014; Thamnarathip et al., 2016).

Oat bran

Oat bran is a by-product during oat milling process and is also a good source of soluble dietary fibre. The composition of oat bran includes 17.1 % protein, 67.9 % carbohydrates, 8.6 % fat, 15 – 22 % dietary fibre and vitamins and minerals. The main dietary fibre component of oat bran is β -glucan, which is a natural polymer composed of the glucose molecules joined by β -(1-3) and β -(1-4) glycosidic bonds (Butt, Tahir-Nadeem, Khan, Shabir, & Butt, 2008; Chatuevedi, Yadav, & Shukla, 2011). As a water-soluble fibre, β -glucan can easily form the viscous solutions, thus slows the intestinal transit, delays gastric emptying and slows glucose and sterol absorption in the intestine. Oat β -glucan has outstanding functional and nutritional properties due to its viscosity properties (Katongole, 2012). Previous studies have illustrated oat bran β -glucan has many beneficial effects, such as attenuation of postprandial blood glucose, reduction in insulin responses and a decrease in serum LDL cholesterol levels (El Khoury, Cuda, Luhovyy, & Anderson, 2011; Wood, 2010).

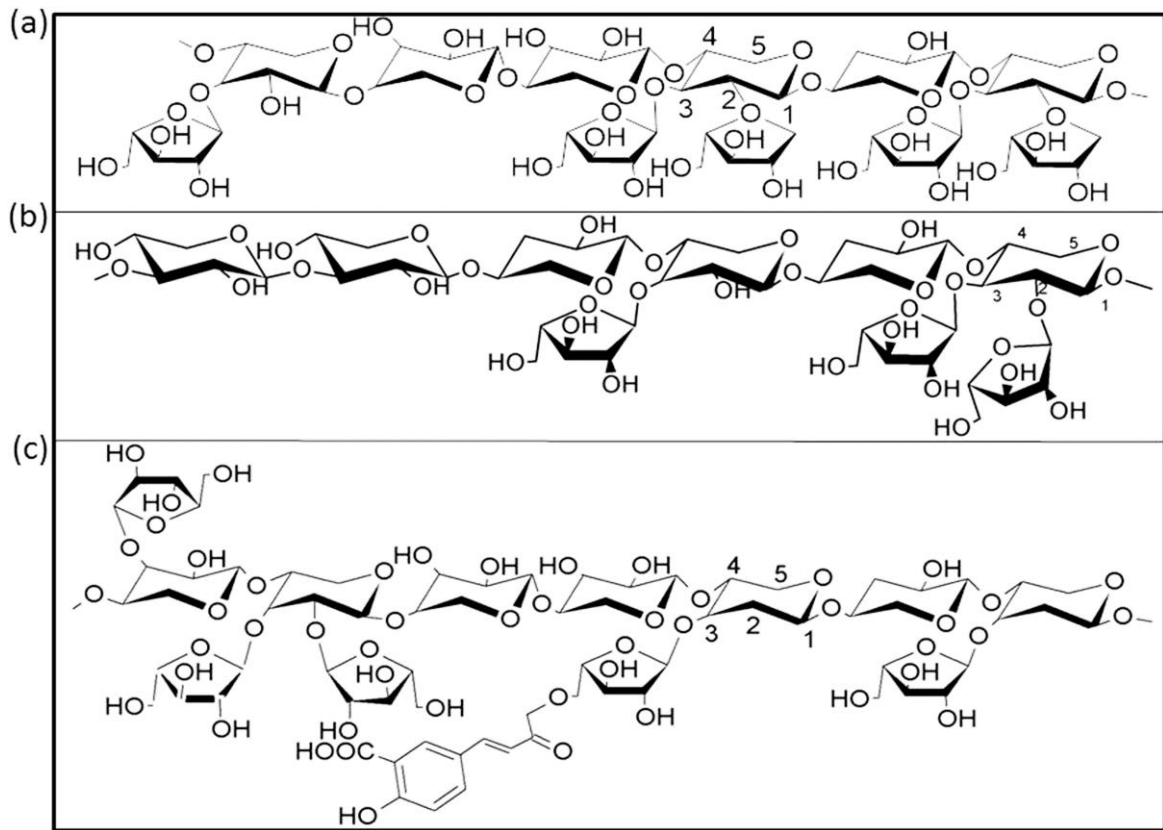


Figure 2.3 Structure of arabinoxylans. (a) hard wheat fine bran (b) durum wheat fine bran (c) wheat flour

Adapted from (Pavlovich-Abril et al., 2016).

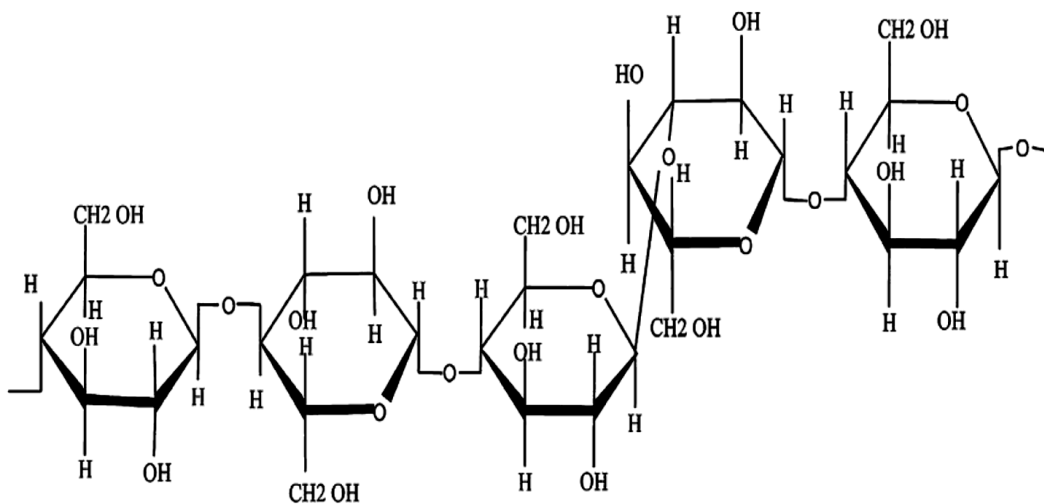


Figure 2.4 Chemical structure of β -glucan

Buckwheat

Buckwheat (*Fagopyrum*) belongs to the family of *Polygonaceae*, which is usually classified as a pseudocereal and widely grown in many countries (China, Russia, Canada, USA and Italy) (Rosenthal et al., 2014). According to research, buckwheat grains are rich in numerous nutritional components, such as dietary fibre, proteins, lipids, and polyphenols (Christa & Soral-Śmietana, 2008; Peng, Zou, Su, Fan, & Zhao, 2015; Wiczowski, Szawara - Nowak, Dębski, Mitrus, & Horbowicz, 2014). Tolaini et al. (2016) have indicated that crackers and biscuits made with buckwheat flour have high total polyphenols amount and antioxidant activity. Choy, Morrison, Hughes, Marriott, and Small (2013) also found the incorporation of buckwheat flour into instant noodle can improve the quality of noodle. Pseudocereals, such as buckwheat, together with bran fractions from other grain crops, are a good source of dietary fibre which can be used in food products such as breads, noodles, cakes and extruded snack products (Oliveira et al., 2015; Robin, Théoduloz, & Srichuwong, 2015; Steadman, Burgoon, Lewis, Edwardson, & Obendorf, 2001)

2.5 Enzymes

Enzymes are natural components of many ingredients in baking as technological aids, and are classified endogenous and exogenous enzymes. Grains contain a large number of various enzymes and the levels of enzyme activity depends on the cultivar variation, environmental conditions, pre-harvest sprouting, storage conditions, milling fractionation, and processing conditions (Poutanen, 1997). There are a large number of endogenous enzymes stored in the outer, aleurone and bran layers of the kernel, and in the germ. In phyto-physiology, endogenous enzymes are considered in the context of the stage of the life cycle of the grain. Therefore, most endogenous enzyme activity is concerned with the synthesis of storage products during maturation. Some hydrolytic enzymes involved in the breakdown of starch and protein stored in the pericarp are found before maturity and may persist (Evers et al., 1999). The enzyme levels

are considerably low in sound, dry grain and enzyme activity is lowest after milling. At the onset of germination, the production of enzymes concerned with solubilization increases rapidly (Evers et al., 1999; Simoinen & Tenkanen, 2000).

Enzymes have been widely used in food industry, especially amylase, protease, lipoxygenase, lipase, and xylanase, which is used in bread making as bread improver (Hamer, 1995). Amylase is essential in large quantities for successful malting and brewing, as well as being necessary in smaller quantities for bread making. The addition of enzymes in bread has been shown reduction of the fermentation time, improve the bread volume, and increase crumb softness (Gupta, Gigras, Mohapatra, Goswami, & Chauhan, 2003). However, many negative effects have been reported on the overuse of enzymes. For example, excessive α -amylase in milling wheat will induce the formation of dextrin in baking, resulting in the bread crumb being sticky (Martínez-Anaya, 1996). Moreover, excessive xylanase also leads to dough that is too soft and sticky, and too many polyphenol oxidases can lead to formation of dark specks in flour products (Martínez-Anaya & Jiménez, 1997). Therefore, current research focus on the optimal complex of enzymes to improve the bread quality, such as combining α -amylase and xylanase/ or pentosanase.

Amylases (α and β)

The action of amylase is to catalyze the hydrolysis of α -1, 4-glycosidic linkages into starch molecules (amylose and amylopectin), at a lower rate, maltodextrins and oligosaccharides (Martínez-Anaya, 1996). Both α - and β -amylase are defined as α -(1 \rightarrow 4)-D-glucanases, which act synergistically due to β -amylase being able to gain greater access to its substrate through the activity of α -amylase (Ishikawa, Nakatani, Katsuya, & Fukazawa, 2007). The main sources of amylases are from plants, animals, and microorganisms (Gupta et al., 2003). However, fungal amylases are less stable than those from cereal under conditions of raised temperature (Martínez-Anaya, 1996).

In the baking industry, α -amylases are the most common enzymes used in bread making as anti-staling agents, which can randomly damage starch and reduce its water binding ability, thus increasing the gluten hydration. Current research has shown that starch granules break down in dough with α -amylase, as a result of extensive hydration and swelling (Patel, Ng, Hawkins, Pitts, & Chakrabarti-Bell, 2012). In addition, depolymerization can catalyze the production of dextrin or fermentable sugars and promote the production of carbon dioxide from yeast thereby increasing the loaf volume (Gupta et al., 2003; Yoo, Feng, Kim, & Yagonia, 2017).

Xylanases

Xylanase is a hydrolase, which can randomly attack the AX backbone and break the glycosidic linkages in AX, result in changing the functional and physicochemical properties of AX (Hilhorst et al., 1999; Pollet, Delcour, & Courtin, 2010; Z. Zhang, Smith, & Li, 2014). Currently, the application of xylanases is being increased in food industry. For example, xylanases are widely used in bread making industry, due to the function of the xylanase. According to current research, xylanase can improve the rheological properties of dough, the loaf volume and crumb structure (Stojceska & Ainsworth, 2008). In addition, there are other improvements, such as dough machinability, dough stability, oven spring and shelf life. Due to the hydrolysis by xylanases, the amount of free sugars (such as pentoses) that are released, are increased and these can be used in fermentation. However, addition of too much xylanases will induce the dough to be too soft and sticky, because xylanase will influence the moisture content of bread (Courtin & Delcour, 2002; Hilhorst et al., 1999; Simoinen & Tenkanen, 2000).

Lipases

Lipases have a long history of being used in breadmaking to catalyze the hydrolysis of triglycerides into mono- or diglycerides, glycerol and free fatty acids (Moayedallaie, Mirzaei, & Paterson, 2010). The main sources of lipases are derived from microorganisms, animals, plants, fungi, and bacteria. Lipases are widely used in food industry such as oil industry, dairy

industry, pharmaceuticals and bakery industry (Aravindan, Anbumathi, & Viruthagiri, 2007). According to current research, the hydrolysis of lipids produce amount of free fatty acids, which can increase the intensity of the oxidation reactions and finally reduce the quality of the gluten network (Erickson, 2002; Vaclavik & Christian, 2014). In bread making, lipases can increase the bread volume and improve the rheological properties of the dough (Andualema & Gessesse, 2012; Aravindan et al., 2007).

Proteases

Protease is a traditional baking enzyme used in food industry, which can hydrolyze peptide bonds in proteins, polypeptides, oligopeptides, peptides and amino acids. Similar to amylase, proteases are also classified as endo-acting and exo-acting (Martínez-Anaya, 1996). Endo-acting proteases randomly disintegrate the protein polymer along the chain, which cause the dough to be extensible, preventing dough shrink back, creating better bread volume, pan flow and faster throughput rate. In addition, protease is inexpensive, which contributes to a major impact on profits (Moodie, 2001).

Lipoxygenases

Lipoxygenases are abundant in plants, fungi, and animals, which can catalyze the oxidative reaction of unsaturated fatty acids containing a series of cis, cis-1,4-pentadiene bonds in the presence of molecular oxygen (Brash, 1999). The oxidative action of lipoxygenases contributes to the production of free radicals that result in the formation of peroxides and hydroperoxides. According to current research (Martínez-Anaya, 1996; Simoinen & Tenkanen, 2000), the main effects of the addition of lipoxygenases in bread are:

1. Bleach flour and dough making the crumb bread whiter.
2. Strengthen gluten structure increasing mixing tolerance and loaf volume.

3. Produce carbonyl compounds that affect bread flavor.
4. Break down liposoluble vitamins and essential fatty acids.

Cellulase

Cellulase is produced by a number of microorganisms, which can hydrolyze cellulose (β -1,4-D-glucan linkages) and produce as primary products glucose, cellobiose and cellooligosaccharides. There are three major types of cellulase enzymes including 1,4- β -D-glucan cellobiohydrolase-CBH (EC 3.2.1.91), Endo- β -1,4-glucanase-EG (EC 3.2.14) and β -glucosidase-BG (EC 3.2.1.21). Enzymes within these classifications can be separated into individual components, such as microbial cellulase compositions may consist of one or more CBH components, one or more EG components and possibly β -glucosidases. The synergistic action of complete cellulase system (CBH, BG and EG) is to convert crystalline cellulose to glucose. The exo-cellobiohydrolases and the endoglucanases act together to hydrolyze cellulose to small cellooligosaccharides. The oligosaccharides are subsequently hydrolyzed to glucose by a major β -glucosidase (Sukumaran et al., 2005). The addition of cellulase to bread making process lead to an increase in bread volume, better crumb structure and a slow starch retrogradation (Monica Haros et al., 2002).

2.6 Dough rheology

Dough rheology, the science of deformation and flow of dough, has as its specific objective the investigation of the behaviour of different flours and additives (reducing agents, oxidizing agents, enzymes, emulsifiers, sugar and salt) that govern their flow and deformation under external forces (Faridi & Faubion, 2012). In the bakery industry, a better understanding of the rheological properties of flour dough during processing is significant, due to the relationships between those properties and quality attributes of the final products. Recently, several types of instruments have been employed to characterize the rheology of dough, such as Farinograph,

DoughLAB, Alveograph, Extensograph and Texture Analyzer. In particular, the doughLAB is an evolution of the current flour analysis equipment, which provides enhanced functions compared with common analysis with its higher speed and higher torque capabilities. According to these measurements, the rheological characteristics of dough are described as mixing time, mixing tolerance, stickiness, extensibility and resistance to extension (Janssen, Van Vliet, & Vereijken, 1996; Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007).

2.7 Glycaemic response to foods

Glycaemic response to foods reflect the balance between glucose load into, and its clearance from the blood (Monro, Mishra, & Venn, 2010a). There are many ways to quantify the glycaemic response, such as glycaemic index (GI), glycaemic load (GL) and glycaemic glucose equivalent (GGE). GI is defined as the indexing of the glycaemic response to a fixed amount of available carbohydrate from foods to the same amount of available carbohydrate from a standard food consumed. In fact, GI of foods is a method of classifying food based on glycaemic response in relation to a known carbohydrate quantity within standard food. The value of GI is based on the incremental area under the blood glucose response curve (AUC) measured over a 2 h period of consumed a fixed amount of available carbohydrate expressed as percentage of the area after intake of a standard quantity of glucose (Bornet, Jardy-Gennetier, Jacquet, & Stowell, 2007; Brennan, 2005; Monro & Shaw, 2008).

The concept of GL was introduced by Salmeron et al. (1997) to quantify the overall glycaemic response to a portion of food. Thus, the values of GL are calculated by multiplying the amount of carbohydrate contained in each consumed food by its GI (Kim, Yun, Choi, & Kim, 2008). Compared to GI and GL, GGE is a practical measurement of relative glycaemic impact, defined as the weight of food equals a given weight of glucose in its glycaemic impact (Brennan, 2005; Monro & Shaw, 2008; Mulholland, Murray, Cardwell, & Cantwell, 2008). In addition, GGE could represent effects of food composition and food intake on relative glycaemic impact,

because it is not restricted equi-carbohydrate comparisons. Therefore, GGE values may be applied to accurately control postprandial glycaemia, because they meet the need to combine GI with carbohydrate dose in diets of varying composition and intake, to obtain a realistic indication of relative glycaemic impact (Monro, 2002; Monro et al., 2010a; Monro & Shaw, 2008). Current research has developed *in vitro* digestion methods for predicting relative glycaemic response which are rapidly available (Monro et al., 2010a).

Chapter 3

Materials and Methods

3.1 Materials

Standard wheat flour was used in all general baking (Champion Flour Milling, Christchurch, New Zealand), as were wheat bran (Goodman Fielder, Auckland, New Zealand), oat bran (Sun Valley Foods, Auckland, New Zealand), buckwheat flour (Ceres Organics Ltd, Auckland, New Zealand), yeast powder (Edmonds Ltd, Auckland, New Zealand) and salt (Pams Products Ltd, Auckland, New Zealand).

Three commercial enzymes were used: Fungamyl 2500 SG (fungal α -amylase EC 3.2.1.1); Pentopan Mono BG (fungal xylanase EC 3.2.1.8) and Cellulast BG (endo-glucanase EC 3.2.1.4) supplied by Novozymes (Novozymes, Australia). Activities of enzymes are presented in Table 3.1.

Table 3.1 Function of enzymes used in this study

Enzymes	Component name	Activity	Fuction
Cellulast BG	endo-Glucanase	3500 EGU/g	hydrolyze (1,4)- β -D-glucosidic linkages in cellulose and other β -D-glucans
Fungamyl 2500 SG	α -Amylase	2500 FAU-F/g	hydrolyze (1,4)- α -D-glucosidic linkages in starch polysaccharides
Pentopan Mono BG	Xylanase (endo-1,4-)	2500 FXU-W/g	hydrolyze (1,4)- β -D-xylosidic linkages in xylans

EGU – Endo-Glucanase Units; FAU – Fungal Amylase Units; FXU – Fungal Xylanase Units.

Enzymes obtained from Novozymes Biotechnology Company.

3.2 Kits

Megazyme total starch and dietary fibre assay kits (Megazyme International Ireland Ltd, Wicklow, Ireland): Thermostable α -amylase (10 mL, 3000 U/mL on Ceralpha reagent), Amyloglucosidase (20 mL, 3300 U/mL on soluble starch), D-Glucose standard solution (5 mL, 1.0 mg/mL), Standardized regular maize starch control (93 % starch and 8.3 % moisture) and Purified protease (10 mL, 50 mg/mL; ~ 350 tyrosine U/mL) were used to determine total starch, total dietary fibre content of CSB.

Enzymes were used to analyze *in vitro* starch digestion including pepsin (EC 3.4.23.1), pancreatin (EC 232-468-9) and invertase (EC 3.2.1.26).

3.3 Chemicals and reagents

Ethanol (95 %), Acetone (reagent grade), Celite (Megazyme cat. No. G-CELITE), 2-(N-morpholino) ethanesulfonic acid (MES) (Megazyme cat. No. B-MES250) and tris(hydroxymethyl)aminomethane (TRIS) (Megazyme cat. No. B-TRIS500), Sodium acetate buffer (100 mM, pH 5.0) plus calcium chloride (5 mM), GOPOD reagent buffer (*p*-hydroxybenzoic acid and sodium azide), Hydrochloric acid solution (0.561 N), Acetate buffer (0.1 M, pH 5.2), NaOH (4 mol/L) and dinitrosalicylic acid (DNS) were obtained from BioLab, New Zealand.

3.4 Rheological properties of dough

3.4.1 Mixing properties analysis

A DoughLAB (Perten Instruments Australia, Macquarie Park, Australia) equipped with a 300 g mixing bowl was used to measure the rheological properties following the AACC 54-21.02 standard method. Flour samples were weighed according to display on the DoughLAB screen, which corrected for moisture content of the flour sample (300 ± 0.1 g for 300 g bowl). Then, the flour was added to the 300 g bowl, where the flour was mixed at speed (63 rpm) and

temperature (30 °C) for 20 min. The DoughLAB automatically added the required amount of water based on the entered water absorption, sample moisture content, and the bowl size used. Finally, the following parameters were automatically recorded by DoughLab software (ver. 1.3.0.185): water absorption (percentage of water required to yield dough consistency of 500 FU), dough development time (the time taken for the dough to reach the 500 FU peak resistance), departure time (the time required for the top curve to fall below the peak resistance), stability (the difference between the arrival and departure times), softening (the difference in torque between the peak resistance and the middle curve at a specified time after the development time), and mixing tolerance index (MTI). Analysis was performed in triplicate (Atwell, 2016).

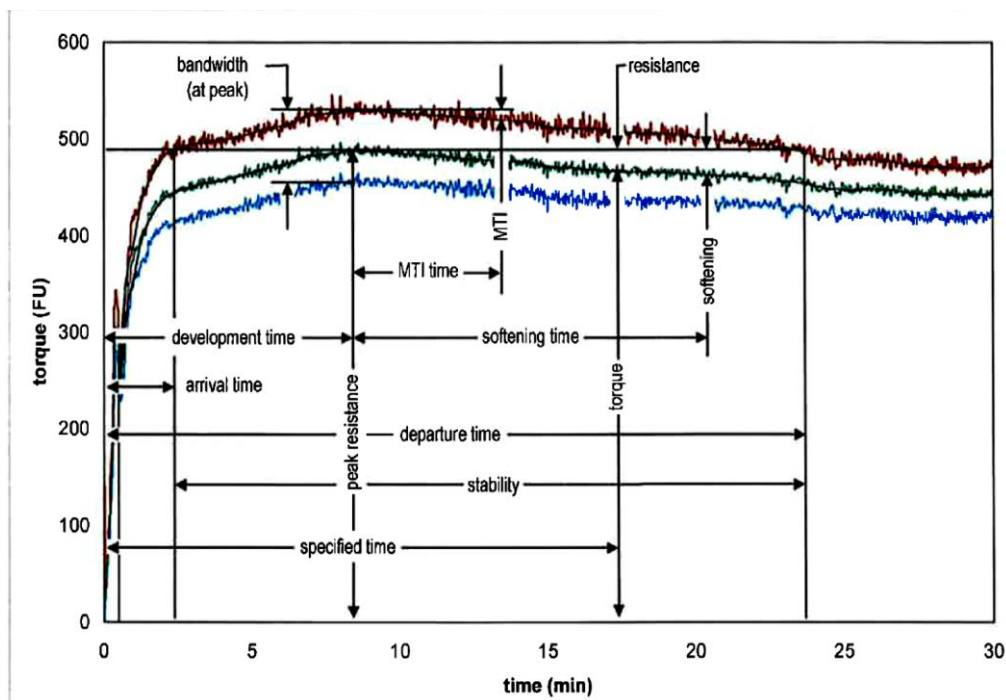


Figure 3.1 Results graph of dough rheology

3.4.2 Dough extension analysis

Dough extension test were conducted by a TA-XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK). The Texture Analyzer equipped with Kieffer dough and gluten extensibility rig was used to perform the extension tests. The resistance to extension (g) and extensibility (mm) was determined in tension mode by recording the peak force and the distance at the maximum and the extension limit. Firstly, dough test samples were placed onto the grooved base of teflon form. Then, cover the upper block of the form on top of the dough samples and push down firmly until the two blocks come together for 40 mins. Scrape off any excess dough sample that is forced out from the sides of the form. Lossen the dough press and carefully slide the upper form backwards over the grooved base to uncover the dough sample strip. Finally, place the strip of dough onto grooved region of sample plate and insert the plate into the Kieffer rig. Start the tensile test. The test settings were: pre-test speed: 2.0 mm/s; test speed: 3.3 mm/s; post-test speed: 10.0 mm/s; distance: 75 mm; trigger force: 5 g (5 kg load cell).

3.4.3 Dough stickiness

Analysis of dough stickiness was carried out by Chen-Hoseney's method and the dough was placed into the chamber of Stable Micro system/Chen–Hoseney Dough Stickiness Cell, and then closed with a die by screwing for test. A small amount of dough sample was placed into the chamber of the cell and excess dough was removed with a spatula so that the dough was flush with the top of the chamber. The chamber was then screwed a little way to extrude a small amount of dough through the lid holes and remove the first extrusion. Finally, rotate the screw again to extrude a 1 mm high dough sample for test. The test settings were: pre-test and test speed: 0.5 mm/s; post-test speed: 10.0 mm/s; distance: 4 mm; time: 0.1 s; trigger force: 5 g (5 kg load cell).

3.5 Preparation of Chinese steamed bread (CSB)

Chinese steamed bread loaves were produced using the formulation of Lin et al. (2012) with some modifications. The recipe consisted of wheat flour (300 g), yeast powder (4 g), salt (1 g), and water (to give a maximum consistency of 500 FU). The dough and steamed bread were prepared by replacing wheat flour with different levels of cereal bran (5 g, 10 g and 15 g / 100 g w/w based on wheat flour dry weight). The dough was formed by using stand mixer (BBEK1092, Briscoes New Zealand Ltd) for 5 min and kneading by hand for 5 min, then it was rested at 28 °C for 5 min. After that, the dough pieces were kneaded for a further 8 min before fermentation in an incubator at 30 °C for 30 min. After fermentation, the dough was rolled out, and allowed to rise at 30 °C for 25 min. Finally, the dough pieces were placed in a Convotherm mini easyTouch oven (CONVOTHERM Elektrogeräte GmbH, Germany) and steamed for 20 min. Steamed bread loaves were cooled to room temperature and then analyzed.

3.6 Determination of the physical characteristics of CSB

3.6.1 Loaf volume and specific volume

Volume of steamed bread loaves were measured using the rapeseed displacement method, following the AACC International Approved Method 10-05. 01 (AACC 2000). The measurements were carried out in triplicate.

The specific volume of steamed bread was calculated by dividing loaf volume by loaf weight, according to the AACC International Approved Method 10-05. 01 (AACC 2000). The measurements were performed in triplicate.

3.6.2 Moisture content

Moisture content of the dough and bread was determined by an oven drying method (105 ± 2 °C overnight) described by AACC International Approved Method 44-16.01 (AACC 2000). The

analysis was performed in triplicate and the results were expressed as g water / 100 g sample.

Analysis was performed in triplicate.

$$\text{Moisture (g water/100 g sample)} = \frac{(\text{loss of weight})}{(\text{sample weight})} \times 100$$

3.6.3 Textural properties of CSB

The textural properties of steamed bread were determined using TA-XT2 Texture Analyser (Stable Micro Systems, Surrey, England) equipped with a 25mm diameter cylinder probe. Steamed bread loaf was cut into slices of 25 mm thickness. The bread samples were compressed twice by probe to provide insight into how samples behave during chewed. The following texture profile analysis (TPA) parameters were automatically recorded by Exponent software: hardness (the peak force of the first compression), springiness (the distance of the detected height during the second compression divided by the original compression distance), cohesiveness (the area of the second compression divided by the area of the first compression), gumminess (hardness \times cohesiveness), chewiness (hardness \times cohesiveness \times springiness), resilience (by dividing the upstroke energy of the first compression by the down stroke energy of the first compression). For each loaf measurement, three slices were used. The test settings were as follows: pre-test speed: 1.0 mm/s; test speed: 1.7 mm/s; post-test speed: 10.0 mm/s; strain: 40 %; trigger force: 5 g (Lin et al. 2012). Analysis was performed in triplicate.

3.6.4 Crumb structure image analysis

Image analysis was carried out following the method described by Pescador-Piedra, Garrido-Castro, Chanona-Pérez, Farrera-Rebollo, Gutiérrez-López, and Calderon-Dominguez (2009) with some modifications. Briefly, a colour video camera (Sony, Digital 8 DRC-TRV-120, Japan) was located above the bread slices at a distance of 5 cm inside a dark room. The images were stored in a bit map (bmp) colour and graphics format of 24 bits, with a resolution of 640 \times 480

pixels and prior to analysis. Then, images were converted to a 256-gray scale (0-255) in 8-bit format, and segmentation process was performed manually by the threshold tool of ImageJ 1.51j8 image analysis software (National Institutes of Health, USA). Three characteristics of the crumb were measured: the number of cells per square centimetre (cells/cm²), the overall mean cell area (mm²), the size of cell (mm).

3.7 Determination of chemical characteristics of CSB

3.7.1 Total starch analysis

The total starch determination of the steamed bread was carried out with the Megazyme Total Starch analysis kit (Megazyme International Ireland Ltd, Wicklow, Ireland) following AACCI Approved Method 76-13. 01 (AACC 2000) as used by Brennan et al. (2008).

Samples were milled and added into glass test tubes. Then, 0.2 mL aqueous ethanol was (80 % v/v) added to the tubes, and test tubes were stirred vigorously. Meanwhile, 3 mL thermostable α -amylase was added and incubated in a boiling water bath for 12 min with stirring after 4, 8 and 12 min. After that, the samples were placed in a bath at 50 °C for 30min and added 0.1 mL amyloglucosidase. The samples were transferred quantitatively to a 100 mL volumetric flask and centrifuged at 3000 g for 10 min. Subsequently, 0.1 mL duplicate aliquots of the diluted solution was added to glass test tubes and each tube was added 3.0 mL GOPOD reagent , then incubated in a bath at 50 °C for 20 min. At last, each sample was determined by spectrophotometer at 510 nm.

$$\text{Starch \%} = \Delta A \times \left(\frac{F}{W}\right) \times FV \times 0.9$$

3.7.2 Total, Soluble and Insoluble Dietary Fibre Analysis

The determination of total (TDF), soluble (SDF) and insoluble (IDF) dietary fibre in the steamed bread incorporated with cereal bran was performed by the Megazyme Dietary Fibre analysis kit following the AACC (AACC, 2001) standard method.

Samples were weighed accurately (1.000 ± 0.005 g) into 400 mL tall-form beakers. MES-TRIS blend buffer solution 40 mL (pH 8.2) and magnetic stirring bar was added to each beaker. Beakers were stirred on magnetic stirrer until sample is completely dispersed in solution. Meanwhile, 50 μ L heat-stable α -amylase solution was added to beakers. Then, the covered samples were placed in shaking water bath at 98-100 °C and incubated for 30 min with continuous agitation. After that, all sample beakers were removed from hot water bath to 60 °C water bath. 10 mL distilled water was used to rinse side wall of beaker. After cooling to 60 °C, 100 μ L protease solution was added to each beaker and beakers were incubated in shaking water bath again at 60 °C, with continuous agitation for 30 min. Before the third incubation at 60 °C for 30 min, 5 mL of 0.561 N HCl solution was added to each beaker for adjusting pH 4.1 - 4.8.

When the three steps of incubation were completed, the sample solution was filtered through crucible into a filtration flask. The residue containing IDF in crucible was washed with 20 mL of 95 % ethanol and acetone. The filtrate and water washings were transferred to 600 mL tall-form beaker and precipitated at room temperature for 60 min for determination of SDF. For the SDF, the precipitated solution was filtered through a crucible and the residue was washed successively with 15 mL of 78 % ethanol, 95 % ethanol and acetone.

Finally, all crucibles containing residue were placed in 105 ± 2 °C oven to dry for overnight. One residue from each type of fibre was analyzed for protein and the second residue of the duplicate was analyzed for ash

Protein determination

The protein content of residue is a significant parameter of fibre analysis, which was measured by the Dumas combustion method (AOAC 992.23). The principle of Dumas method is nitrogen freed by pyrolysis and subsequent combustions at high temperature in pure oxygen is quantified by thermal conductivity detection. The total protein present was calculated from the nitrogen content.

$$\text{Protein \%} = \text{N} \times 5.70$$

Ash determination

The crucible containing residue was incinerated in muffle furnace at 525 °C for 8 h. After that, crucible was placed in desiccator to cool down and weighed to nearest 0.1 mg.

$$\text{Dietary fibre (\%)} = \frac{\frac{R1 + R2}{2} - p - A - B}{\frac{m1 + m2}{2}} \times 100$$

R1 = residue weight 1 from m1; R2 = residue weight 2 from m2; m1 = sample weight 1;

m2 = sample weight 2; A = ash weight from R1; p = protein weight from R2; B = blank weight.

3.8 Glycaemic Response Analysis in Steamed Bread Samples

***In vitro* method analysis:** An *in vitro* glycaemic measurement as described by Brennan et al. (2013). Briefly, Samples were milled with a coffee grinder and weighed accurately by balance in triplicate 2.5g food sample, references, blank. Then, 30 mL water was added to sample which was placed on a heated stirrer. Meanwhile, 0.8 mL 1 M HCl was added to tubes and mixed well. After mixing, pH was measured and adjusted to pH 2.5 ± 0.2 . The samples were incubated at 37 °C. After that, 1 mL of 10 % pepsin dissolved in 0.05 M HCl was added, and the mixture stirred slowly and constantly for 30 min at 37 °C to mimic gastric digestion. Then 2 mL 1 M NaHCO₃ and 5 mL of 0.1 M Na maleate buffer (pH 6) were added to the tubes and 1 mL digesta sample was removed to 4 mL ethanol at 0 min. The starch digestion was started by adding, in

quick succession, 0.1 mL amyloglucosidase and 5 mL of 2.5 % pancreatin in 0.1 M Na maleate buffer pH 6 and tubes were incubated in water bath at 37 °C for 120 min with steady constant mixing. Duplicate 1 mL samples were each removed to 4 mL ethanol at 20, 60 and 120 min.

Sugars released during digestion were measured after an invertase + amyloglucosidase secondary digestion as glucose equivalents (GE) by DNS (Dinitrosalicylic acid) method, using glucose references. The above tubes were centrifuged at 1000 G for 10 min. A 0.05 mL aliquot of ethanolic sample from the *in vitro* digestion above was added to 0.25 mL of 0.2 M acetate buffer (pH 5.2) (1 % invertase + 1 % amyloglucosidase) and incubated at room temperature for 20 min. Then reducing sugars were measured by adding 0.75 mL DNS mixture and heated for 15 min at 95-100 °C. The tubes were cooled and added 4 mL water, and finally read absorbances at 530 nm.

3.9 Statistical Analysis

3.9.1 One way – ANOVA

The analysis of variance (ANOVA) was conducted in Minitab 17 statistical software. The ANOVA illustrated if significant differences exist among the various means. If there was a significant difference, multiple comparisons using the Tukey's comparison test was used to identify which of the means were significantly different at Tukey's significant differences level $p < 0.05$ (Myers, Montgomery, & Anderson-Cook, 2016).

3.9.2 Factorial 2³ design

Two experimental designs were performed: the first one to evaluate the effect of single enzyme on the dough rheology and CSB quality, and the second one to investigate the effect of adding mixtures of enzymes on the dough rheology and CSB quality. Firstly, the effect of single enzyme on the rheological properties of regular dough and dough incorporated with 15 % of

cereal bran as bran dough control was analyzed by analysis of variance (ANOVA). According to the manufacture recommendations of Novozymes and previous publications (Caballero, Gómez, & Rosell, 2007a, 2007b; Serventi, Jensen, Skibsted, & Kidmose, 2016; Shafisoltani, Salehifar, & Hashemi, 2014), the dosage of the Cellulast BG, Fungamyl 2500 SG and Pentopan Mono BG was added with 35 ppm, 10 ppm and 70 ppm, respectively.

Second, in order to investigate the effect of mixtures of enzymes on the rheological properties of dough incorporated with 15 % of cereal bran, the full factorial 2^3 design of experiment in triplicate was used to evaluate all single effects and second-order interactions between factors. Generally, there are three factors (α -amylase, xylanase and cellulase) at two levels (-1, 1) resulted in 8 different combinations of experiments and the coded values per each level of each factor are presented in Supplementary Table 3.2 (Haros, Ferrer, & Rosell, 2006). According to the estimated coefficients (β_i , β_{ij} & β_{ijk}), the theoretical response function (W) was calculated as following polynomial linear regression model:

$$W = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC + \beta_{123}ABC$$

Factors: A – α -amylase; B – xylanase; C – cellulase; AB – α -amylase*xylanase; AC – α -amylase*cellulase; BC – xylanase*cellulase; ABC – α -amylase*xylanase*cellulase.

W – The theoretical response variable; β_0 – The global mean; β_i – The regression coefficient corresponding to main factor; β_{ij} and β_{ijk} –The regression coefficient corresponding to the interactions.

This multiple linear regression model with three independent variables describes the rheological property of dough is related to the α -amylase, xylanase and cellulase.

Table 3.2 Description of experimental factors at two level

Factor	(A) α – Amylase	(B) Xylanase	(C) Cellulase
Regular	-	-	-
Control	0	0	0
F1	-1	-1	-1
F2	1	-1	-1
F3	-1	1	-1
F4	1	1	-1
F5	-1	-1	1
F6	1	-1	1
F7	-1	1	1
F8	1	1	1

Amylase (-1, 1) – (6 ppm, 10 ppm); Xylanase (-1, 1) – (70 ppm, 120 ppm);
Cellulase (-1, 1) – (35 ppm, 60 ppm).

Chapter 4

Effect of wheat bran on the dough rheology and quality of Chinese steamed bread

- This part of research has been published in *Cereal Chemistry* (Liu, W., Brennan, M., Serventi, L., & Brennan, C. (2017). Effect of wheat bran on dough rheology and final quality of Chinese steamed bread. *Cereal Chemistry*, 94(3), 581-587.).

4.1 Introduction

Wheat bran has a high fibre content, which consists of 44-50 % of dietary fibre. Currently, consumers have a growing awareness of the link between diet and nutrition, thus there has been an increased demand for healthier products with a consequent rise in interest in functional and nutritional items by the food industry (Brennan et al. 2008; Chareonthaikij et al. 2016; Grigor et al. 2016; McGill and Devareddy 2015). Dietary fibre is the edible part of plants or analogous carbohydrate that resists digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine (Camire et al. 2001). A previous study by Brennan (2005) illustrated that dietary fibre has many beneficial effects on human health, for example decreased intestinal transit time, reduction of blood cholesterol levels (total and LDL), and a reduction in glycaemic response and insulin levels. Additionally, dietary fibre has a potential role for disease prevention and control, such as diabetes, obesity, heart disease, diseases of the large bowel, and colon cancer (Birkett and Cho 2013; Grigor et al. 2016; Slavin et al. 2016).

The major dietary fibre component of wheat bran is arabinoxylan (AX), which has many health benefits including immunomodulatory activity, cholesterol lowering activity, attenuation of type II diabetes, enhanced absorption of certain minerals, fecal bulking, and a prebiotic effect (Aprich et al. 2014; Gómez et al. 2011; Mendis and Simsek 2014; Thamnarathip et al. 2016). In the past, the main utilization of wheat bran was in the feed industry as livestock feed and only a small part was used as additive in foods. Currently, with an expanding market for health

food, wheat bran is considered as a nutritionally valuable ingredient in food processing due to the potential health benefits (Chinma et al. 2015; Coda et al. 2015; Thamnarathip et al. 2016). As a by-product derived from wheat flour production, wheat bran is widely used as a source of dietary fibre for incorporation into processed foods, particularly bread. However, the addition of wheat bran into bread generally results in poor dough rheology properties and poor textural properties of final products, for example reducing loaf volume, darkening the crumb, and increasing the firmness (Heiniö et al. 2016; Hemdane et al. 2016; Mastromatteo et al. 2015; Oliveira et al. 2015; Rashid et al. 2015).

Chinese steamed bread (CSB) is a traditional fermented food and widely consumed as a staple food in China (Wang, Guo, & Zhu, 2016; Zhu, 2014). The main ingredients of CSB include wheat flour, yeast and water with processing by steam cooking. Due to the differences in processing, the properties of CSB and western style baked bread are significantly different. For instance, the flavours of CSB are formed during the fermentation, while the flavours of western style breads are derived during baking by Maillard reaction (Lin et al. 2012). In addition, the lower processing temperature (100 °C) may result in the greater retention of nutrients in CSB (Zhu et al. 2016).

The objective of this study was to investigate the effects of incorporating wheat bran into the CSB at various levels (5 %, 10 % and 15 %) on the product from physical and nutritional perspectives with reference to the glycaemic response.

4.2 Materials and Methods

4.2.1 Materials

Ingredients used for this chapter were described in 3.1.

Table 4.1 Nutrition information for wheat Bran used in this study

Per 100g	Wheat flour (g)	Wheat bran (g)
Protein	11.0	14.6
Fat, total	1.4	5.4
- saturated	<1	<1
Carbohydrate	77.3	24.0
- sugars	<1	3.6
Dietary fibre	3.1	44.4
Sodium	0.005	0.02

Supplied by Champion Flour

4.2.2 Chemicals and reagents

Chemicals and reagents used for analysis were described in 3.3.

4.2.3 Dough rheological analysis

Rheological properties of CSB dough were determined according to the method described in 3.4.

4.2.4 Preparation of CSB incorporated with wheat bran

Chinese steamed breads samples were prepared as described in 3.5.

4.2.5 Moisture content analysis

Moisture content of dough and CSB samples were determined according to AACC standard method described in 3.6.2.

4.2.6 Physical properties of CSB incorporated with wheat bran

All CSB samples were assessed for their physical characteristics specific volume and texture as described in 3.6.

4.2.7 Total starch analysis

Total starch of CSB was performed as described in 3.7.1.

4.2.8 Glycaemic response analysis in CSB

Glycaemic response of CSB was measured using *in vitro* digestion method described by Brennan et al. (2013) as outlined in 3.8.

4.2.9 Statistical analysis

One-way ANOVA was used to compare the characteristics of each sample as described in 3.9.1.

4.3 Results and discussion

4.3.1 Dough rheological analysis

The effect of the addition of wheat bran on the rheological properties of dough is presented in Figure 4.1. There was a significant ($p < 0.05$) increase in water absorption as the addition of wheat bran levels increased from 0 to 15 % in Figure 4.3. The trend observed was that increased fibre content increases the water absorption of the dough due to the high hydration property of dietary fibre has been noted by others (Comino, Collins, Lahnstein, & Gidley, 2016; Robertson et al., 2000). According to the report by Chaplin (2003), the structure of dietary fibre comprises of a large number of hydroxyl groups, which can interact with hydrogen bonds of water. The highest stability value (15.10 min) was observed when the substitution level of 5 % wheat bran. However, there was a significant decrease in the dough stability after the addition of 5 % wheat bran. This observation is consistent with the research of Gómez, Jiménez, Ruiz, & Oliete (2011) where the stability increased as the addition levels of bran increased from 0 to 2.5 %, and stability values decreased with levels of inclusion higher than 5 %. Previous studies have found the incorporation of dietary fibre into flour can disrupt the starch-gluten network structure, thus affecting dough viscoelastic behaviour and constraining dough machinability (Bonnand-Ducasse et al. 2010; Gómez et al. 2011). The addition of wheat bran increased the time taken for the dough to reach the peak resistance (500 FU), although no significant differences were observed between the substitution levels of 5 %, 10 % and 15 %. Similar results were found by

Penella, Collar, & Haros (2008), the increase in development time could be attributed to the competition between fibre and protein for the available water, and this prevents adequate hydration of the proteins. Figure 4.1 shows that the addition of wheat bran resulted in decreased softening and increased departure time. In addition, there was an increase in MTI due to the impact on the disruption of gluten network.

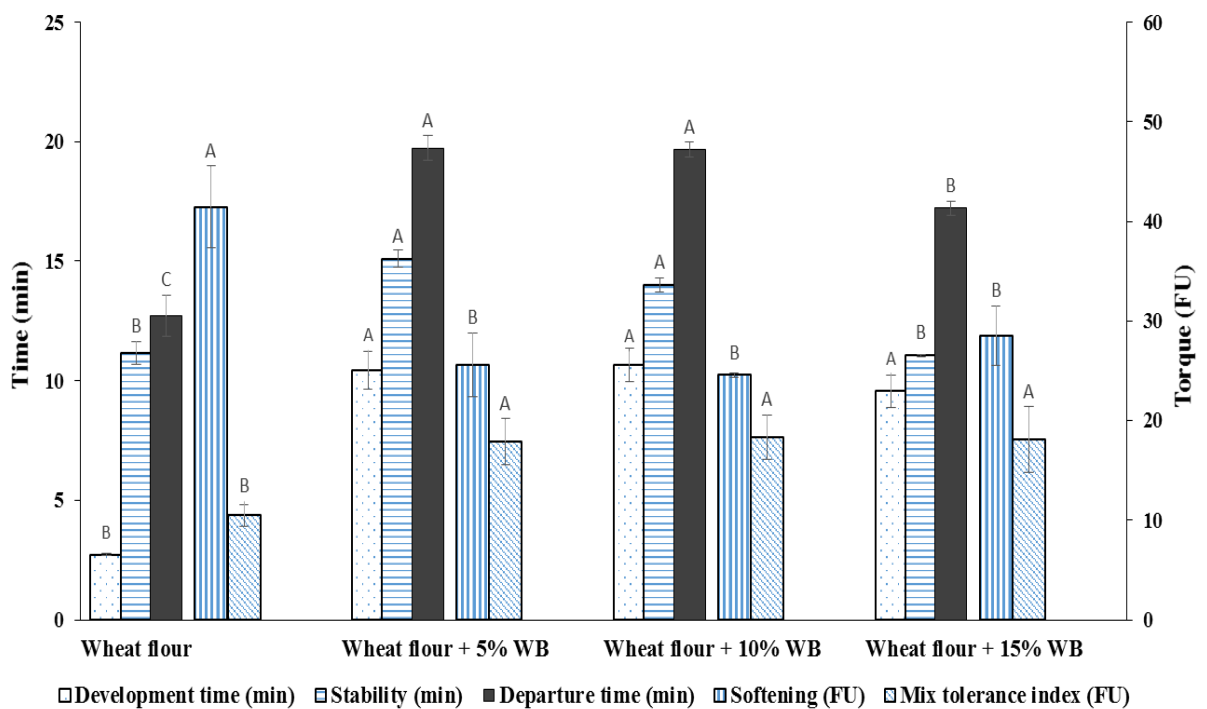


Figure 4.1 Rheological properties of dough with different levels of wheat bran.

Error bars represent standard deviation of replicates, and there is no significant ($p < 0.05$) difference among the bars with the same letter. WB = wheat bran.

The effects of wheat bran on the textural properties of dough are presented in Figure 4.2. Dough stickiness decreased significantly ($p < 0.05$) from 46.22 to 41.59 g when the wheat bran substitution increased from 0 to 15 %. However, there are no significant differences in the cohesiveness / strength of the dough. A similar result was observed by Sangnark and Noomhorm (2004b), dough stickiness decreased as sugarcane bagasse substitution increased from 0-15 %. Sangnark and Noomhorm (2004a) also indicated the addition of fibre decreased the stickiness of dough. These observations could also be attributed to the water absorption, particle size, the mixing time, ingredient formulation, and the level of enzymes addition (Le Bleis, Chaunier, Chiron, Della Valle, & Saulnier, 2015).

Regarding the dough extensibility, a significant ($p < 0.05$) decrease was observed with the addition of wheat bran increased. Comparable results have been reported by Gómez, Jiménez, Ruiz, & Oliete (2011), the addition of wheat bran reduced dough extensibility due to the interactions between fibre and gluten. According to the research of Rieder, Holtekjølen, Sahlstrøm, and Moldestad (2012), the addition of oat bran into wheat flour led to a significant reduction of extensibility, due to the disruption of starch-gluten network by bran. In addition, the resistance to extension was decreased by the addition of wheat bran, although there was no significant difference between 5 %, 10 % and 15 %. Skendi et al. (2010) illustrated that the addition of bran negatively affects the gluten network due to the disruption of starch-gluten matrix by bran. The effect could also be related to the percentage of bran added, the bran source, and particle size (Pieter J. Jacobs, Hemdane, Dornez, Delcour, & Courtin, 2015; Le Bleis et al., 2015).

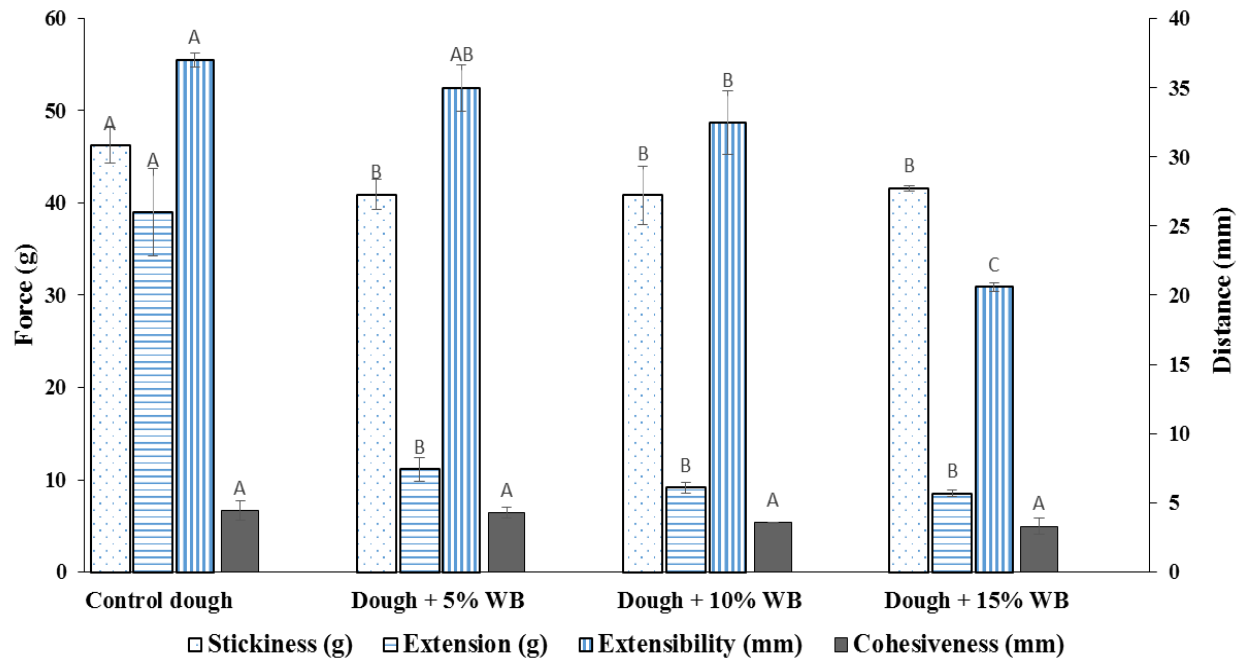


Figure 4.2 Textural properties of dough with different levels of wheat bran. Error bars represent standard deviation of replicates, and there is no significant ($p < 0.05$) difference among the bars with the same letter. WB = wheat bran.

4.3.2 Physical and Textural Properties of Steamed Bread

The effect of wheat bran addition on the physical properties of CSB from the loaf volume, moisture and texture, with the texture, the hardness, springiness, cohesiveness, gumminess, chewiness and resilience of CSB is shown in Figure 4.3, Figure 4.4 and Figure 4.5. It can be seen that the loaf height of CSB decreased significantly ($p < 0.05$) as wheat bran levels increased. Additionally, loaf volume and specific volume decreased significantly ($p < 0.05$) with the addition of wheat bran. Foschia, Peressini, Sensidoni, & Brennan (2015) reported that the addition of dietary fibre resulted in poor quality of baking products, such as a decrease in loaf volume and height. According to the Chinese standard SBT 10139-93 (wheat flour for CSB production) launched by the Ministry of Commerce of China 1993, the criteria of specific volume should be around 2.4 mL/g (Zhu 2014). Compared with the Chinese standard, the specific volume of CSB samples with wheat flour only were slightly higher than 2.4 mL/g.

However, the values of the CSB samples fortified with different levels of wheat bran were lower than the standard. These observations may be attributed to the dilution of gluten in wheat flour-based dough as well as disruption of the gluten hydration, which leads to the reduction of gas retention capacity. Additionally, the moisture of CSB increased slightly with increasing wheat bran substitution from 5 % to 15 % possibly due to increased dietary fibre content (Wang et al. 2002).

The influence of wheat bran on the hardness, springiness, cohesiveness, gumminess, chewiness and resilience of CSB. In the food industry, excellent quality products are usually associated with desirable textural properties. It can be seen that hardness, gumminess, and chewiness of CSB were significantly increased as the substitution levels increased. However, the addition of wheat bran tended to significantly ($p < 0.05$) reduce the springiness and cohesiveness. Resilience is how well a product can restore the original height, especially, the resilience increased significantly ($p < 0.05$) from 0.57 to 0.73, as the addition increased by 15 %. Therefore, the lower value of resilience shows that the bread has better ability to restore the original height. Gómez et al. (2003) pointed out that the addition of dietary fibre affects the dough rheology and bread quality because of the interaction between fibre and gluten. Brennan and Kuri (2006) also indicated that the hardness, gumminess and chewiness may be related to the degree of starch gelatinization. Therefore, the addition of wheat bran may disrupt the reaction between starch and protein resulting in the CSB being harder and less springy (Lin et al. 2012; Penella et al. 2008; Rosell et al. 2006).

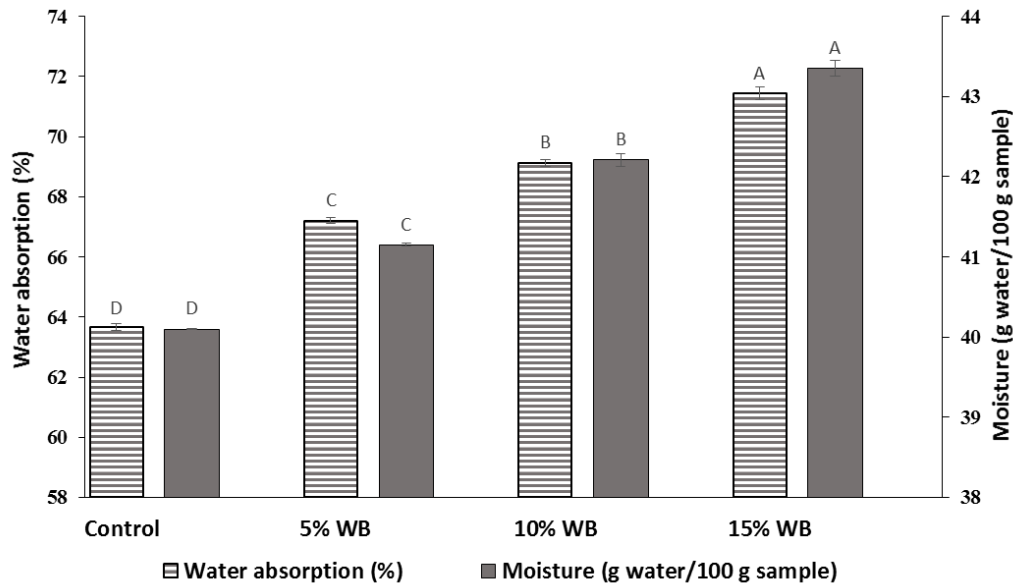


Figure 4.3 Water absorption of dough and bread moisture.

Error bars represent standard deviation of replicates, and there is no significant ($p < 0.05$) difference among the bars with the same letter. WB = wheat bran.

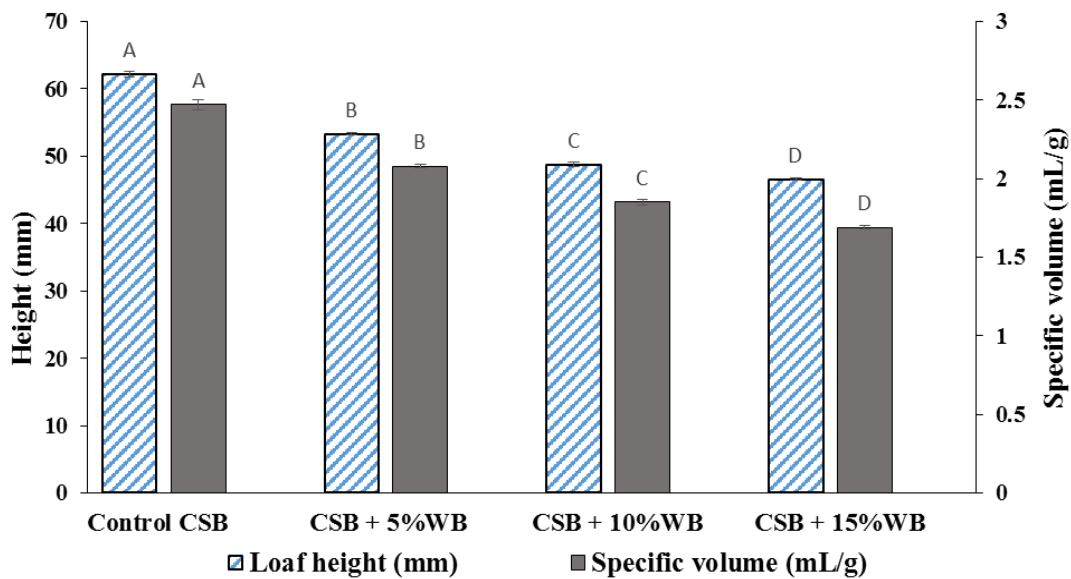


Figure 4.4 The physical properties of CSB with different levels of wheat bran.

Error bars represent standard deviation of replicates, and there is no significant ($p < 0.05$) difference among the bars with the same letter. WB = wheat bran.

4.3.3 Total starch and glycaemic response analysis

The total starch content of CSB samples is investigated in this study and there was a significant decrease ($p < 0.05$) as the addition of wheat bran increased. The reducing sugar released during the in vitro digestion of all CSB samples over 120 min are shown in Table 4.2. There was a rapid starch degradation during the first 20 min. Starch digested between 20 and 120 min is slowly digested starch. There was a trend to reduce the starch degradation as the substitution level of wheat bran increased. The addition of wheat bran at different levels revealed a significant decrease in the amount of reducing sugar released compared to the control sample. The area under the glucose release curve is a measurement of glycaemic response for 2 hours after food consumed (Monro 2002; Monro et al. 2010; Monro and Shaw 2008). The values of area under the glycaemic response curve (AUC) shown in Figure 4.6 demonstrate that the addition of wheat bran significantly decreased the AUC values from around 491 to 299. Steamed bread with 15 % wheat bran had the lowest AUC value (299) among all samples. These observations are consistent with Brennan (2005) and Brennan, Kuri, and Tudorica (2004) that the fibre enriched bread showed a significant reduction of sugars release. According to previous research, there are several factors that influence the rate of starch digestion, such as size of starch granules, extent of damage or gelatinization, composition and structure, and non-starch components content of starch-protein matrix (Wolter et al. 2013). Foschia, Peressini, Sensidoni, Brennan, and Brennan (2015) has also found that dietary fibre can combine with proteins and form a matrix barrier surrounding the starch granules to reduce the enzymes activity.

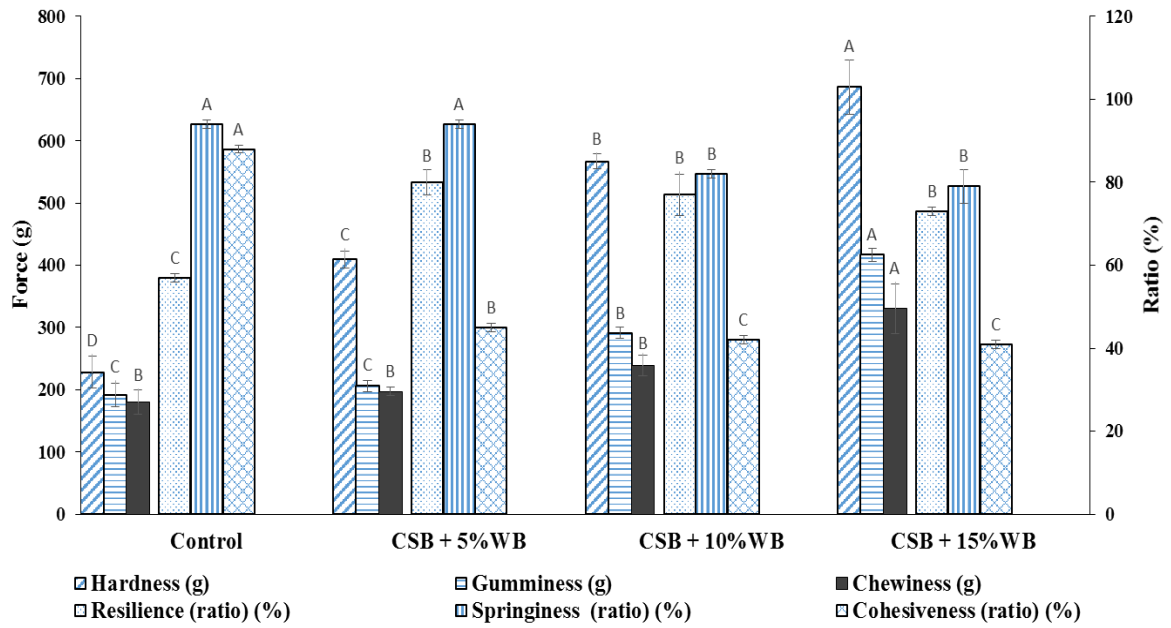


Figure 4.5 Textural properties of CSB with different levels of what bran. Error bars represent standard deviation of replicates, and there is no significant ($p < 0.05$) difference among the bars with the same letter. WB = wheat bran

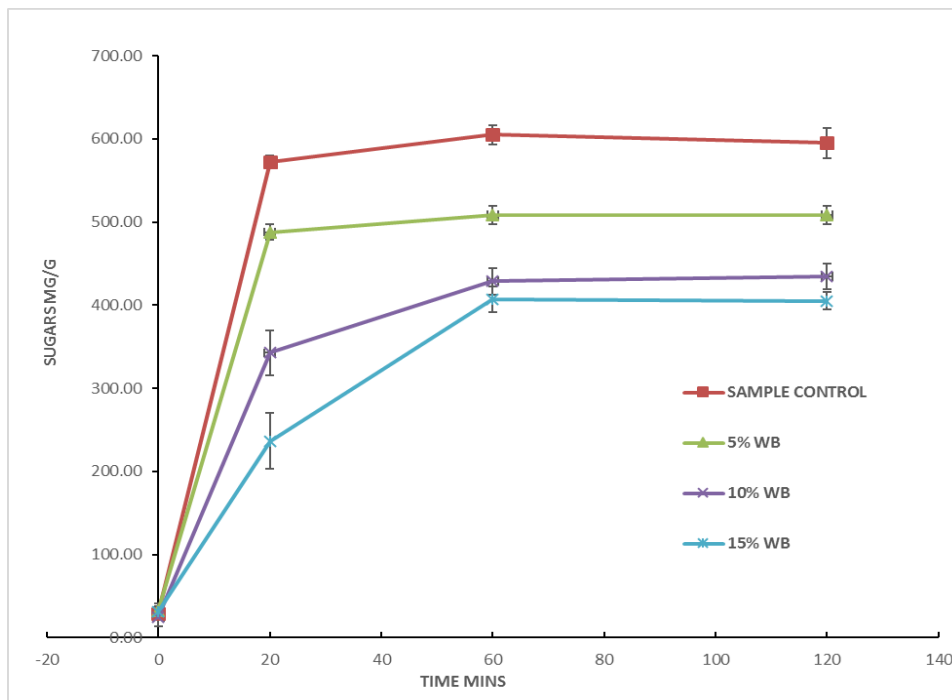


Figure 4.6 Reducing sugar released during *in vitro* digestion.

Table 4.2 Total starch content and glycaemic response of steamed bread

Samples	Total starch (dwd)	AUC values
Control	43.82 ± 1.30 ^A	491.77 ± 7.19 ^A
CSB+5% WB	39.45 ± 0.31 ^B	417.25 ± 7.11 ^B
CSB+10% WB	36.92 ± 0.68 ^C	337.49 ± 12.62 ^C
CSB+15% WB	36.67 ± 1.25 ^C	299.26 ± 12.27 ^D

Means ± standard deviations (n=3). Values in the same column with different letters differ significantly ($p < 0.05$).

4.4 Conclusion

This study found that incorporation of wheat bran into wheat flour can influence the nutritional quality of Chinese steamed bread. As revealed by *in vitro* method, the addition of wheat bran has the potential reduction of the sugar release, and consequently control the glycaemic response. However, the addition of wheat bran altered the rheological properties of the dough, and consequently the physical properties of CSB due to the high hydration property of dietary fibre and the disruption of gluten network. In addition, the current results show that the extensibility of the dough decreases with the levels of wheat bran increasing from 5 % to 15 %. In terms of physical properties of CSB, the specific volume was reduced significantly as the addition of wheat bran increased. For textural properties of CSB, the addition of wheat bran increased the hardness, gumminess and chewiness but there was no significant difference revealed between 5 % and 10 % levels.

Overall, the substitution level of wheat bran into wheat flour can be increased up to 15 % resulting in a reduced predicted glycaemic response which may confer significant health benefits to the consumer.

Chapter 5

Effect of buckwheat flour on the dough rheology and quality of Chinese steamed bread

- This part of research has been published in European Food Research and Technology (Liu, W., Brennan, M., Serventi, L., & Brennan, C. (2017). Buckwheat flour inclusion in Chinese steamed bread: Potential reduction in glycaemic response and effects on dough quality. *European Food Research and Technology*, 243(5), 727-734.)

5.1 Introduction

Buckwheat (*Fagopyrum*) belongs to the family of *Polygonaceae*, which is usually classified as a pseudocereal and widely grown in many countries (China, Russia, Canada, USA and Italy) (Rosenthal et al., 2014). According to research, buckwheat grains are rich in numerous nutritional components, such as dietary fibre, proteins, lipids, and polyphenols (Christa & Soral-Śmietana, 2008; Peng et al., 2015; Wiczowski et al., 2014). Tolaini et al. (2016) have indicated that crackers and biscuits made with buckwheat flour have high total polyphenols amount and antioxidant activity. Choy et al. (2013) also found the incorporation of buckwheat flour into instant noodle can improve the quality of noodle. Pseudocereals, such as buckwheat, together with bran fractions from other grain crops, are a good source of dietary fibre which can be used in food products such as breads, noodles, cakes and extruded snack products (Oliveira et al., 2015; Robin et al., 2015; Steadman et al., 2001).

In recent years, consumers have become more aware of the link between diet and health and there has been an increased demand for healthier foods with a consequent rise in interest in functional and nutritional foods by the food industry (Brennan & Tudorica, 2008; Chinma et al., 2015; Martins et al., 2015; Mastromatteo et al., 2015). Dietary fibre has been proven to be a functional ingredient that can be added to food (Elleuch et al., 2011; Grigor et al., 2016; Oliveira et al., 2015). Dietary fibre is a complex compound, which is defined as the edible part

of plants or analogous carbohydrate that are resistant to the digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine (Camire et al., 2001). Accordingly, the chemical nature of dietary fibre is composed of non-digestible carbohydrates, including oligosaccharides, polysaccharides and lignin, e.g., cellulose, β -glucan, hemicelluloses, arabinoxylan, gums, mucilage, pectin, inulin, resistant starch (Lunn & Buttriss, 2007). The major sources of dietary fibre include cereal, fruit, vegetable, and legume. Current research has shown that dietary fibre has many beneficial physiological effects, such as increase in stool bulk, decrease in intestinal transit time, reduction of blood cholesterol levels, and reduction in glycaemic impact (GI) and insulin levels (C. S. Brennan, 2005; Marlett, McBurney, & Slavin, 2002). According to Burkitt's research (Burkitt, Walker, & Painter, 1972), dietary fibre has a potential role for disease prevention and control, such as diabetes, obesity, heart disease, diseases of the large bowel, and colon cancer.

Chinese steamed bread (CSB), also known as mantou, is a Chinese traditional fermented food, which has been widely consumed as a staple food in China (Liu et al., 2014; Wu et al., 2012). The main ingredients of CSB include wheat flour, yeast and water with processing by steam cooking. Due to the differences in processing, the properties of CSB and western style baked bread are significantly different. For instance, the flavours of CSB are formed during the fermentation, while the flavours of western style breads are derived during baking by Maillard reaction (Lin et al., 2012). Recent studies have indicated that the addition of BW into normal bread doughs could decrease the quality of the bread, such as reducing the volume of bread, darkening the crumb appearance, and increasing the firmness of bread. There is a paucity of information regarding the effect of BW on the quality of the Chinese steamed bread.

Hence, the present study investigated the effects of incorporating buckwheat flour (BW) into Chinese steamed bread (CSB). Different levels (0 %, 5 %, 10 % and 15 %) of BW were added into wheat flour. The physical quality of dough was measured as moisture and textural properties of the dough. Quality of CSB was analysed from two perspectives; physical

properties and nutritional quality. For physical properties, specific volume, loaf height, moisture, and texture were measured by AACC methods. The nutritional quality of the bread was analysed using the glycaemic response determined by an *in vitro* digestion method.

5.2 Materials and Methods

5.2.1 Materials

Ingredients used for this chapter were described in 3.1.

Table 5.1 Nutrition information of wheat flour and buckwheat flour

Per 100g	Wheat flour (g)	Buckwheat flour (g)
Protein	11.0	13.3
Fat, total	1.4	3.4
- saturated	<1	0.7
Carbohydrate	77.3	71.5
- sugars	<1	3.0
Dietary fibre	3.1	10.0
Sodium	0.005	0.011

Supplied by Ceres Organics and Champion Flour Milling Ltd.

5.2.2 Chemicals and reagents

Chemicals and reagents used for analysis were described in 3.3.

5.2.3 Dough rheological analysis

Rheological properties of CSB dough were determined according to the method described in 3.4.

5.2.4 Preparation of CSB with buckwheat

Chinese steamed breads samples were prepared as described in 3.5.

5.2.5 Moisture content analysis

Moisture content of dough and CSB samples were determined according to AACC standard method described in 3.6.2.

5.2.6 Physical properties of CSB incorporated with buckwheat

All CSB samples were assessed for their physical characteristics specific volume and texture as described in 3.6.

5.2.7 Total starch analysis

Total starch of CSB was performed as described in 3.7.1.

5.2.8 Glycaemic response analysis

Glycaemic response of CSB was measured using in vitro digestion method described by Brennan et al. (2013) as outlined in 3.8.

5.2.9 Statistical analysis

One-way ANOVA was used to compare the characteristics of each sample as described in 3.9.1.

5.3 Results and discussion

5.3.1 The rheology of the dough

The effects of BW addition at different levels on the rheological properties of dough were measured by DoughLAB. Table 5.2 shows the parameters of rheological properties of the addition of 5 % - 15 % BW in wheat flour. The water absorption increased from 63.67 % to 66.50 % with the increase in BW levels from 0 to 15 %. The same trend was also observed by other authors that high fibre content increases the water absorption of the dough due to the water holding capacity of dietary fibre (Lin et al., 2012; Nikolić, Sakač, & Mastilović, 2011). The development time is the time taken for the dough to reach the peak resistance (500 FU). The addition of BW caused an increase in development time from 2.73 to 7.1 min. Sedej et al. (2011) indicated that the dough development time increased significantly by increasing the addition of BW, due to the high fibre and lipid content of BW. According to the study of Gómez et al.

(2003), the addition of different fibres increased the dough development time. Table 5.2 shows that the addition of BW resulted in increased softening and mixing tolerance index (MTI). Lin et al. (Lin et al., 2012) also reported that the MTI was increased by blending of wheat flour with barley flour because of the dilution of gluten content. A decrease in dough stability was observed with an increase in substitution level of BW (Table 5.2), similar results were reported by previous studies (Atalay, Bilgicli, Elgün, & Demir, 2013; Sedej et al., 2011). Previous research has shown that gluten is a major functional component of the dough for binding water and developing the viscoelastic behaviour (Delcour et al., 2012; Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007; Veraverbeke & Delcour, 2002). However, the proteins of BW mainly consist of albumin and globulin, and the properties of BW proteins are very different from gluten proteins (Nikolić et al., 2011; Sedej et al., 2011). Therefore, the decrease in dough stability is probably due to the lack of structure forming ability of buckwheat proteins and the decrease in the concentration of gluten.

Table 5.2 Rheological properties of dough incorporated with buckwheat flour

Sample	WA (%)	Stability (min)	Development time (min)	Softening (FU)	Departure time (min)	MTI (FU)
Wheat flour	63.67 ± 0.11 ^c	11.17 ± 0.47 ^A	2.73 ± 0.06 ^C	41.46 ± 4.11 ^C	12.72 ± 0.85 ^A	10.51 ± 1.10 ^B
Wheat flour+5%BW	64.32 ± 0.02 ^B	10.07 ± 0.11 ^B	5.63 ± 0.58 ^B	66.83 ± 0.21 ^A	11.67 ± 0.21 ^A	30.01 ± 6.00 ^A
Wheat flour+10%BW	64.31 ± 0.11 ^B	10.17 ± 0.21 ^B	6.76 ± 0.15 ^A	59.23 ± 1.62 ^{AB}	12.13 ± 0.15 ^A	31.36 ± 0.50 ^A
Wheat flour+15%BW	65.07 ± 0.03 ^A	8.72 ± 0.45 ^C	7.12 ± 0.13 ^A	53.37 ± 5.31 ^B	12.21 ± 0.36 ^A	31.31 ± 0.11 ^A

Means ± standard deviations (n=3). Values in the same column with different letters differ significantly ($p < 0.05$)

5.3.2 Extensibility and stickiness of dough

The stickiness, cohesiveness, extension and extensibility of the dough are shown in Table 5.3, which revealed the handling properties of the dough during the bread making processing (Angioloni & Dalla Rosa, 2007; Tseng & Lai, 2002). The stickiness of dough increased significantly ($p < 0.05$) with the additional levels of BW from 5-15 %. However, there is a significant decrease in the cohesiveness / strength of the dough. Previous research suggested that the water absorption, the mixing time, ingredient formulation, and the level of enzymes addition all contribute to the stickiness of dough (Chen & Hosney, 1995; Peressini & Sensidoni, 2009; Sudha et al., 2007).

Dough resistance to extension was decreased significantly from 38.99 g to 17.54 g with the addition of BW increasing to 15 %. Additionally, the extensibility of the dough had a slight decrease from 37 mm to 34.6 mm as the substitution of BW increased. Nikolić, Sakač, & Mastilović (2011) illustrated that dough formulated with BW had lower values of extensibility and resistance in comparison to dough with wheat flour only. Similar results were reported by Pruska-Kędzior et al. (2008) who pointed out that buckwheat, rice and maize are gluten free and are unable to form the same structure as a gluten containing product. Several investigations have also showed that the combination of wheat flour with BW results in the dilution of gluten and disruption of the gluten network structure (Gómez, Ronda, Blanco, Caballero, & Apesteguía, 2003; J. Zhu, Huang, & Khan, 2001).

Table 5.3 Textural properties of dough incorporated with buckwheat flour

Dough samples	Stickiness (g)	Cohesiveness (mm)	Extension (g)	Extensibility (mm)
Control	46.22 ± 1.94 ^C	4.46 ± 0.72 ^A	38.99 ± 4.78 ^A	37.06 ± 0.51 ^A
Dough+5%BW	51.76 ± 5.25 ^C	2.32 ± 0.45 ^B	35.72 ± 2.42 ^A	36.14 ± 0.46 ^{AB}
Dough+10%BW	62.01 ± 5.72 ^B	1.35 ± 0.87 ^{BC}	22.63 ± 1.93 ^B	34.90 ± 0.42 ^B
Dough+15%BW	77.59 ± 5.39 ^A	0.76 ± 0.36 ^C	17.54 ± 1.24 ^B	34.60 ± 0.21 ^B

Means ± standard deviations (n=3). Values in the same column with different letters differ significantly ($p < 0.05$). BW-buckwheat flour.

5.3.3 The physical and textural properties of steamed bread

Table 5.4 and Table 5.5 illustrate that the addition of buckwheat to CSB influenced the physical properties of products from the loaf volume, moisture and texture, with the texture, the hardness, springiness, cohesiveness, gumminess, chewiness and resilience of CSB. The loaf height of CSB decreased significantly ($p < 0.05$) as BW levels increased. Similarly, loaf volume and specific volume significantly ($p < 0.05$) decreased as the content of BW increased. Foschia et al. (2015) reported the addition of dietary fibre resulted in inferior quality of baking products, such as a decrease in loaf volume and height. According to the Chinese standard SBT 10139-93 (wheat flour for CSB production) launched by the Ministry of Commerce of China 1993, the criteria of specific volume should be around 2.4 mL/g (Zhu, 2014). Compared with the Chinese standard, the specific volume of CSB samples with wheat flour only were slightly higher than 2.4 mL/g. However, the values of the CSB samples fortified with different levels of BW were lower than the standard. The addition of BW may cause the dilution of gluten in wheat flour-based dough and disrupting the hydration of gluten, which lead to the reduction of the gas retention capacity (Day, Augustin, Batey, & Wrigley, 2006; Veraverbeke & Delcour, 2002; Zhu et al., 2001). Additionally, the moisture of CSB increased slightly with the levels of BW substitution increasing from 5 % to 15 % possibly due to increased dietary fibre content (Wang, Rosell, & de Barber, 2002).

In terms of texture, Table 5.5 shows the influence of BW on the textural properties of CSB, hardness, springiness, cohesiveness, gumminess, chewiness and resilience. In the food industry, good quality products are usually associated with desirable textural properties. It can be seen that hardness, gumminess, and chewiness of CSB were significantly increased as the level of BW increased. However, the addition of BW tended to reduce the springiness, cohesiveness, and resilience. Gómez et al. (Gómez et al., 2003) pointed out that the addition of dietary fibre

affects the dough rheology and bread quality because of the interaction between fibre and gluten. Brennan and Kuri (Brennan & Kuri, 2006) also indicated that the hardness, gumminess and chewiness may be related to the degree of starch gelatinization. Therefore, the addition of BW may disrupt the reaction between starch and protein resulting in the CSB being harder and less springy. Previous studies indicated that many factors may affect the loaf volume and texture, such as gluten content, moisture content, yeast and enzymes. Kang et al. (Kang, Sohn, Yoon, Lee, & Ko) illustrated the larger loaves were soft and had a looser loaf structure, resulting in lower hardness, gumminess and chewiness values.

Table 5.4 The physical properties of steamed bread

CSB samples	Loaf height (mm)	Specific volume (mL/g)	Moisture (g water/100 g)
Control	62.14 ± 0.38 ^A	2.47 ± 0.03 ^A	40.10 ± 0.01 ^C
CSB+5%BW	59.65 ± 0.14 ^B	2.36 ± 0.03 ^B	40.15 ± 0.02 ^{BC}
CSB+10%BW	58.37 ± 0.37 ^C	2.25 ± 0.02 ^C	40.31 ± 0.08 ^B
CSB+15%BW	57.65 ± 0.30 ^C	2.19 ± 0.01 ^D	41.15 ± 0.10 ^A

Means ± standard deviations (n=3). Values in the same column with different letters differ significantly ($p < 0.05$). CSB-Chinese steamed bread; BW-buckwheat flour.

Table 5.5 The textural properties of steamed bread

Samples	Hardness (g)	Springiness (ratio)	Cohesiveness (ratio)	Gumminess (g)	Chewiness (g)	Resilience (ratio)
Control	228.24 ± 25.92 ^C	0.94 ± 0.01 ^A	0.88 ± 0.01 ^A	191.75 ± 19.15 ^C	179.83 ± 19.34 ^C	0.57 ± 0.01 ^A
CSB+5%BW	287.95 ± 19.01 ^{BC}	0.93 ± 0.01 ^{AB}	0.87 ± 0.02 ^{AB}	249.35 ± 22.22 ^{BC}	229.44 ± 24.05 ^{BC}	0.57 ± 0.03 ^A
CSB+10%BW	333.99 ± 56.38 ^{AB}	0.91 ± 0.01 ^B	0.86 ± 0.01 ^{AB}	289.81 ± 50.98 ^{AB}	244.82 ± 41.47 ^B	0.54 ± 0.02 ^{AB}
CSB+15%BW	401.31 ± 61.49 ^A	0.89 ± 0.01 ^C	0.85 ± 0.01 ^B	345.79 ± 55.80 ^A	307.97 ± 49.46 ^A	0.53 ± 0.01 ^B

Means ± standard deviations (n=3). Values in the same column with different letters differ significantly ($p < 0.05$). CSB-Chinese steamed bread; BW-buckwheat flour.

5.3.4 Total starch and glycaemic response analysis

The total starch of CSB samples is presented in Table 5.6. There was a slight decrease among 0 %, 5 %, 10 % and 15 %, but no significant differences were observed. The reducing sugar released during the *in vitro* digestion of all CSB samples over 120 min are shown in Figure 5.1. It can be seen that there was a rapid starch degradation during the first 20 min. After 20 min, the starch was digested slowly between 20 and 120 min digestion. There was a trend to reduce the starch degradation as the substitution level of BW increased. Steamed bread with BW at 10 % and 15 % levels revealed a significant decrease in the amount of reducing sugar released compared to the control sample. Zhu et al. (2016) reported that bread and noodles with BW had a lower enzyme susceptibility and glycaemic index than that with wheat flour only. The area under the glucose release curve is a measurement of glycaemic response for 2 hours after food consumed (Monro, 2002; Monro, Mishra, & Venn, 2010b; Monro & Shaw, 2008). The values of area under the glycaemic-response curve (AUC) shown in Figure 5.2 demonstrates that the addition of BW significantly decreased the AUC values. Steamed bread with 15 % BW had the lowest AUC value (337.27) among all samples (Table 5.6). According to previous research, there are several factors that influence the rate of starch digestion, such as size of starch granules, extent of damage or gelatinization, composition and structure, and non-starch components content of starch-protein matrix (Wolter, Hager, Zannini, & Arendt, 2013). Foschia et al. (2015) has also found that dietary fibre can combine with proteins and form a matrix barrier surrounding the starch granules to reduce the enzymes activity. Similar results were reported by Zhu (2016), BW products had the lower glycaemic response due to the non-starch components (such as lipids, proteins, dietary fibre, protease inhibitors, and tannins) and food matrix effects.

Table 5.6 The total starch and AUC of steamed bread

Samples	Total starch (%)	AUC values
Control	43.82 ± 1.30 ^A	431.51 ± 31.80 ^A
CSB+5%BW	42.75 ± 1.85 ^A	403.08 ± 7.36 ^A
CSB+10%BW	42.55 ± 0.32 ^A	393.09 ± 23.08 ^{AB}
CSB+15%BW	42.07 ± 0.21 ^A	337.27 ± 21.11 ^B

Means ± standard deviations (n=3). Values in the same column with different letters differ significantly ($p < 0.05$). CSB-Chinese steamed bread; BW-buckwheat flour.

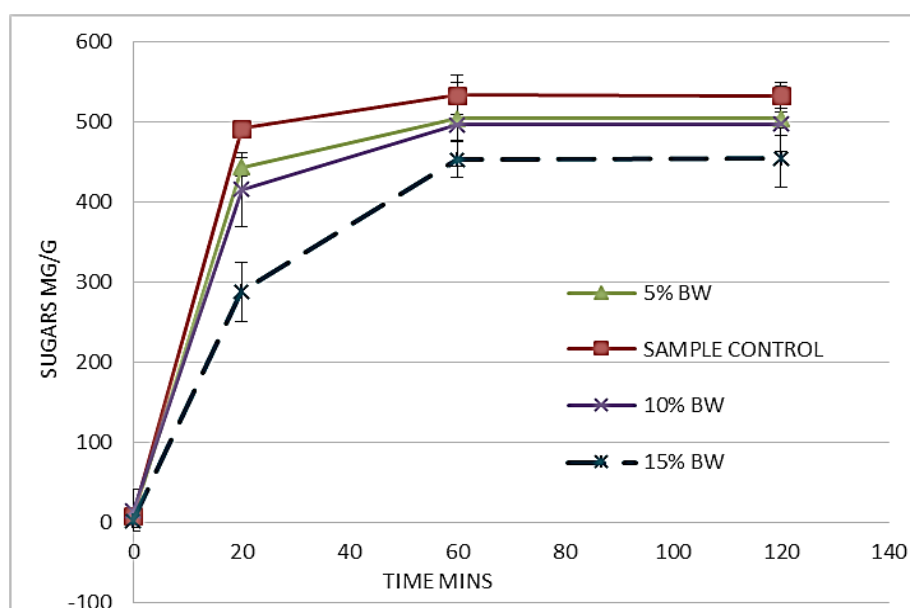


Figure 5.1 Reducing sugar released during *in vitro* digestion
BW-buckwheat flour

5.4 Conclusion

This study is one of the first research publications to illustrate the incorporation of BW into wheat flour can influence the nutritional quality of Chinese steamed bread. As revealed *in vitro* method, the addition of BW may reduce the sugar release, and consequently control the glycaemic response. Brennan (2005) illustrated that dietary fibre plays an important role in reducing the risk of diabetes and obesity. Moreover, the work of Mann and Cummings (2009) showed that the dietary fibre had positive effects on the disease prevention, such as heart disease and colon cancer.

However, the addition of BW altered the rheological properties of the dough, and consequently the physical properties of CSB due to the dilution of gluten (Gómez et al., 2003; Lin et al., 2012; Zhu, 2014). The current results show that the extensibility of the dough decreases with the levels of BW increasing from 5 % to 15 %, but there is no significant difference between 5 % and 10 % levels. From physical properties of CSB, it can be seen that the specific volume reduced significantly with the addition of BW increasing. However, according to the Chinese standard SBT 10139-93 (wheat flour for CSB production), the criteria of specific volume should be around 2.4 mL/g. Thus, the control and 5 % levels of CSB are acceptable on the basis of criteria (Zhu, 2014). In textural properties of CSB, the addition of BW increased the hardness, gumminess and chewiness but there was no significant difference revealed between 5 % and 10 % levels. In order to maintain the high quality of the bread, hydrocolloids are used as aids, being added to flour during bread making. Peressini, Pin, and Sensidoni (2011) pointed out the addition of propylene glycol alginate can significantly increase the specific volume and decrease the firmness.

In conclusion, the substitution level of BW into wheat flour can be increased up to 15 % resulting in a reduced predicted glycaemic response which may confer significant health benefits to the consumer. This research indicates that buckwheat flour can be used as a functional ingredient in food industry with many health benefits for consumer.

Chapter 6

Effect of oat bran on the dough rheology and quality of Chinese steamed bread

6.1 Introduction

Oat bran (OB) is a low-cost by-product produced during oat milling, which is a good source of dietary fibre (Zhang, Bai, & Zhang, 2011). The main dietary fibre component of oat bran is β -glucan, which is a natural polymer composed of the glucose molecules joined by β -(1-3) and β -(1-4) glycoside bonds (Butt, Tahir-Nadeem, Khan, et al., 2008; Chatuevedi et al., 2011). As a water-soluble fibre, β -glucan can easily form the viscous solutions, thus slows the intestinal transit, delays gastric emptying and slows glucose and sterol absorption in the intestine. Oat bran β -glucan has outstanding functional and nutritional properties due to its viscosity properties (Katongole, 2012). Previous studies have illustrated oat bran β -glucan has many beneficial effects, such as attenuation of postprandial blood glucose, reduction in insulin responses and a decrease in serum LDL cholesterol levels (El Khoury et al., 2011; Wood, 2010). Additionally, oat bran has a potential role for disease prevention and control such as coronary heart disease, diabetes and obesity (Cavazos & Gonzalez de Mejia, 2013; Hasler, 1998).

Currently, consumers have a growing awareness of the link between diet and nutrition, thus there has been an increased demand for healthier products with a consequent rise in interest in functional and nutritional items by the food industry (Brennan et al., 2008; Chareonthaikij et al., 2016; Grigor et al., 2016; McGill & Devareddy, 2015). As a functional ingredient, dietary fibre has been proven to have many health beneficial effects and a potential role for disease prevention (Aune et al., 2011; Dhingra et al., 2012; Elleuch et al., 2011; Threapleton et al., 2013). Therefore, the current major goal of scientists is to incorporate the cereal brans into cereal-based and other food products in order to increase the intake of dietary fibre. Oat bran is an important source of dietary fibre and common ingredient added to food products, especially

bread. Most of studies in this area focus on western bread, due to the addition of bran is detrimental to the baking performance (Campbell et al., 2008). Previous research has reported that the addition of OB led to higher water absorption, sticker dough and lower loaf volume (Campbell et al., 2008; Krishnan, Chang, & Brown, 1987). However, there is a paucity of information regarding the effect of OB on the quality of the CSB.

Chinese steamed bread (CSB), also known as mantou, is a traditional fermented food and widely consumed as a staple food in China (Ma et al. 2014). Currently, steamed bread has widely spread to other Asian countries, North America and some European countries, and accounted for around 15 % of the wheat consumption (Wu et al., 2012). The main ingredients of CSB include wheat flour, yeast and water with processing by steam cooking. Due to the differences in processing, the properties of CSB and western style baked bread are significantly different. For instance, the flavours of CSB are formed during the fermentation, while the flavours of western style breads are derived during baking by Maillard reaction (Lin et al., 2012). In addition, the lower processing temperature (100 °C) may result in the greater retention of nutrients in CSB (Zhu et al., 2016).

Hence, the present study investigated the effects of incorporating oat bran (OB) into Chinese steamed bread (CSB). Different levels (0 %, 5 %, 10 % and 15 %) of OB were added into wheat flour. The physical quality of dough was measured as moisture and textural properties of the dough. Quality of CSB was analysed from two perspectives; physical properties and nutritional quality. For physical properties, specific volume, loaf height, moisture, and texture were measured by AACC methods. The nutritional quality of the bread was analysed using the glycaemic response determined by an *in vitro* digestion method.

6.2 Materials and Methods

6.2.1 Materials

Ingredients used for this chapter were described in 3.1.

Table 6.1 Nutrition information for wheat flour and oat bran

Per 100g	Wheat flour (g)	Oat bran (g)
Protein	11.0	10.5
Fat, total	1.4	9.9
- saturated	<1	2.0
Carbohydrate	77.3	44.3
- sugars	<1	2.9
Dietary fibre	3.1	10.0
Sodium	0.005	0.003

Supplied by Sun Valley Foods Ltd and Champion Flour Milling Ltd.

6.2.2 Chemicals and reagents

Chemicals and reagents used for analysis were described in 3.3.

6.2.3 Dough rheological analysis

Rheological properties of CSB dough were determined according to the method described in 3.4.

6.2.4 Preparation of Chinese steamed bread with oat bran

Chinese steamed breads samples were prepared as described in 3.5.

6.2.5 Moisture content analysis

Moisture content of dough and CSB samples were determined according to AACC standard method described in 3.6.2.

6.2.6 Physical properties of CSB incorporated with oat bran

All CSB samples were assessed for their physical characteristics specific volume and texture as described in 3.6.

6.2.7 Total starch analysis

Total starch of CSB was performed as described in 3.7.1.

6.2.8 Glycaemic response analysis

Glycaemic response of CSB was measured using *in vitro* digestion method described by Brennan et al. (2013) as outlined in 3.8.

6.2.9 Statistical analysis

One-way ANOVA was used to compare the characteristics of each sample as described in 3.9.1.

6.3 Results and discussion

6.3.1 Rheological properties of dough incorporated with OB

Table 6.2 The rheology of dough with oat bran incorporated

Sample	WA (%)	Stability (min)	Development time (min)	Softening (FU)	Departure time (min)	MTI (FU)
Wheat flour	63.67 ± 0.11 ^D	11.17 ± 0.47 ^C	2.73 ± 0.06 ^C	41.46 ± 4.11 ^A	12.72 ± 0.85 ^B	10.51 ± 1.10 ^B
Wheat flour+5%OB	64.73 ± 0.11 ^C	14.70 ± 0.60 ^A	7.73 ± 0.21 ^B	37.83 ± 1.34 ^A	16.60 ± 0.53 ^A	21.83 ± 1.27 ^A
Wheat flour+10%OB	66.30 ± 0.17 ^B	12.73 ± 0.11 ^B	9.26 ± 0.31 ^A	37.90 ± 1.04 ^A	16.93 ± 0.11 ^A	20.06 ± 0.25 ^A
Wheat flour+15%OB	67.77 ± 0.45 ^A	10.50 ± 0.36 ^C	9.50 ± 0.20 ^A	38.63 ± 0.38 ^A	16.13 ± 0.06 ^A	22.20 ± 0.91 ^A

Means ± standard deviations (n=3). Values in the same column with different letters differ significantly ($p < 0.05$). OB-oat bran, WA-water absorption, MTI-mixing tolerant index.

The effects of OB on the rheological properties of CSB dough are presented in Table 6.2. In terms of water absorption, there was a significant ($p < 0.05$) increase from 63.67 to 67.77 % with the addition of OB levels increased from 0 to 15 %. A similar result was observed by Rieder et al. (2012) who illustrated that substitution of wheat with OB significantly increased

the WA due to the high fibre content. Dhinda, Prakash, and Dasappa (2012) illustrated the addition of OB significantly increased the WA of wheat flour dough because of the high hydration property of dietary fibre. These observations can be attributed to the large number of hydroxyl groups in the structure of dietary fibre, which can interact with the hydrogen bonds of water (Robertson et al., 2000; Rosell et al., 2006). The development time is the time taken for mixing the dough to reach the peak consistency (500 FU). The addition of OB increased the development time from 2.73 to 9.5 min, whereas there is no significant difference between 10 % and 15 % OB. This observation is consistent with Rieder et al. (2012), who pointed out the inclusion of OB led to an increase in development time due to high water binding capacity of dietary fibre and disturbance of gluten network by dietary fibre. Krishnan et al. (1987) have already illustrated that the addition of 10 % and 15 % oat bran to wheat flour can increase the dough development time. With regards to MTI and departure time, dough incorporated with oat bran has higher values than wheat flour dough. This effect could be related to the high content of soluble fibre in oat bran. Similar effects on MTI and departure time were observed by Sudha et al. (2007) who reported that the addition of oat bran significantly increased the MTI. The interesting results in dough stability is presented in Table 6.2. The highest stability value (14.70 min) was observed when the substitution level of 5 % wheat bran. However, there was a significant decrease in the dough stability after the addition of 5 % oat bran. This observation is consistent with the research of Gómez, Jiménez, Ruiz, & Oliete (2011) where the stability increased as the addition levels of bran increased from 0 to 2.5 %, and stability values decreased with levels of inclusion higher than 5 %.

6.3.2 Extensibility and stickiness of dough

Table 6.3 Extensibility and stickiness of dough with oat bran incorporated

Dough samples	Stickiness (g)	Cohesiveness (mm)	Extension (g)	Extensibility (mm)
Control	46.22 ± 1.94 ^C	4.46 ± 0.72 ^A	38.99 ± 4.78 ^A	37.06 ± 0.51 ^A
Dough+5%OB	48.63 ± 0.78 ^{BC}	2.73 ± 0.14 ^B	37.80 ± 0.28 ^A	17.35 ± 0.28 ^B
Dough+10%OB	51.26 ± 0.31 ^B	2.63 ± 0.03 ^B	37.51 ± 1.17 ^A	14.36 ± 0.23 ^C
Dough+15%OB	54.87 ± 0.53 ^A	3.12 ± 0.21 ^B	21.55 ± 0.24 ^B	11.27 ± 0.16 ^D

Means ± standard deviations (n=3). Values in the same column with different letters differ significantly ($p < 0.05$). OB-oat bran.

The effect of oat bran on the dough extensibility and stickiness are presented in Table 6.3. The extensibility decreased significantly ($p < 0.05$) from 37.06 to 11.27 mm when the substitution of oat bran increased from 0 to 15 %. This effect could be related to the disruption of the starch-gluten network by oat bran. Similar results were observed by Rieder et al. (2012) who illustrated the addition of oat bran significantly decreased the extensibility and resistance to extension due to dilution of wheat gluten. In addition, Liu et al. (2017a) pointed out the addition of bran reduced the dough extensibility owing to the interactions between fibre and wheat gluten. For the extension, a decrease was observed as the substitution of 15 % oat bran, although there was no significant difference between 5 % and 10 %. Sudha, Rajeswari, and Venkateswara Rao (2012) and Skendi et al. (2010) both illustrated that the addition of bran negatively affects the gluten network due to the disruption of starch-gluten matrix by bran. The effect could also be related to the percentage of bran added, the bran source, and particle size (Pieter J. Jacobs et al., 2015; Le Bleis et al., 2015).

Dough stickiness increased significantly ($p < 0.05$) from 46.22 to 54.87 g when the oat bran substitution increased from 0 to 15 %. Campbell et al. (2008) reported that the addition of oat bran increased the dough stickiness due to the higher content of soluble fibre (β -glucan). This effect could be attributed to the unbound water in β -glucan and starch-gluten system. The excess

water in the β -glucan and wheat gluten dough that is not bound by proteins and this seems to be responsible for the increased stickiness (Ahmed & Thomas, 2015).

6.3.3 The physical and textural properties of steamed bread

Table 6.4 The physical properties of CSB with oat bran incorporated

CSB samples	Loaf height (mm)	Specific volume (mL/g)	Moisture (g water/100 g)
Control	62.14 \pm 0.38 ^A	2.47 \pm 0.03 ^A	40.10 \pm 0.01 ^D
CSB+5%OB	54.03 \pm 0.52 ^B	2.17 \pm 0.01 ^B	44.88 \pm 0.07 ^C
CSB+10%OB	50.29 \pm 0.16 ^C	2.05 \pm 0.01 ^C	45.14 \pm 0.09 ^B
CSB+15%OB	41.08 \pm 0.56 ^D	1.80 \pm 0.01 ^D	45.55 \pm 0.16 ^A

Means \pm standard deviations (n=3). Values in the same column with different letters differ significantly ($p < 0.05$). CSB-Chinese steamed bread, OB-oat bran.

Table 6.4 shows that the addition of oat bran to CSB influenced the physical properties of CSB from the specific volume, loaf height and moisture. In terms of specific volume and loaf height, a significant ($p < 0.05$) decrease was observed as the substitution of oat bran increased from 0 to 15 %. These observations are consistent with Rieder et al. (2012) who reported that the addition of wheat and oat bran led to a significant decrease in bread volume compared to bread made with wheat flour. Additionally, Liu et al. (2017a) illustrated that the addition of wheat bran resulted in poor quality of CSB, such as a reduction of specific volume and loaf height. These observations can be attributed to the dilution of gluten in wheat flour-based dough and disrupting the hydration of gluten (Dhinda et al., 2012; Lin et al., 2012; Liu et al., 2017a). The moisture of CSB significantly increased when the substitution of oat bran increased due to the water holding capacity of dietary fibre (Güler-Akın, Ferliarslan, & Akın, 2016; Sette, Calvache, Soria, Pla, & Gerschenson, 2016).

Table 6.5 The textural properties of CSB with oat bran incorporated

Samples	Hardness (g)	Springiness (ratio)	Cohesiveness (ratio)	Gumminess (g)	Chewiness (g)	Resilience (ratio)
Control	228.24 ± 25.92 ^D	0.94 ± 0.01 ^B	0.88 ± 0.01 ^A	191.75 ± 19.15 ^D	179.83 ± 19.34 ^D	0.57 ± 0.01 ^A
CSB+5%OB	326.19 ± 6.26 ^C	0.98 ± 0.01 ^A	0.88 ± 0.01 ^A	288.15 ± 2.22 ^C	279.91 ± 8.98 ^C	0.59 ± 0.01 ^A
CSB+10%OB	425.81 ± 13.19 ^B	0.98 ± 0.01 ^A	0.88 ± 0.01 ^A	370.83 ± 5.98 ^B	365.85 ± 10.70 ^B	0.59 ± 0.01 ^A
CSB+15%OB	521.12 ± 10.79 ^A	0.98 ± 0.01 ^A	0.85 ± 0.01 ^B	469.59 ± 15.80 ^A	446.98 ± 19.76 ^A	0.56 ± 0.01 ^B

Means ± standard deviations (n=3). Values in the same column with different letters differ significantly ($p < 0.05$). CSB-Chinese steamed bread, OB-oat bran.

The influence of OB on the textural properties of CSB from hardness, springiness, cohesiveness, gumminess, chewiness and resilience are presented in Table 6.5. There was a significant ($p < 0.05$) increase in hardness, gumminess and chewiness as the substitution of OB increased from 0 to 15 %. Compared to control CSB, a slight increase was observed among 5, 10 and 15 %, whereas there was no significant difference. In terms of cohesiveness and resilience, only CSB incorporated with 15 % OB had lower values than control CSB, while no significant differences were observed among 0, 5 and 10 %. Similar trend was observed by Liu et al. (2017a), who illustrated that the addition of bran increased the hardness, gumminess and chewiness of CSB, whereas reduced the springiness and cohesiveness. In addition, Rieder et al. (2012) reported that bread incorporated with oat bran had a higher crumb firmness than wheat bread owing to the β -glucan content and molecular weight. Previous research pointed out the oat bran had a higher molecular weight of β -glucan than oat flour, which may disrupt the reaction between starch and protein in wheat flour, resulting in the CSB being hard and less springy (Åman, Rimsten, & Andersson, 2004; Kerckhoffs, Hornstra, & Mensink, 2003; Lin et al., 2012; W. Liu et al., 2016, 2017a).

6.3.4 Predicted glycaemic impact of CSB incorporated with OB

The total starch content of CSB with OB is presented in Table 6.6. There was a decrease in total starch content when the substitution of OB increased from 0 to 15 %. Compared to the control, CSB incorporated with 15 % OB had a lower starch content (37.52 %). The values of reducing sugars released during the *in vitro* digestion of all CSB samples over 120 min is shown in Figure 6.1. The results give a good indication of the rate of starch degradation and allow the calculation of a predictive glycaemic index of pasta samples. From the results, there was a trend to reduce the starch degradation as the substitution level of OB increased. Similar results were observed by Foschia et al. (2015) and Lindström et al. (2015) who pointed out food products containing oat bran can reduce the postprandial blood glucose response. The area under the glucose release curve is a calculation of predicted glycaemic response for 2 h after food consumption (Monro, 2002; Monro et al., 2010b; Monro & Shaw, 2008). Table 6.6 shows that the addition of OB decreased the AUC values and CSB substituted with 15 % OB had the lowest value (344.61). This effect can be related to β -glucan, as a water-soluble fibre, which can easily form the viscous solutions, thus slows the intestinal transit, delays gastric emptying and slows glucose and sterol absorption in the intestine (AbuMweis, Thandapilly, Storsley, & Ames, 2016; Pérez-Quirce, Lazaridou, Biliaderis, & Ronda, 2017). In addition, Foschia et al. (2015) has found that dietary fibre can combine with proteins and form a matrix barrier surrounding the starch granules to reduce the enzymes activity.

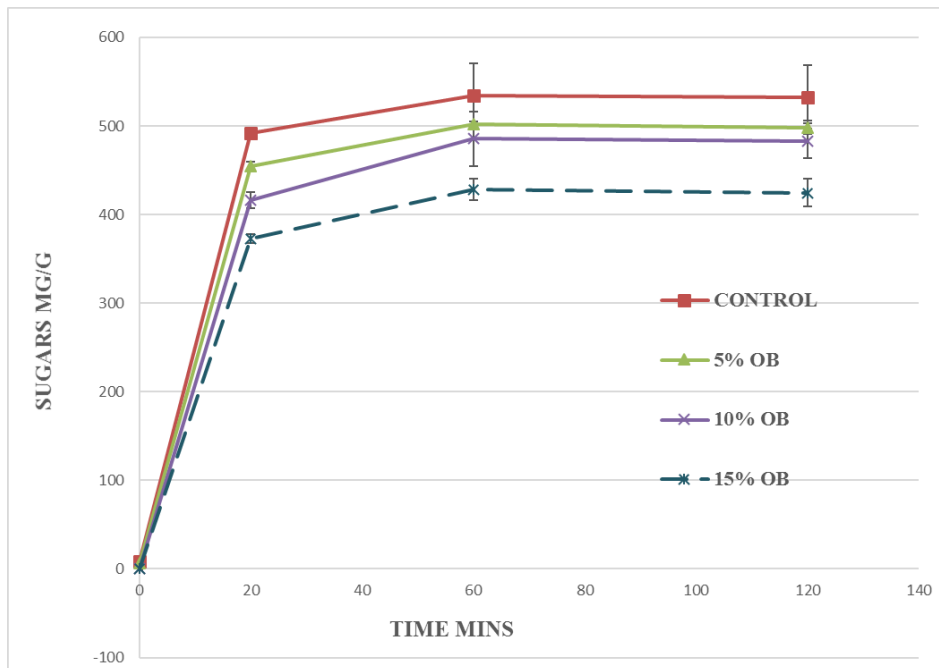


Figure 6.1 Reducing sugar released during *in vitro* digestion of CSB with oat bran inclusion
OB-oat bran, CSB-Chinese steamed bread

Table 6.6 Total starch content and predicting glycaemic response (AUC) of CSB with oat bran

Samples	Total starch (%)	AUC values
Control	43.82 ± 1.30 ^A	431.51 ± 31.80 ^A
CSB+5%OB	40.65 ± 0.05 ^B	399.13 ± 3.31 ^{AB}
CSB+10%OB	39.06 ± 0.38 ^{BC}	383.29 ± 17.40 ^{BC}
CSB+15%OB	37.52 ± 0.26 ^C	344.61 ± 2.81 ^C

Means ± standard deviations (n=3). Values in the same column with different letters differ significantly ($p < 0.05$). CSB-Chinese steamed bread, OB-oat bran.

6.4 Conclusion

This study illustrates that addition of OB into wheat flour can influence the dough rheology and final quality of Chinese steamed bread due to the high hydration properties of dietary fibre and the disruption of the starch-gluten network by dietary fibre. For instance, the addition of OB significantly increased water absorption, development time and stickiness, whereas decreased extensibility. In consequence, the specific volume of Chinese steamed bread decreased significantly, whereas hardness, gumminess and chewiness increased significantly as the substitution increased from 0 to 15 %. In addition, the addition of oat bran into Chinese steamed bread led to a reduction of predicted glycaemic response. Because as a water-soluble fibre, oat bran β -glucan can easily form the viscous solutions, thus slows the intestinal transit, delays gastric emptying and slows glucose and sterol absorption in the intestine.

In general, the substitution level of OB into wheat flour can be increased up to 15 % resulting in a reduced predicted glycaemic response which may confer significant health benefits to the consumer. This research indicates that oat bran can be used as a functional ingredient in food industry with many health benefits for consumer.

Chapter 7

Effect of α -amylase, xylanase and cellulase combinations on the rheological properties of Chinese steamed bread dough enriched in wheat bran

- This part of research has been published in Food Chemistry (Liu, W., Brennan, M. A., Serventi, L., & Brennan, C. S. (2017). Effect of cellulase, xylanase and α -amylase combinations on the rheological properties of Chinese steamed bread dough enriched in wheat bran. *Food chemistry*, 234, 93-102.)

7.1 Introduction

Dough rheology, the science of deformation and flow of dough, is concerned with the complex interrelationship of different flours and additives (reducing agents, oxidizing agents, enzymes, emulsifiers, sugar and salt) that govern the flow and deformation of dough systems under external forces. In the bakery industry, a better understanding of the rheological properties of flour dough during processing is significant, due to the relationships between those properties and quality attributes of the final products (Peressini et al., 2016). Several types of instruments have been employed to characterize the rheology of cereal products, such as Farinograph, DoughLAB, Alveograph, Extensograph and Texture Analyzer (Chinma et al., 2015; Li et al., 2016). In particular, the doughLAB is an evolution of the current flour analysis equipment, which provides enhanced functions compared with common analysis with its higher speed and higher torque capabilities. According to these measurements, the rheological characteristics of dough are described as mixing time, mixing tolerance, stickiness, extensibility and resistance to extension (Janssen, Van Vliet, & Vereijken, 1996; Lazaridou et al., 2007).

In recent years, consumers have a growing awareness of the link between diet and nutrition, thus there has been an increased demand for healthier foods with a consequent rise in interest in functional and nutritional foods by the food industry (Brennan et al., 2008; Liu et al., 2016).

Previous studies by Brennan (2005) have illustrated that dietary fibre has many beneficial effects on human health, e.g. decrease in intestinal transit time, reduction of blood cholesterol levels (total and LDL), and reduction in glycaemic response and insulin levels. As a by-product derived from roller milling of wheat flour production, wheat bran has high dietary fibre content, which contains 44-50 % of fibre. However, the addition of wheat bran into bread dough generally results in poor rheological properties, poor baking performance, and poor texture properties of final products, such as reducing the extensibility, increasing the dough stickiness, reducing loaf volume, darkening the crumb, and increasing the firmness (Boita et al., 2016; Hemdane et al., 2016; Pieter J Jacobs, Bogaerts, Hemdane, Delcour, & Courtin, 2016; Veraverbeke & Delcour, 2002).

In order to maintain high quality in bread, enzymes are used as aids, being added to the flour during the baking process (Sanz Penella, Collar, & Haros, 2008). There are three main commercial enzymes preparations that are used in baking industry (Linko, Javanainen, & Linko, 1997). Fungal α -amylase is an enzyme with widespread application in food industry. The action of amylase is to catalyze the hydrolysis of α -1, 4-glycosidic linkages into starch molecules (amylose and amylopectin), at a lower rate, maltodextrins and oligosaccharides (Antonia Martínez-Anaya, 1996). Xylanase is a hydrolase, which can attack the AX backbone and break the glycosidic linkages in AX, resulting in changing the functional and physicochemical properties of AX (Hilhorst et al., 1999). Cellulase belongs to the glycoside hydrolase family, which can catalyze the hydrolysis of (1,4)-beta-D-glucosidic linkages in cellulose and other beta-D-glucans. Due to their particular action mechanism, these enzymes may produce positive effects during breadmaking, such as rheological behaviour of dough and the quality of final products (Caballero et al., 2007b). However, reports on the effects of enzymes combination, especially, the combination of cellulase, xylanase and α -amylase on the dough rheology are limited.

Hence, this research investigates the effect of α -amylase, xylanase and cellulase on the rheological properties of the bread dough with 15 % content of wheat bran compared to regular bread dough without wheat bran. The rheological properties of dough were carried out by DoughLAB and Texture Analyzer.

7.2 Materials and Methods

7.2.1 Materials

The ingredients for the bread dough were described in 3.1.

7.2.2 Rheological analysis of dough

Rheological properties of CSB dough treated with enzymes were determined according to the method described in 3.4.

7.2.3 Design of experiment

Two experimental designs were performed: the first one to evaluate the effect of single enzyme on the dough rheology, and the second one to investigate the effect of adding mixtures of enzymes on the dough rheology. Firstly, the effect of single enzyme on the rheological properties of regular dough and dough incorporated with 15 % of wheat bran as wheat bran dough was analyzed by analysis of variance (ANOVA). According to the manufacture recommendations of Novozymes and previous publications (Caballero, Gómez, & Rosell, 2007a; Caballero et al., 2007b; Serventi, Jensen, Skibsted, & Kidmose, 2016; Shafisoltani, Salehifar, & Hashemi, 2014), the dosage of the Cellulast BG, Fungamyl 2500 SG and Pentopan Mono BG was added with 35 ppm, 10 ppm and 70 ppm, respectively.

Second, in order to investigate the effect of mixtures of enzymes on the rheological properties of dough incorporated with 15 % of wheat bran, a full factorial 2^3 design of experiments with central point in triplicate was used to evaluate all single effects and second-order interactions

between factors. Generally, there are three factors (α -amylase, xylanase and cellulase) at two levels (-1, 1) resulted in 8 different combinations of experiments and the coded values per each level of each factor are presented in Table 7.2 (Haros, Ferrer, & Rosell, 2006). According to the estimated coefficients (β_i , β_{ij} & β_{ijk}), the theoretical response function (W) was calculated as following polynomial linear regression model:

$$W = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC + \beta_{123}ABC$$

Factors: A – α -amylase; B – xylanase; C – cellulase; AB – α -amylase*xylanase; AC – α -amylase*cellulase; BC – xylanase*cellulase; ABC – α -amylase*xylanase*cellulase.

W – The theoretical response variable; β_0 – The global mean; β_i – The regression coefficient corresponding to main factor; β_{ij} and β_{ijk} – The regression coefficient corresponding to the interactions.

This multiple linear regression model with three independent variables describes the rheological property of dough is related to the α -amylase, xylanase and cellulase.

7.2.4 Statistical analysis

All data were treated by ANOVA and factorial design analysis as described in 3.9.

7.3 Results and discussion

7.3.1 Effect of single enzyme on the rheological properties of dough

The effects of single enzyme inclusion on the rheological properties of wheat flour dough (regular) and dough replaced with 15 % wheat bran as wheat bran dough are presented in Table 7.1. Previous research has indicated that the addition of wheat bran can change the rheology of the dough due to the disruption of starch-gluten network and high hydration properties of dietary fibre (Penella, Collar, & Haros, 2008; Wang et al., 2002). As a result, Table 7.1 shows that the substitution level of wheat bran at 15 % results in a significant ($p < 0.05$) increase of water absorption, dough stickiness, development time, departure time and mixing tolerance

index (MTI). On the contrary, the dough with 15 % bran had lower extension and extensibility than the regular dough. In terms of dough stability and softening, no significant differences were observed between regular dough and dough with 15 % wheat bran.

With regards to the xylanase, the addition of xylanase increased water absorption, development time, stability, stickiness and MTI of wheat flour dough (regular) significantly ($p < 0.05$). The results also show xylanase addition increased ($p < 0.05$) the dough extensibility from 31.31 to 39.88 mm and decreased resistance to extension. Similar results were observed by Pescador-Piedra et al. (2009), the addition of xylanase increased the water absorption, stability and development time of wheat flour dough. Selinheimo, Kruus, Buchert, Hopia, and Autio (2006) indicated that the addition of xylanase to the wheat flour dough resulted in a significant increase in extensibility, due to the solubilization and degradation of pentosans. According to the scanning electron microscopic studies of Indrani, Prabhasankar, Rajiv, and Rao (2003), the addition of xylanase leads to a distortion of some starch granules with thinning of protein film. This change results in an increase in dough development time, stability and MTI. In comparison, the addition of xylanase to the dough with 15 % wheat bran did not significantly affect the water absorption, development time, stability and departure time of wheat bran dough. Significant increase in extensibility and stickiness of dough replacing with 15 % wheat bran is presented in Table 7.1. This observation is consistent with the study of Laurikainen, Härkönen, Autio, and Poutanen (1998) where xylanase addition had little influence on water absorption, development time and stability of dough with 5 % rye bran. Therefore, the addition of xylanase had great effects on the rheological properties of dough due to the disruption of gluten network (Bombara, Anon, & Pilosof, 1997; Indrani et al., 2003; Weegles & Hamer, 1992). Caballero et al. (2007a) illustrated xylanase can reduce the number of total pentosans associated with the gluten matrix and counteract the over-aggregation of gluten.

In terms of α -amylase, the results show that α -amylase addition significantly ($p < 0.05$) decreased the stability and resistance to extension of regular dough and increased the softening, MTI, extensibility and stickiness of regular dough. No significant differences in water absorption and development time was observed between regular dough and dough incorporated with α -amylase. The same result was observed by Kim et al. (2006) in that the addition of α -amylase to wheat flour significantly decreased the stability due to the dextrinization of starch by α -amylase. Previous research has illustrated that α -amylase can break down the damaged starch to dextrans and improve the handling properties of dough (Indrani et al., 2003). These changes can make the dough weaker and sticky (Shafisoltani et al., 2014). With respect to dough with 15 % wheat bran, the addition of α -amylase has no significant effect on mixing properties, except an increase in MTI. The trend of extension of wheat bran dough is consistent with the trend of regular dough. In the case of dough stickiness, no significant difference was observed when adding the α -amylase to wheat bran dough. The same results were proved by Penella et al. (2008), single fungal α -amylase addition did not show significant effects on development and stability of dough with wheat bran, whereas increased the MTI. Kim et al. (2006) also reported the α -amylase addition did not significantly affect the water absorption of dough substituted with polished flour.

Table 7.1 shows that the addition of cellulase significantly changed the rheological properties of regular dough in addition to water absorption. As a result, the cellulase addition significantly ($p < 0.05$) increased the development time, stability, departure time, MTI, extensibility and stickiness of regular dough, and decreased both softening and resistance to extension. These observations are similar to those reported by Lu, He, and Liu (2015), who pointed out development time increased when the addition of cellulase increased from 40 to 100 mg / kg and the dough has highest value of stability when the addition level was 80 mg / kg. However, the softening and extensibility of wheat flour dough reduced as the cellulase level increased

from 80 to 100 mg / kg. Harada, Lysenko, Edwards, and Preston (2005) also indicated that the mixing time of dough increased with the increasing content of cellulase from 0 to 50 ECU / 100 g. Regarding to the dough with 15 % wheat bran, the addition of cellulase increased the development time, whereas decreased water absorption and stability. Similar results were reported by Maeda and Morita (2000), the cellulase increased the development time of wheat flour substituted with polished flour, but decreased the stability.

7.3.2 Effect of enzymes combination on rheological properties of regular dough

The effects of α -amylase, xylanase and cellulase at two levels each, on the rheological properties of regular dough were analyzed using 2^3 full factorial design (Table 7.2), and analytical results obtained from the factorial design in dough rheology are presented in Table 7.3. It can be seen that any enzyme and interaction of the enzymes significantly ($p < 0.05$) affects the water absorption of regular dough in Table 7.3 and Figure 7.1. From Table 7.3, the α -amylase had a quadratic positive effect on the water absorption of regular dough, and the interaction of α -amylase and xylanase also shown a significantly synergistic effect ($R^2 = 0.98$). In addition, the final empirical model for the water absorption follows:

$$W (\text{WA } \%) = 61.02 + 0.22A - 0.16B - 0.71C + 0.12AB - 0.83AC - 0.11BC \quad (R^2 = 0.98)$$

Factors: A – α -amylase; B – xylanase; C – cellulase; AB – α -amylase*xylanase; AC – α -amylase*cellulase; BC – xylanase*cellulase.

Table 7.1 Effect of single enzyme application on the rheology of regular dough and dough incorporated with 15 % wheat bran

	No enzyme		Xylanase		Amylase		Cellulase	
	regular	wheat bran	regular	wheat bran	regular	wheat bran	regular	wheat bran
WA %	63.86 ± 0.12 ^D	71.43 ± 0.21 ^A	65.20 ± 0.10 ^C	71.50 ± 0.36 ^A	63.93 ± 0.15 ^D	70.16 ± 0.88 ^{AB}	63.20 ± 0.70 ^D	70.73 ± 0.15 ^B
Development time (min)	2.73 ± 0.06 ^D	9.23 ± 0.73 ^{BC}	8.60 ± 0.61 ^C	9.73 ± 0.68 ^{ABC}	2.80 ± 0.10 ^D	10.13 ± 0.55 ^{AB}	8.76 ± 0.45 ^{BC}	10.86 ± 0.31 ^A
Stability (min)	11.07 ± 0.55 ^C	11.06 ± 0.06 ^C	18.03 ± 0.06 ^A	10.23 ± 0.78 ^C	8.67 ± 0.51 ^D	11.20 ± 0.20 ^C	16.63 ± 0.55 ^B	10.76 ± 0.06 ^C
Softening (FU)	43.13 ± 1.63 ^{BC}	38.56 ± 1.46 ^{CD}	23.40 ± 1.75 ^F	45.76 ± 3.78 ^B	60.30 ± 1.48 ^A	40.03 ± 3.28 ^{BCD}	31.00 ± 2.46 ^E	36.63 ± 0.55 ^{DE}
Departure time (min)	12.67 ± 0.91 ^E	17.27 ± 0.23 ^{BCD}	20.00 ± 0.01 ^A	16.33 ± 0.38 ^D	10.50 ± 0.46 ^F	17.10 ± 0.46 ^{CD}	18.56 ± 0.66 ^B	17.86 ± 0.35 ^{BC}
MTI (FU)	10.37 ± 0.92 ^E	17.76 ± 1.85 ^{BCD}	15.73 ± 1.16 ^D	21.73 ± 1.13 ^A	20.80 ± 0.20 ^{AB}	22.03 ± 1.53 ^A	16.63 ± 1.56 ^{CD}	20.36 ± 1.51 ^{ABC}
Resistance to extension (g)	44.59 ± 0.68 ^A	34.14 ± 0.28 ^B	25.73 ± 1.05 ^D	28.89 ± 0.66 ^C	35.37 ± 0.52 ^B	25.62 ± 0.50 ^D	19.96 ± 0.22 ^F	23.53 ± 0.15 ^E
Extensibility (mm)	31.31 ± 0.23 ^D	20.73 ± 0.17 ^G	39.88 ± 0.78 ^C	21.76 ± 0.26 ^F	45.27 ± 0.53 ^A	23.59 ± 0.32 ^E	43.53 ± 0.40 ^B	19.84 ± 0.19 ^G
Stickiness (g)	44.62 ± 0.36 ^F	46.62 ± 0.95 ^E	66.72 ± 0.88 ^A	51.58 ± 0.41 ^D	58.43 ± 0.32 ^B	45.38 ± 0.06 ^{EF}	53.86 ± 0.32 ^C	46.39 ± 0.25 ^E

Means ± standard deviations (n=3). Values in the same row with different letters differ significantly ($p < 0.05$). WA %-water absorption, MTI-mixing tolerance index.

The positive synergistic effects meant that water absorption of regular dough increased with an increase of these factors (α -amylase and α -amylase*xylanase). Whereas, the factors i.e. xylanase, cellulase, α -amylase*cellulase and xylanase*cellulase had a negative effect on the water absorption of the regular dough. Figure 7.1 clearly shows that the water absorption decreased with the level of xylanase and cellulase increasing. Compared to the positive effect of single enzyme (Table 7.1), the combination of enzymes decreased the water absorption by 59.6 % (Table 7.2) when the enzymes were added with the level (10, 120 and 60 ppm). As a result, the dough had the minimum value of water absorption when xylanase and cellulase were added with the highest level in the combination situation. A similar result was observed by Pescador-Piedra et al. (2009), the addition of glucose oxidase, peroxidase and xylanase combination has lower water absorption than the addition of single enzyme. No studies have explained the effects of α -amylase, xylanase and cellulase on the water absorption. From these results, the decrease in water absorption may be attributed to the antagonistic effect of xylanase and cellulase against the positive effect of single α -amylase. Song et al. (2016) have evaluated the synergistic effect of xylanase and cellulase on the degradation of lignocellulosic substrates. On the other hand, these observations could be related to the synergetic mechanism of xylanase and α -amylase. Hemalatha, Leelavathi, Salimath, and Rao (2014) found that the combination of α -amylase and xylanase decreased soluble starch and soluble amylose contents and resulted in low moisture content.

In terms of development time (DT), both α -amylase and xylanase had an antagonistic effect on the dough development time. It is clearly shown in Figure 7.1 that the development time decreased when the dosage of amylase increased, and there was no effects of xylanase or cellulase addition on the development time. Shafisoltani et al. (2014) found that the combination of glucose oxidase and xylanase had a significant effect on development time of dough, and lower dosage of xylanase resulted in a longer development time. According to

Pescador-Piedra et al. (2009), who pointed out the mixing time increased when the xylanase, glucose oxidase and peroxidase were added separately, whereas decreased significantly as adding the enzymes combination. These results are similar to our results, which show that the addition of single enzyme increased the dough development time, while the α -amylase and xylanase had a negative effect when they were combined in the dough. This observation could be due to the presence of endogenous inhibitors in wheat flour (De Gobba, Olsen, & Skibsted, 2016). Sancho et al. (2003) and Juge, Payan, and Williamson (2004) also illustrated that the cereal protein inhibitors had cross-inhibitory activity against α -amylase and xylanase.

For the stability, the α -amylase had a negative effect on the dough stability, whereas cellulase and α -amylase*cellulase had a positive effect. In addition, the dough with 10 ppm α -amylase has lower stability (8.67 min) than that of regular dough (Table 7.1). It can be seen from Figure 7.1 the stability of the regular dough decreased as the level of α -amylase increased from 6 to 10 ppm. On the contrary, the stability increased when the dosage of cellulase increased by 60 ppm. Table 7.2 shows that the value of dough stability is minimum (6.3) as adding the highest concentration of α -amylase (10 ppm) and the lowest concentration of cellulase (35 ppm). In contrast, the maximum value of stability was observed when the concentrations of α -amylase and cellulase were 6 ppm and 60 ppm, respectively. J. H. Kim et al. (2006) illustrated that the addition of single α -amylase resulted in a reduction of stability. According to Lu et al. (2015), the dough incorporated with high concentration cellulase had the high value of stability.

Table 7.2 and 7.3 shows the combination of α -amylase, xylanase and cellulase resulted in the dough softening. It can be seen that both α -amylase and xylanase had a positive effect on the dough softening. However, the negative effect of cellulase and interaction of enzymes on dough softening was presented in Table 7.3 and Figure 7.1. The result of single enzyme experiment shows that only the dough with 10 ppm α -amylase had a higher value of softening than regular dough, and dough with xylanase or cellulase had a lower value. Compared to the use of the single enzyme, the effect of enzymes combination on the dough softening was more effective.

According to Shafisoltani et al. (2014), the combination of enzymes led to an increase in dough softening. Eugenia Steffolani, Ribotta, Pérez, and León (2012) reported that the combination of α -amylase, xylanase and glucose oxidase had positive effect on dough softening. With regarding to MTI, the results show that the addition of single or combined enzymes increased significantly ($p < 0.05$) the MTI of dough. The maximum value (31.2) of MTI was observed when the concentration of α -amylase, xylanase and cellulase was 10, 70 and 35 ppm, respectively.

Resistance to extension (g) decreased significantly ($p < 0.05$) when enzymes were added alone or as combinations. In contrast, a significant increase in extensibility was observed (Table 7.2 and Figure 7.1) when adding single enzyme or combined enzymes. Similar results were reported by previous study, the single enzyme or combined enzymes decreased resistance to extension and increased extensibility, due to the modifications in starch and arabinoxylans fractions (Katina, Salmenkallio-Marttila, Partanen, Forssell, & Autio, 2006; Selinheimo et al., 2006).

Previous research has reported that dough with high stickiness leads to malleability problems during bread making, whereas low stickiness of dough can be dry and hard (Eugenia Steffolani et al., 2012). In order to maintain good performance, the bread dough should have an appropriate stickiness (Eugenia Steffolani et al., 2012). According to the results, the addition of single enzyme and combined enzymes resulted in dough stickier than the regular dough. Table 7.2 shows the dough has the minimum stickiness when the α -amylase, xylanase and cellulase was added with the lowest concentration (6, 70 and 35 ppm). Eugenia Steffolani et al. (2012) found that dough added with enzyme mixture (α -amylase, xylanase and glucose oxidase) had the intermediate stickiness.

Table 7.2 Effect of enzymes combination on regular dough and wheat bran dough rheology

Blocks	A	B	C	WA %	DT (min)	Stability (min)	Softening (FU)	Departure time(min)	MTI (FU)	Extension (g)	Extensibility (mm)	Stickiness (g)
regular	0	0	0	63.86	2.73	11.07	43.13	12.67	10.37	44.58	34.14	44.62
1	6	70	35	60.8	3.4	9.8	55.9	11.3	26.9	20.91	56.89	54.22
2	6	70	60	61.2	5.5	11	59.8	12.3	22	21.87	55.59	65.05
3	6	120	60	60.5	3.6	9.7	63	11.2	18.1	21.02	58.42	62.51
4	6	120	35	60.6	6.1	10.2	65.2	11.6	29.8	28.93	43.2	56.69
5	10	70	35	62.6	3.1	7	74.2	8.8	31.2	18.71	44.23	66.88
6	10	120	35	62.9	2.6	6.3	70.9	8.1	27	23.68	48.56	68.89
7	10	120	60	59.6	3.2	9.5	65.9	10.8	28.8	21.12	51.89	64.06
8	10	70	60	59.8	3.2	9	63.9	11	25.7	19.65	53.86	70.23
wheatbran	0	0	0	71.43	9.23	11.06	38.56	17.27	17.26	34.14	20.73	46.62
1	6	70	35	67.8	9.4	8.0	71.12	14	43.5	21.5	22.86	52.13
2	6	70	60	68.3	8.9	7.9	81.22	13.8	44.2	21.33	25.23	47.08
3	6	120	60	67.9	8.9	7.0	80.33	13.6	45.6	25.22	21.66	51.45
4	6	120	35	68.0	9.5	7.6	77.3	13.3	46.7	22.32	20.05	53.15
5	10	70	35	69.9	9.5	8.7	71.2	14.9	30.3	26.6	23.26	52.13
6	10	120	35	70.3	10.2	8.1	69.87	14.8	39.3	20.10	22.52	56.98
7	10	120	60	67.1	10.5	8.0	68.33	14.3	41.03	28.67	19.07	48.74
8	10	70	60	67.3	9.7	8.1	65.5	15.3	35.6	20.66	26.22	56.65

All values are means (n=3). A (factor) – α -amylase; B (factor) – xylanase; C (factor) – cellulase. Regular – wheat flour dough; Bran – wheat flour dough with 15 % wheat bran. WA %-water absorption, DT-development time, MTI-mixing tolerance index. (Data is obtained from Factorial Design, it is not ANOVA)

Table 7.3 Estimated coefficients of the factors of the rheological properties of regular dough

Coefficient estimate	WA%	DT (min)	Stability (min)	Softening (FU)	Departure time (min)	MTI (FU)	Extension (g)	Extensibility (mm)	Stickiness (g)
Constant (β_0)	61.02	3.88	9.03	66.2	10.51	26.98	21.95	51.61	63.72
Amylase (β_1)	0.22	-0.86	-1.08	4.39	-0.96	1.24	-1.08	-2.21	3.88
Xylanase (β_2)	-0.16	-0.05	NS	1.65	NS	NS	1.89	-0.92	-0.57
Cellulase (β_3)	-0.71	NS	0.66	-2.21	0.5	-3.9	-0.8	3.18	1.83
Amylase*Xylanase(β_{12})	0.12	NS	NS	-1.68	NS	NS	NS	1.25	-0.32
Amylase*Cellulase(β_{13})	-0.83	NS	0.49	-1.65	0.45	1.78	0.78	NS	-2.01
Xylanase*Cellulase(β_{23})	-0.11	-0.43	NS	NS	NS	NS	-1.46	0.67	-1.51
Amylase*Xylanase*Cellulase	NS	0.51	0.37	2.58	NS	2.82	0.63	-3.21	-0.55
R ²	98.43%	96.76%	98.08%	90.66%	88.93%	90.83%	98.69%	96.29%	99.73%

NS – no significant effect at level ($p < 0.05$); R² – adjusted square coefficient (describes the percentage of variability for which the model accounts); β_0 – global means of parameters; β_1 , β_2 and β_3 – regression coefficients corresponding to main factors; β_{12} , β_{13} , β_{23} and β_{123} – regression coefficients corresponding to interactions; ‘+’ – positive effect; ‘-’ – negative effect; WA %-water absorption, DT-development time, MTI-mixing tolerance index.

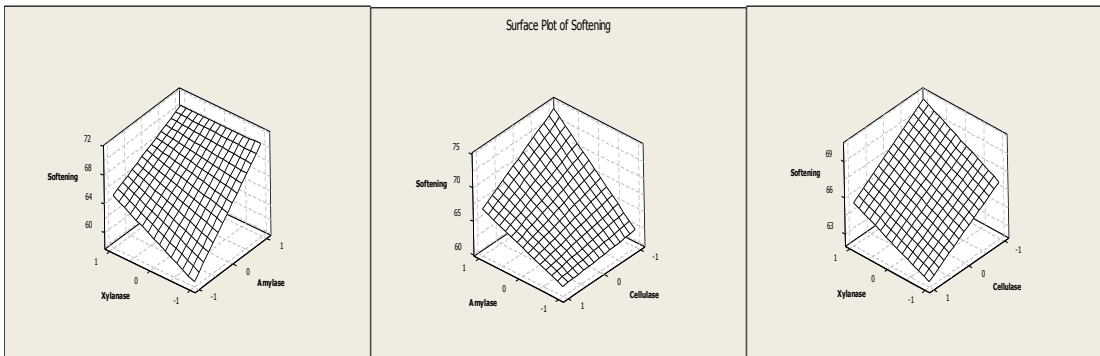
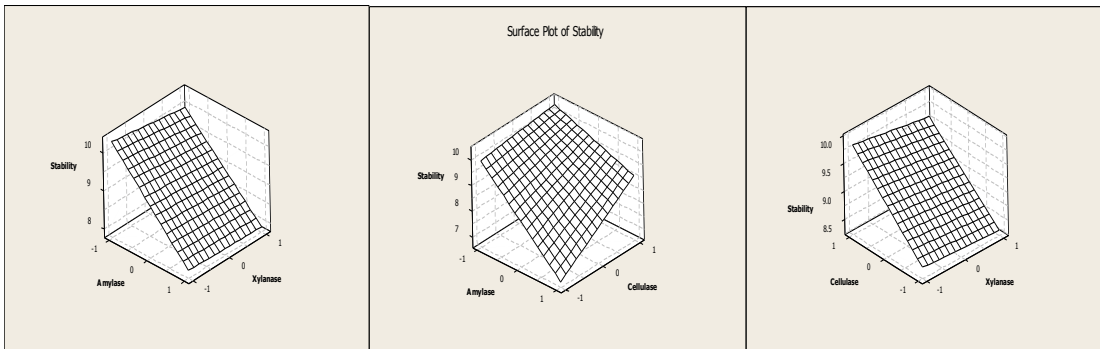
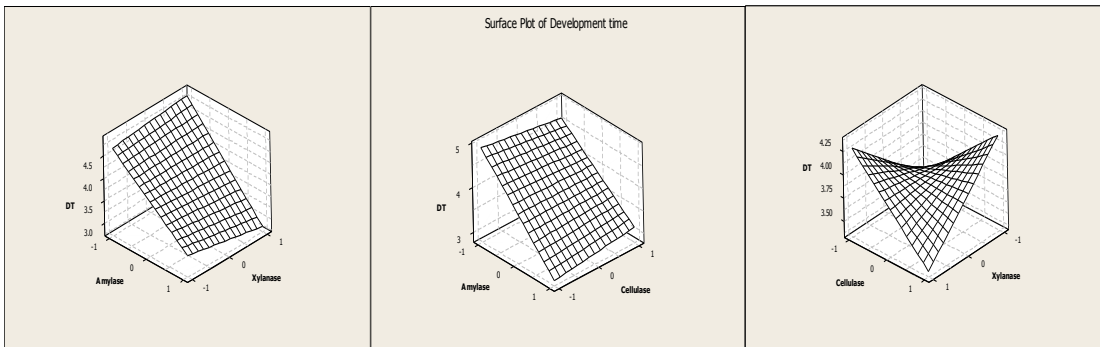
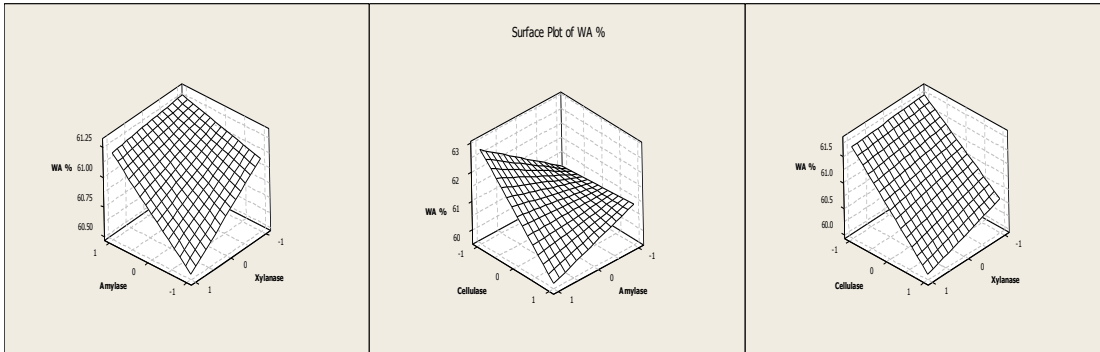
7.3.3 Effect of enzymes combination on rheological properties of dough with 15 % wheat bran

The effect of combined enzymes on rheology of dough with 15 % bran is presented in Table 7.2 and 7.4, and the interaction of enzymes is shown in Figure 7.2. It can be seen that addition of enzymes combination to the wheat bran dough has significant effects on the dough rheology. In comparison with single enzyme, the combinations are more effective in the dough incorporated with 15 % bran. There is limited research about the effect of combination enzymes on wheat bran dough rheology. However, several studies have pointed out the mixture enzymes had a beneficial effect on high-fibre baking due to the changes in cell wall polysaccharides of wheat flour. For instance, Laurikainen et al. (1998) and Katina et al. (2006) pointed out the enzyme mixture can improve the quality of bran bread due to the degradation of cell wall components and higher water absorption of bran resulting in altered the water distribution among starch, protein and bran particles.

Table 7.4 Estimated regression coefficients of the factors of the rheological properties of dough incorporated with wheat bran.

Coefficients	WA%	DT (min)	Stability (min)	Softening (FU)	Departure time (min)	MTI (FU)	Extension (g)	Extensibility (mm)	Stickiness (g)
Constant	68.31	9.64	7.95	72.2	14.27	40.38	23.46	22.69	52.30
Amylase	0.32	0.45	0.33	-5.28	0.56	-4.35	0.55	0.09	1.18
Xylanase	NS	NS	-0.25	NS	NS	2.51	0.65	-1.81	0.31
Cellulase	-0.7	NS	NS	NS	NS	NS	0.44	0.46	-1.38
Amylase*Xylanase	NS	NS	NS	NS	NS	NS	-0.22	-0.36	-1.04
Amylase*Cellulase	-0.75	0.21	NS	-3.93	NS	NS	NS	-0.33	0.36
Xylanase*Cellulase	-0.15	NS	NS	NS	NS	NS	2.38	-0.82	-1.12
Amylase*Xylanase*Cellulase	NS	NS	NS	NS	NS	NS	1.43	-0.65	-2.01
R ²	98.13%	80.76%	78.08%	80.66%	85.93%	80.3%	98.52%	96.04%	99.57%

NS – no significant effect at level ($p < 0.05$); R² – adjusted square coefficient (describes the percentage of variability for which the model accounts); β_0 – global means of parameters; β_1 , β_2 and β_3 – regression coefficients corresponding to main factors; β_{12} , β_{13} , β_{23} and β_{123} – regression coefficients corresponding to interactions; ‘+’ – positive effect; ‘-’ – negative effect; WA %-water absorption, DT-development time, MTI-mixing tolerance index.



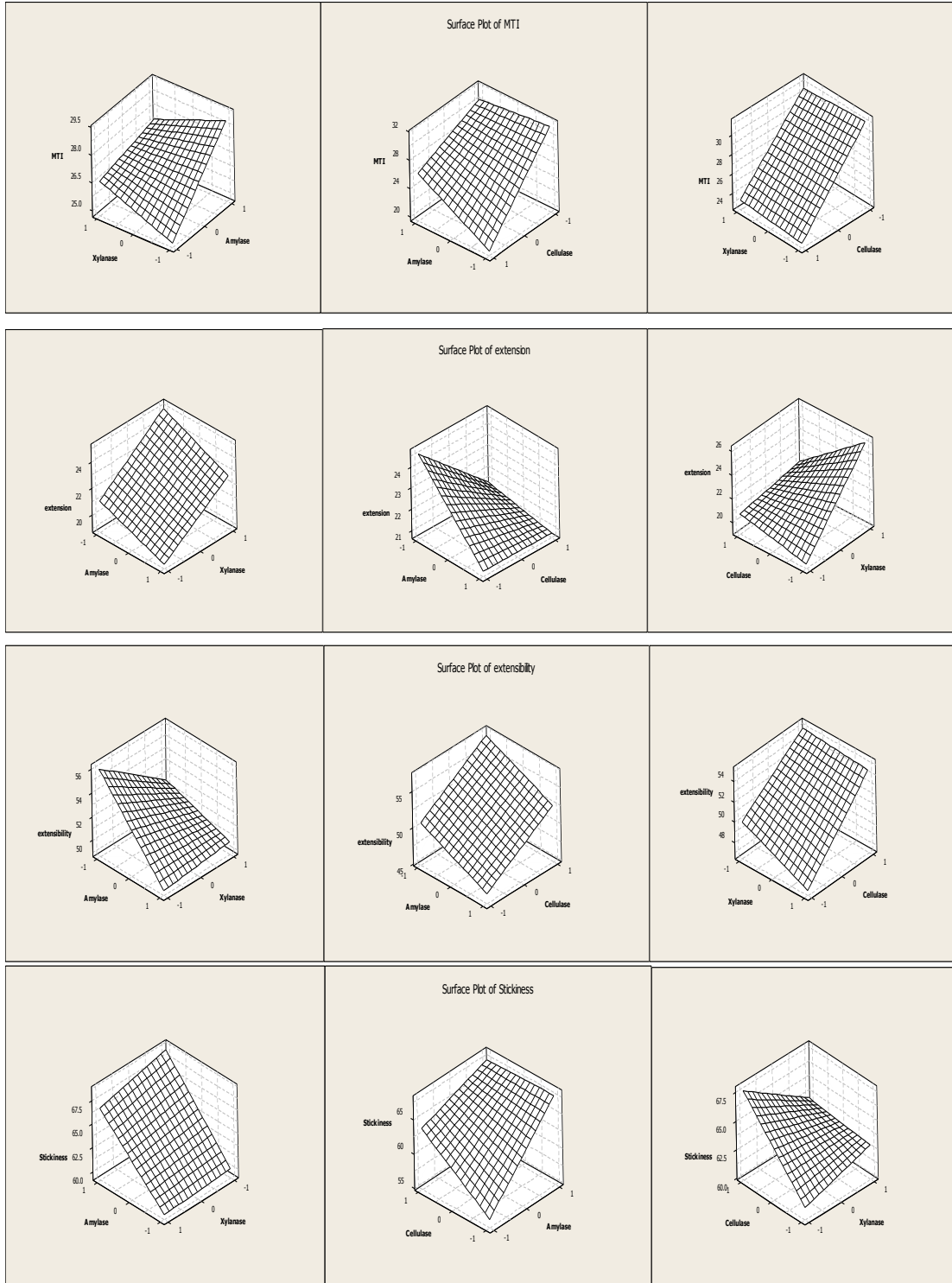
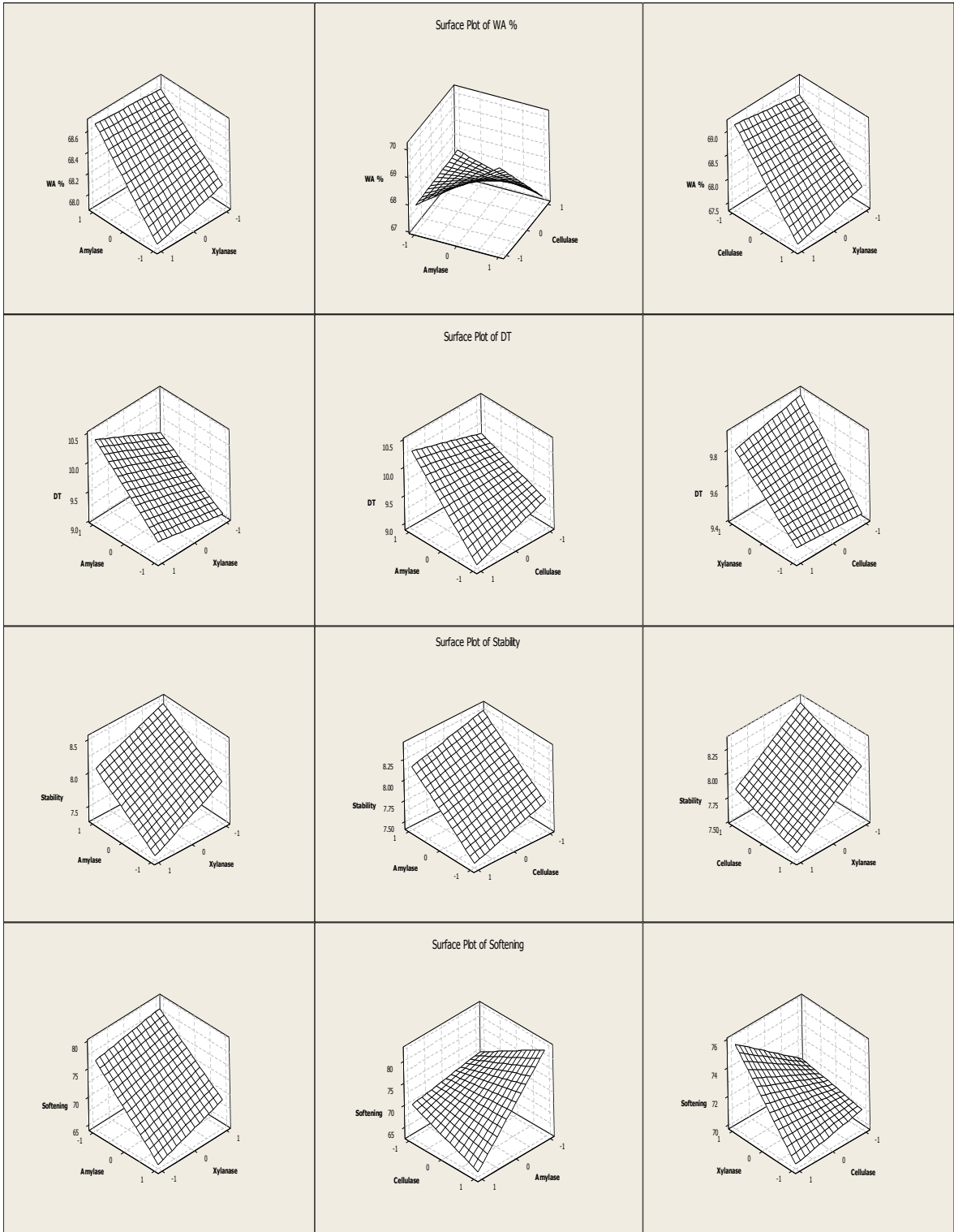


Figure 7.1 Response surface plots showing the effect of α -amylase, xylanase and cellulase on the rheology of regular dough
 Regular dough-wheat flour dough; WA %-water absorption; MTI-mixing tolerance index.



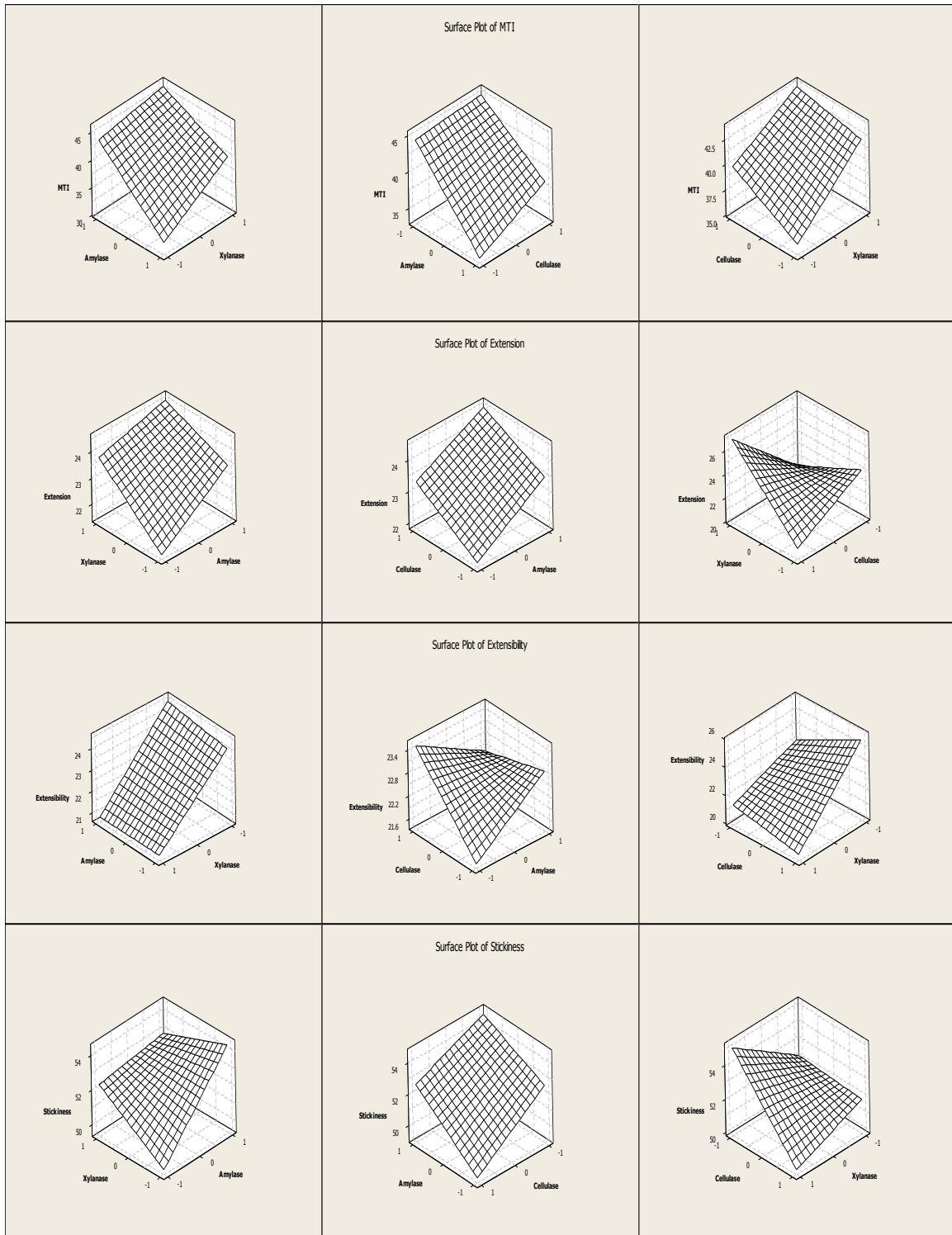


Figure 7.2 Response surface plots showing the effect of α -amylase, xylanase and cellulase on wheat bran dough rheology

Wheat bran dough-wheat flour dough with 15 % wheat bran inclusion; WA %-water absorption; MTI-mixing tolerance index. DT-development time.

7.4 Conclusion

The addition of α -amylase significantly ($p < 0.05$) decreased the stability and resistance to extension of regular dough and increased the softening, MTI, extensibility and stickiness of regular dough. Xylanase can increase water absorption, development time, stability, extensibility, stickiness and MTI of wheat flour dough significantly ($p < 0.05$). Cellulase addition significantly ($p < 0.05$) increased the development time, stability, departure time, MTI, extensibility and stickiness of regular dough, and decreased both softening and resistance to extension. Compared to the single enzyme, the blended enzymes can reduce the water absorption of the dough to the minimum value when the enzyme combination were added with the high concentrations (10, 120 and 60 ppm). Both single enzyme and enzyme combination can increase the extensibility, softening, MTI and stickiness, whereas decrease the resistance to extension. In particular, the combinations of α -amylase, xylanase and cellulase can increase the extensibility, softening, MTI and stickiness to higher value than the single enzyme.

In conclusion, the combination of α -amylase, xylanase and cellulase had a synergetic effect on the dough rheology due to the interactions among enzyme activities and their coupled reactions.

Chapter 8

Effect of α -amylase, xylanase and cellulase combinations on the rheological properties of Chinese steamed bread dough enriched in oat bran

8.1 Introduction

Over the past decade, there has been a rapidly increasing demand for healthier foods with a consequent rise in interest in functional and nutritional foods by the food industry. As a by-product of oat flour milling, oat bran is a good source of dietary fibre, which mainly contains water soluble β -glucan and can be incorporated into food products to alter the nutritional value of products. As a water-soluble fibre, β -glucan can easily form the viscous solutions, thus slows the intestinal transit, delays gastric emptying and slows glucose and sterol absorption in the intestine. Oat β -glucan has functional and nutritional properties due to its viscosity properties (Katongole, 2012). Previous studies have illustrated oat bran β -glucan has many beneficial effects, such as attenuation of postprandial blood glucose, reduction in insulin responses and a decrease in serum LDL cholesterol levels (El Khoury et al., 2011; Wood, 2010).

However, the addition of oat bran had a detrimental effect on the dough rheology and final food quality. For instance, Rieder et al. (2012) illustrated that the addition of oat bran increased the water absorption and dough development time, and decreased dough stability time and extensibility. Additionally, the research pointed that the substitution of wheat flour with oat bran significantly decreased the bread volume accompanied by an increase in crumb firmness (Rieder et al., 2012). Lee and Inglett (2006) also indicated that the addition of oat bran to cookies led to a decrease in dynamic viscoelastic properties of cookies dough and an increase in moisture content and hardness of cookies. According to the research of Dhinda et al. (2012), who reported that replacement of wheat flour with oat bran, chickpea flour and soy protein isolate decreased dough stability and quality of bread.

A better understanding of the rheological properties of flour dough during processing is required to understand the complexity of the relationships between those properties and quality attributes of the final products (Peressini et al., 2016). Enzymes such as α -amylase and xylanase have been widely used to improve the dough handling and breadmaking in the bakery industry (Butt, Tahir-Nadeem, Ahmad, & Sultan, 2008; Courtin & Delcour, 2002; Goesaert et al., 2005; Goesaert, Slade, Levine, & Delcour, 2009). *Alpha*-amylase is endo-acting amylase, which randomly hydrolyzes α -1, 4-glycosidic linkages into starch molecules (amylose and amylopectin), at a lower rate, maltodextrins and oligosaccharides (Antonia Martínez-Anaya, 1996). Xylanase is a hydrolase, which can attack the AX backbone and break the glycosidic linkages in AX, resulting in changing the functional and physicochemical properties of AX (Hilhorst et al., 1999). Celluloses are widely used for extraction and clarification of fruit and vegetable juices to increase the yield of juices (Kuhad et al., 2011). Cellulase belongs to the glycoside hydrolase family, which can catalyze the hydrolysis of (1, 4)-beta-D-glucosidic linkages in cellulose and other beta-D-glucans. However, the reports on the effects of enzymes combination, especially, the combination of cellulase, xylanase and α -amylase on the dough rheology are limited. Thus, this research investigates the effect of α -amylase, xylanase and cellulase on the rheological properties of the bread dough with 15 % content of wheat bran compared to regular bread dough without wheat bran. The rheological properties of dough were carried out by DoughLAB and Texture Analyser.

8.2 Materials and Methods

8.2.1 Materials

The ingredients for the bread dough were described in 3.1.

8.2.2 Rheological analysis of dough

Rheological properties of CSB dough treated with enzymes were determined according to the method described in 3.4.

8.2.3 Design of experiment

Two experimental designs were performed: the first one to evaluate the effect of single enzyme on the dough rheology, and the second one to investigate the effect of adding mixtures of enzymes on the dough rheology. Firstly, the effect of single enzyme on the rheological properties of regular dough and dough incorporated with 15 % of oat bran as oat bran dough was analyzed by analysis of variance (ANOVA). According to the manufacture recommendations of Novozymes and previous publications (Caballero et al., 2007a, 2007b; Serventi et al., 2016; Shafisoltani et al., 2014), the dosage of the Cellulast BG, Fungamyl 2500 SG and Pentopan Mono BG was added with 35 ppm, 10 ppm and 70 ppm, respectively.

Second, in order to investigate the effect of mixtures of enzymes on the rheological properties of dough incorporated with 15 % of oat bran, a full factorial 2^3 design of experiments with central point in triplicate was used to evaluate all single effects and second-order interactions between factors. Generally, there are three factors (α -amylase, xylanase and cellulase) at two levels (-1, 1) resulted in 8 different combinations of experiments (Haros, Ferrer, & Rosell, 2006). According to the estimated coefficients (β_i , β_{ij} & β_{ijk}), the theoretical response function (W) was calculated as following polynomial linear regression model:

$$W = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC + \beta_{123}ABC$$

Factors: A – α -amylase; B – xylanase; C – cellulase; AB – α -amylase*xylanase; AC – α -amylase*cellulase; BC – xylanase*cellulase; ABC – α -amylase*xylanase*cellulase.

W – The theoretical response variable; β_0 – The global mean; β_i – The regression coefficient corresponding to main factor; β_{ij} and β_{ijk} – The regression coefficient corresponding to the interactions.

This multiple linear regression model with three independent variables describes the rheological property of dough is related to the α -amylase, xylanase and cellulase.

8.2.4 Statistical analysis

All data were treated by ANOVA and factorial design analysis as described in 3.9.

8.3 Results and discussion

8.3.1 Effect of single enzyme on the rheological properties of dough

Individual effects of enzymes on the rheological properties of wheat flour dough (regular) and dough replacing with 15 % oat bran as oat bran dough are showed in Table 8.1. Compared to the regular dough, the oat bran dough has higher value of water absorption, development time, departure time and MTI accompanied by lower value of resistance to extension and extensibility. However, there was no significant difference in stability, softening and stickiness between regular dough and oat bran dough. According to previous research, the substitution of oat bran had a significant effect on the rheology of dough due to the disruption of starch-gluten network and high hydration properties of β -glucan (Lee & Inglett, 2006; Rieder et al., 2012). The effects of α -amylase, xylanase and cellulase on the rheology of regular dough have been described and discussed in 7.3.1.

This chapter focuses on how the single enzyme affects the rheological properties of dough replacing with 15 % oat bran. Table 8.1 shows that the addition of single enzyme influenced the rheology of oat bran dough. The addition of xylanase to oat bran dough did not significantly affect the water absorption, development time, stability, softening and departure time, whereas significantly ($p < 0.05$) decreased MTI and increased extensibility and stickiness. A similar

result was observed by Laurikainen et al. (1998), addition of xylanase led to an increase in stickiness, but no significant effect on water absorption of wheat flour dough substituted with rye bran. Trogh et al. (2004) illustrated that the dough stickiness increased when adding 60 U/kg xylanase to hull-less barley flour dough. Additionally, Flander et al. (2008) indicated that xylanase treatment increased water-extractable arabinoxylan and water-soluble polysaccharides (β -glucan), which may increase the viscosity and resistance to extension of oat dough. Therefore, this observation may be due to the change of water-soluble polysaccharides (β -glucan) content and the disruption of gluten network.

With regards to the α -amylase, the results show that there was no significant effect on water absorption, development time, stability, departure time and MTI of oat bran dough. Similar result was reported by Penella et al. (2008), single fungal α -amylase addition did not show significant effects on development and stability of dough enriched in wheat bran. Moreover, Kim et al. (2006) indicated that the addition of α -amylase did not significantly affect the water absorption of dough replacing with polished flour. This observation may be attributed to the increasing content of β -glucan, which cannot be hydrolyzed by α -amylase. Table 8.1 also shows that the addition of amylase significantly ($p < 0.05$) increased the softening, resistance to extension, extensibility and stickiness of oat bran dough. Kim et al. (2006) illustrated that the addition of fungal α -amylase resulted in an increase in resistance to extension and the viscosity coefficient of dough incorporated with polished flours. According to the research of Indrani et al. (2003), the addition of α -amylase can break down the starch to dextrin and improve the handling properties of dough. Other research has reported that the addition of α -amylase led to the dough weaker and sticky (Błaszczak, Sadowska, Rosell, & Fornal, 2004; J. H. Kim et al., 2006; Maeda, Hashimoto, Minoda, Tamagawa, & Morita, 2003).

In terms of cellulase, Table 8.1 shows that the cellulase addition decreased water absorption, softening and MTI, whereas increased stability, resistance to extension and extensibility of oat bran dough. No significant differences in development time and stickiness was observed

between oat bran dough and oat bran dough containing cellulase. No research has been reported on effects of cellulase on the rheological properties of oat bran.

8.3.2 Effect of enzymes combination on rheological properties of oat bran dough

The effects of α -amylase, xylanase and cellulase combinations on the rheological properties of oat bran were analyzed using 2^3 full factorial design, and analytical results are presented in Table 8.2. Figure 8.1 illustrates the interaction of α -amylase, xylanase and cellulase on parameters of oat bran dough rheology. Regression coefficients and R^2 obtained from the full factorial design in dough rheology are presented in Table 8.3. Table 8.3 illustrates that any enzyme and interaction of the enzymes significantly ($p < 0.05$) influenced the water absorption, development time, stability, softening, departure time, MTI, resistance to extension, extensibility and stickiness of oat bran. Additionally, the selected coefficients represented in Table 8.3 were fitted to the following empirical model as shown below:

$$W (\text{WA } \%) = 63.79 - 0.17A - 0.12B - 0.71C - 0.08AB - 0.61AC \quad (R^2 = 0.97)$$

$$W (\text{Development time}) = 7.69 - 0.16A - 0.18B + 0.11C - 0.23AB - 0.18ABC \quad (R^2 = 0.89)$$

$$W (\text{Stability}) = 7.25 + 0.16A - 0.32B + 0.25C - 0.10AB \quad (R^2 = 0.81)$$

$$W (\text{Softening}) = 89.10 + 2.90A + 7.11B - 1.99C + 2.58AB - 2.30BC \quad (R^2 = 0.81)$$

$$W (\text{Departure time}) = 11.64 - 0.26A - 0.31B + 0.31C - 0.21AB + 0.16AC \quad (R^2 = 0.95)$$

$$W (\text{MTI}) = 48.19 + 2.77B - 1.72C + 1.61AB - 1.29BC - 1.22ABC \quad (R^2 = 0.81)$$

$$W (\text{Extension}) = 38.02 + 1.98A - 5.99B - 4.10C + 7.09AB + 4.96BC + 1.77ABC \quad (R^2 = 0.98)$$

$$W (\text{Extensibility}) = 14.54 + 0.56A - 0.73C + 1.25AB + 0.46BC + 0.51ABC \quad (R^2 = 0.98)$$

$$W (\text{Stickiness}) = 54.56 + 0.23A + 1.06B + 0.92C - 3.01AB - 2.88AC - 1.41BC - 0.58ABC \quad (R^2 = 0.99)$$

Factors: A – α -amylase; B – xylanase; C – cellulase; AB – α -amylase*xylanase; AC – α -amylase*cellulase; BC – xylanase*cellulase; ABC – α -amylase*xylanase*cellulase.

Enzyme combinations decreased the water absorption of oat bran dough from 67.8 % to 62.1 % when the concentration is 10, 120 and 60 ppm. From Table 8.3, the α -amylase, xylanase and cellulase had a negative effect on water absorption, and the interaction of α -amylase*xylanase and α -amylase*cellulase had a negative effect as well. Compared to the single enzyme, the enzyme combinations reduced the water absorption to the minimum value when α -amylase, xylanase and cellulase were added to the highest level. Figure 8.1 also shows that the water absorption of oat bran dough decreased as level of enzymes increased. Similar observations were reported by Pescador-Piedra et al. (2009), the addition of glucose oxidase, peroxidase and xylanase combination had lower water absorption than the addition of single enzyme. Liu et al. (2017b) suggested that the combination of α -amylase, xylanase and cellulase had a synergetic effect on the dough rheology due to the interactions among enzyme activities and their coupled reactions. Additionally, Hemalatha et al. (2014) illustrated that the mixture of α -amylase and xylanase led to a decrease in starch content and a low moisture content.

The addition of enzyme combination resulted in a decrease from 9.5 min to 7.1 min when the enzymes added with 10, 120, 60 ppm. Both α -amylase and xylanase had a negative effect on the development time, whereas the addition of cellulase had a positive effect. The interaction of α -amylase*xylanase and α -amylase*xylanase*cellulase also showed a negative effect on development time of oat bran dough. Compared with increasing effect of single enzyme, the mixture of enzymes significantly ($p < 0.05$) decreased the development time. The similar observations were reported by Pescador-Piedra et al. (2009) and Liu et al. (2017b), who pointed out mixing time increased when the single enzyme added, whereas decreased significantly as adding enzyme combination.

Table 8.1 The effect of single enzyme application on the rheology of regular dough and dough with 15 % oat bran

	No enzymes		Xylanase		Amylase		Cellulase	
	regular	oat bran	regular	oat bran	regular	oat bran	regular	oat bran
WA %	63.86 ± 0.12 ^D	67.76 ± 0.06 ^A	65.20 ± 0.10 ^C	67.63 ± 0.06 ^A	63.93 ± 0.15 ^D	66.93 ± 0.51 ^{AB}	63.20 ± 0.70 ^D	66.23 ± 0.15 ^B
Development time (min)	2.73 ± 0.06 ^C	9.47 ± 0.49 ^{AB}	8.60 ± 0.61 ^B	10.20 ± 0.35 ^A	2.80 ± 0.10 ^C	9.37 ± 0.32 ^{AB}	8.76 ± 0.45 ^B	10.20 ± 0.10 ^A
Stability (min)	11.07 ± 0.55 ^{CDE}	10.50 ± 0.36 ^{DE}	18.03 ± 0.06 ^A	11.77 ± 0.25 ^{CD}	8.67 ± 0.51 ^F	10.17 ± 0.47 ^E	16.63 ± 0.55 ^B	12.10 ± 0.60 ^C
Softening (FU)	43.13 ± 1.63 ^{BC}	38.63 ± 1.66 ^{CD}	23.40 ± 1.75 ^G	36.13 ± 4.44 ^{DE}	60.30 ± 1.48 ^A	48.86 ± 1.56 ^B	31.00 ± 2.46 ^{EF}	29.60 ± 0.95 ^{FG}
Departure time (min)	12.67 ± 0.91 ^E	16.13 ± 0.55 ^{CD}	20.00 ± 0.01 ^A	17.77 ± 0.15 ^{BC}	10.50 ± 0.46 ^E	14.90 ± 1.65 ^D	18.56 ± 0.66 ^{AB}	17.93 ± 0.50 ^{ABC}
MTI (FU)	10.37 ± 0.92 ^E	22.20 ± 0.92 ^A	15.73 ± 1.16 ^D	19.43 ± 1.13 ^B	20.80 ± 0.20 ^{AB}	21.70 ± 0.75 ^{AB}	16.63 ± 1.56 ^{CD}	19.33 ± 0.25 ^{BC}
Resistance to extension (g)	44.59 ± 0.68 ^A	21.55 ± 0.23 ^F	25.73 ± 1.05 ^E	30.55 ± 0.33 ^D	35.37 ± 0.52 ^C	38.23 ± 0.36 ^B	19.96 ± 0.22 ^G	38.44 ± 0.57 ^B
Extensibility (mm)	31.31 ± 0.23 ^D	11.43 ± 0.11 ^G	39.88 ± 0.78 ^C	13.53 ± 0.20 ^F	45.27 ± 0.53 ^A	15.18 ± 0.05 ^E	43.53 ± 0.40 ^B	15.08 ± 0.02 ^E
Stickiness (g)	44.62 ± 0.36 ^G	45.73 ± 0.58 ^{FG}	66.72 ± 0.88 ^A	60.25 ± 0.11 ^B	58.43 ± 0.32 ^C	52.38 ± 0.26 ^E	53.86 ± 0.32 ^D	46.42 ± 0.50 ^F

Means ± standard deviations (n=3). Values in the same row with different letters differ significantly ($p < 0.05$). Regular-wheat flour dough; Oat bran-wheat flour dough substituted with 15 % oat bran; MTI-mixing tolerance index.

With respect to stability, mixture of enzymes decreased the stability of oat bran dough from 10.5 to 6.6 min. From Table 8.3 and Figure 8.1, both α -amylase and cellulase had a positive effect among the combination on the stability of oat bran dough, whereas xylanase showed a negative effect. Only interaction of α -amylase*xylanase had been observed a negative effect. In comparison with single enzyme, the combination enzymes decreased the stability of oat bran dough significantly. A similar observation was reported in Chapter 7.3.3. Unfortunately, there is a paucity of information regarding the effect of enzyme combination on the oat bran dough rheology. Table 8.3 also shows that the combination enzymes increased the softening and MTI of oat bran dough. When the concentrations of enzyme combination were added at 10, 120, 35 ppm, the value of softening and MTI were maximum, 106.9 FU and 58.1 FU respectively.

The use of blends of enzymes increased extensibility and stickiness to the maximum value, 16.5 mm and 60.8 g respectively. Similar results were reported by previous study, the single enzyme or combined enzymes increased extensibility, due to the modifications in starch and arabinoxylans fractions (Katina et al., 2006; Selinheimo et al., 2006). Moreover, Eugenia Steffolani et al. (2012) found that dough added with enzyme mixture (α -amylase, xylanase and glucose oxidase) had the intermediate stickiness. These observations may be due to the degradation of cell wall components and higher water absorption of bran resulting in altered the water distribution among starch, protein and bran particles (Laurikainen et al., 1998; W. Liu et al., 2017b; Selinheimo et al., 2006).

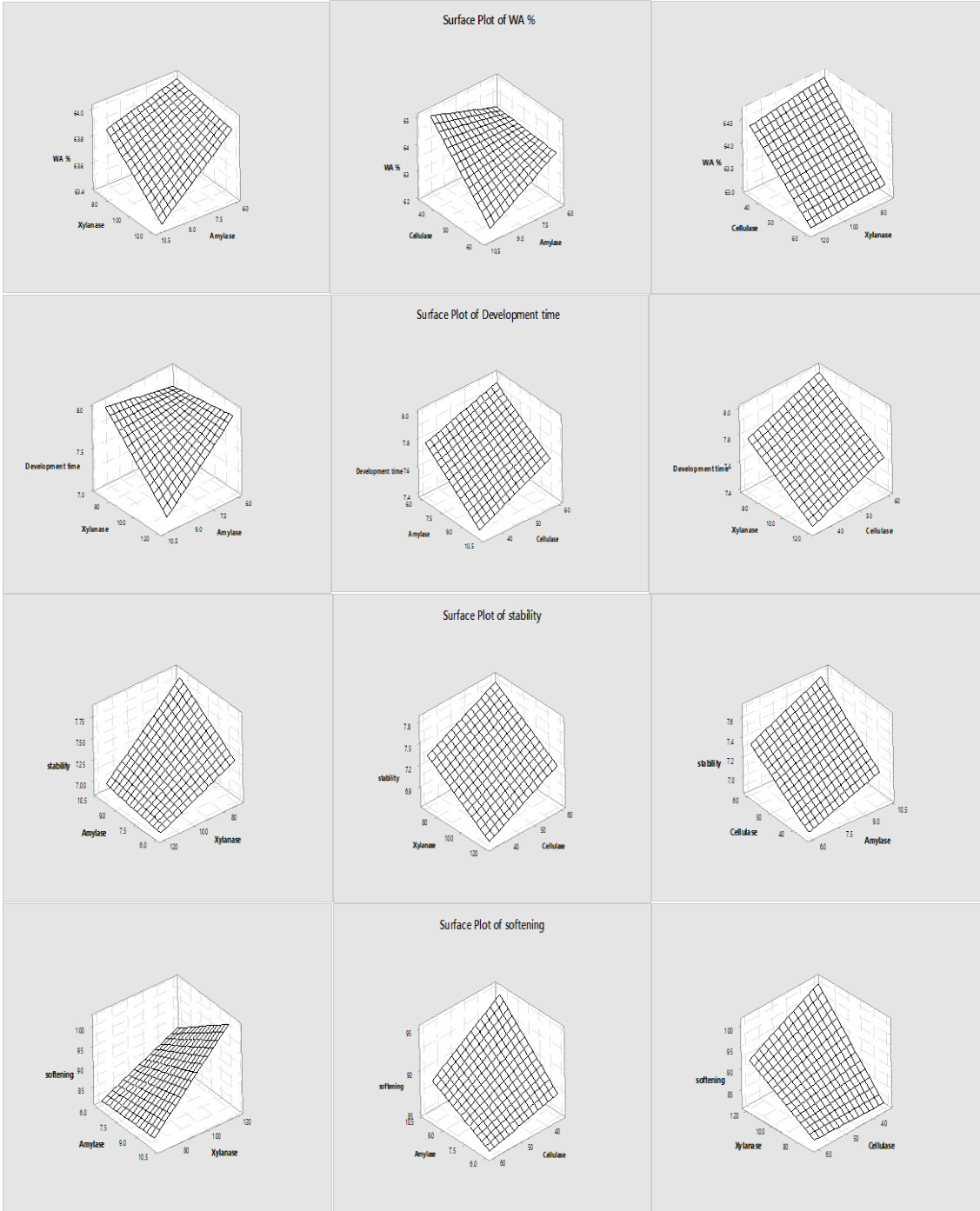




Figure 8.1 Response surface plot showing the effect of α -amylase, xylanase and cellulose on the rheology of dough with 15 % oat bran.
 WA %-water absorption; MTI-mixing tolerance index. DT-development time.

8.4 Conclusion

This chapter investigated the effects of α -amylase (6 and 10 ppm), xylanase (70 and 120 ppm) and cellulase (35 and 60 ppm) on the rheological properties of oat bran dough. As a result, the addition of single enzyme did not significantly affect the water absorption, development time and stability, whereas increased extensibility and stickiness of oat bran dough. Compared to the single enzyme, the blended enzymes can reduce the water absorption, development time and stability of oat bran dough to the minimum value when the enzyme combination were added with the high concentrations (10, 120 and 60 ppm). In particular, the combinations of α -amylase, xylanase and cellulase can increase the extensibility and stickiness of oat bran dough to higher value than the single enzyme. Therefore, the combined enzymes were more efficient than the single enzyme in rheological properties of oat bran dough.

In conclusion, the combination of α -amylase, xylanase and cellulase had a synergistic effect on the dough rheology due to the interactions among enzyme activities and their coupled reactions.

Table 8.2 The effect of different enzyme combinations on the rheology of dough containing 15 % oat bran

Blocks	A	B	C	WA %	DT (min)	Stability (min)	Softening (FU)	Departure time (min)	MTI (FU)	Extension (g)	Extensibility (mm)	Stickiness (g)
Oat bran	0	0	0	67.8	9.5	10.5	38.6	16.1	22.2	21.5	11.4	45.7
1	6	70	35	64.1	7.9	7.1	80.5	11.9	47.4	56.2	15.8	46.2
2	6	70	60	63.9	7.7	7.5	82.7	12.1	45.4	42.2	14.6	54.3
3	6	120	60	63.8	8.2	7.1	87.4	12.0	47.6	22.4	12.1	60.8
4	6	120	35	64.0	7.6	6.6	94.1	11.6	49.9	23.5	13.4	56.0
5	10	70	35	65.2	7.6	7.6	82.8	11.4	44.3	50.1	15.7	56.1
6	10	120	35	64.7	7.2	6.7	106.9	10.4	58.1	38.8	16.2	56.2
7	10	120	60	62.1	7.1	7.3	96.4	11.3	48.3	43.4	16.5	49.5
8	10	70	60	62.5	8.3	8.1	81.9	12.4	44.6	27.7	12.1	57.4

All values are means (n = 3). A (factor) – α -amylase; B (factor) – xylanase; C (factor) – cellulase. Oat bran – wheat flour dough with 15 % oat bran. WA %-water absorption, DT-development time, MTI-mixing tolerance index.

Table 8.3 The estimated coefficients of the factors of the rheological properties of dough containing oat bran

Coefficients	WA%	DT (min)	Stability (min)	Softening (FU)	Departure time (min)	MTI (FU)	Extension (g)	Extensibility (mm)	Stickiness (g)
Constant	63.79	7.69	7.25	89.10	11.64	48.19	38.02	14.54	54.56
Amylase	-0.17	-0.16	0.16	2.90	-0.26	NS	1.98	0.56	0.23
Xylanase	-0.12	-0.18	-0.32	7.11	-0.31	2.77	-5.99	NS	1.06
Cellulase	-0.71	0.11	0.25	-1.99	0.31	-1.72	-4.10	-0.73	0.92
Amylase*Xylanase	-0.08	-0.23	-0.10	2.58	-0.21	1.61	7.09	1.25	-3.01
Amylase*Cellulase	-0.61	NS	NS	NS	0.16	NS	NS	NS	-2.88
Xylanase*Cellulase	NS	NS	NS	-2.30	NS	-1.29	4.96	0.46	-1.41
Amylase*Xylanase*Cellulase	NS	-0.18	NS	NS	NS	-1.22	1.77	0.51	-0.58
R ²	97.58%	89.50%	81.11%	80.66%	95.26%	81.63%	98.92%	98.28%	99.71%

NS – no significant effect at level ($p < 0.05$); R² – adjusted square coefficient (describes the percentage of variability for which the model accounts); β_0 – global means of parameters; β_1 , β_2 and β_3 – regression coefficients corresponding to main factors; β_{12} , β_{13} , β_{23} and β_{123} – regression coefficients corresponding to interactions; ‘+’ – positive effect; ‘-’ – negative effect; WA %-water absorption, DT-development time, MTI-mixing tolerance index.

Chapter 9

Effect of α -amylase, xylanase and cellulase combinations on the rheological properties of Chinese steamed bread dough enriched in buckwheat flour

9.1 Introduction

In Chapter 5, the effect of buckwheat flour (BW) on the Chinese steamed bread and dough were investigated. As a result, the addition of BW significantly influenced rheological properties of the steamed bread dough. For instance, the water absorption increased with the increase in BW levels from 0 to 15 % due to the water holding capacity of dietary fibre (Lin et al., 2012; Nikolić et al., 2011). Buckwheat seeds are rich in dietary fibre (resistant starch), protein, antioxidative substances and trace elements (Bonafaccia, Marocchini, & Kreft, 2003; Steadman et al., 2001). The addition of BW caused an increase in development time probably due to the high fibre and lipid content of BW (Sedej et al., 2011). According to the study of Gómez et al. (Gómez et al., 2003), the addition of different fibres increased the dough development time. Additionally, the addition of BW resulted in increased softening and mixing tolerance index (MTI). Lin et al. (Lin et al., 2012) also reported that the MTI was increased by blending of wheat flour with barley flour because of the dilution of gluten content so that a decrease in dough stability was observed with an increase in substitution level of BW. This observation was probably due to the lack of structure forming ability of buckwheat proteins and the decrease in the concentration of gluten (Nikolić et al., 2011; Sedej et al., 2011). The stickiness of dough increased significantly ($p < 0.05$) with the additional levels of BW from 5 - 15 %. Whereas, the extensibility of the dough had a slight decrease as the substitution of BW increased. Nikolić, Sakač, & Mastilović (2011) illustrated that dough formulated with BW had lower values of extensibility and resistance in comparison to dough with wheat flour only due to dilution of gluten and disruption of the gluten network structure.

Previous research has reported that the use of enzymes can improve the dough rheology and the quality of final product in baking industry (Caballero et al., 2007a, 2007b; Rouau, El-Hayek, & Moreau, 1994; Steffolani, Ribotta, Pérez, & León, 2010). However, there is a paucity of information regarding the effect of α -amylase, xylanase and cellulase on the rheological properties of the buckwheat flour dough. Thus, this chapter investigates the effect of α -amylase, xylanase and cellulase on the rheological properties of the steamed bread dough incorporated with 15 % of buckwheat flour.

9.2 Materials and Methods

9.2.1 Materials

The ingredients for the bread dough were described in 3.1.

9.2.2 Rheological analysis of dough

Rheological properties of CSB dough treated with enzymes were determined according to the method described in 3.4.

9.2.3 Design of experiment

Two experimental designs were performed: the first one to evaluate the effect of single enzyme on the dough rheology, and the second one to investigate the effect of combined enzymes on the dough rheology. Firstly, the effect of single enzyme on the rheological properties of dough incorporated with 15 % of buckwheat flour as buckwheat dough was analysed by analysis of variance (ANOVA). According to the manufacture recommendations of Novozymes and previous publications (Caballero et al., 2007a, 2007b; Serventi et al., 2016; Shafisoltani et al., 2014), the dosage of the Cellulast BG, Fungamyl 2500 SG and Pentopan Mono BG was added with 35 ppm, 10 ppm and 70 ppm, respectively.

Second, in order to investigate the effect of mixtures of enzymes on the rheological properties of dough incorporated with 15 % of buckwheat flour, a full factorial 2^3 design of experiments with central point in triplicate was used to evaluate all single effects and second-order interactions between factors. Generally, there are three factors (α -amylase, xylanase and cellulase) at two levels (-1, 1) resulted in 8 different combinations of experiments and the coded values per each level of each factor are presented in Supplementary Table 2 (Haros, Ferrer, & Rosell, 2006). According to the estimated coefficients (β_i , β_{ij} & β_{ijk}), the theoretical response function (W) was calculated as following polynomial linear regression model:

$$W = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC + \beta_{123}ABC$$

Factors: A – α -amylase; B – xylanase; C – cellulase; AB – α -amylase*xylanase; AC – α -amylase*cellulase; BC – xylanase*cellulase; ABC – α -amylase*xylanase*cellulase.

W – The theoretical response variable; β_0 – The global mean; β_i – The regression coefficient corresponding to main factor; β_{ij} and β_{ijk} – The regression coefficient corresponding to the interactions.

This multiple linear regression model with three independent variables describes the rheological property of dough is related to the α -amylase, xylanase and cellulase.

9.2.4 Statistical analysis

All data were treated by ANOVA and factorial design analysis as described in 3.9.

9.3 Results and discussion

9.3.1 The effect of single enzyme on the rheological properties of buckwheat dough

The effects of single enzyme on the rheology of dough incorporated with 15 % buckwheat flour are presented in Table 9.1. The incorporation of α -amylase to buckwheat dough lowered water absorption, development time, stability, departure time, extensibility and stickiness, whereas

raised the softening, MTI and resistance to extension. Similar results have been observed by Atalay et al. (2013) who reported that the addition of transglutaminase and sodium stearyl-2-lactylate decreased the water absorption and extensibility, while increased maximum resistance of flour blended with buckwheat milling products. Stefano Renzetti and Arendt (2009) reported that the addition of protease reduced the viscosity of buckwheat flour dough. Additionally, J. H. Kim et al. (2006) illustrated that the addition of fungal α -amylase to flour resulted in decrease of the water absorption, development time, stability and extensibility and an increase of resistance due to the presence of a low molecular weight dextrin produced by α -amylase hydrolysis.

The addition of xylanase decreased the water absorption, development time, stability, departure time, extensibility and stickiness, whereas increased softening, MTI and resistance. McCleary, Gibson, Allen, and Gams (1986) reported that addition of xylanase decreased the water absorption and consistency of dough due to the depolymerization of pentosane by xylanase. Jia, Huang, Abdel-Samie, Huang, and Huang (2011) also illustrated that the addition of xylanase to the dough incorporated with almond skin flour led to a decrease in development time and stability. Moreover, Shah, Shah, and Madamwar (2006) demonstrated that xylanase addition reduced the water absorption of whole wheat flour possibly being attributed to the enzymatic hydrolysis of soluble pentosane. Table 9.1 also shows that the effect of cellulase on the buckwheat dough rheology has the same trend with xylanase and α -amylase.

Table 9.1 The effect of a single enzyme on the rheology of dough containing buckwheat flour

Sample	15% buckwheat	+ Amylase	+ Xylanase	+ Cellulase
WA %	65.07±0.03 ^A	62.10±0.17 ^B	62.23±0.06 ^B	62.43±0.15 ^B
Development time (min)	7.12±0.13 ^A	6.53±0.15 ^B	6.83±0.06 ^B	6.23±0.06 ^B
Stability (min)	8.73±0.45 ^A	8.07±0.06 ^B	6.50±0.30 ^C	5.03±0.06 ^D
Softening (FU)	53.37±5.31 ^C	88.07±2.63 ^A	71.63±1.93 ^B	90.46±0.45 ^A
Departure time (min)	12.21±0.36 ^A	10.20±0.35 ^B	10.50±0.10 ^B	9.00±0.10 ^C
MTI (FU)	31.31±0.11 ^D	52.17±2.65 ^B	41.46±1.46 ^C	60.70±0.10 ^A
Extension (g)	17.54±1.24 ^C	38.23±0.36 ^B	42.21±0.39 ^A	42.58±1.18 ^A
Extensibility (mm)	34.60±0.21 ^A	18.43±0.35 ^B	17.15±0.73 ^C	16.07±0.22 ^C
Stickiness (g)	77.59±5.39 ^A	62.35±0.26 ^C	65.48±0.11 ^B	52.93±1.68 ^D

Values are means ± standard deviations (n=3). Values in the same row with different letters differ significantly ($p < 0.05$).

WA-water absorption; MTI-mixing tolerance index

9.3.2 Effect of enzymes combination on rheological properties of buckwheat dough

Full factorial design 2^3 was used to investigate the effect of α -amylase, xylanase and cellulase combinations on the rheological properties of buckwheat dough. The analytical results and the interaction of α -amylase, xylanase and cellulase on parameters of buckwheat dough rheology are presented in Table 9.2 and Figure 9.1. Table 9.3 illustrates regression coefficients and R^2 obtained from the full factorial design in dough rheology. The coefficients that showed significant difference ($p < 0.05$) in Table 9.3 were fitted to the following empirical model:

$$W (\text{WA } \%) = 61.32 + 0.1A - 0.53C + 0.18AB - 0.33AC + 0.09BC + 0.08ABC \quad (R^2 = 0.95)$$

$$W (\text{Development time}) = 6.35 + 0.17AB + 0.15AC \quad (R^2 = 0.66)$$

$$W (\text{Stability}) = 5.07 + 0.12AC + 0.08ABC \quad (R^2 = 0.63)$$

$$W (\text{Softening}) = 108.51 + 2.41A + 5.92B + 1.62AB - 2.85AC - 2.98BC - 2.59ABC \quad (R^2 = 0.91)$$

$$W (\text{Departure time}) = 8.83 - 0.43A - 0.12B + 0.10C + 0.24AC \quad (R^2 = 0.86)$$

$$W (\text{MTI}) = 64.38 + 3.53A - 1.44C + 1.11AB - 3.00AC - 1.04BC \quad (R^2 = 0.86)$$

$$W (\text{Extension}) = 32.63 - 6.82A - 2.31B - 5.42C - 1.85AB + 1.83AC - 1.55BC + 4.93ABC \quad (R^2 = 0.99)$$

$$W (\text{Extensibility}) = 18.45 + 1.24A + 0.55B + 0.21C - 0.32AB - 0.25BC - 0.35ABC \quad (R^2 = 0.98)$$

$$W (\text{Stickiness}) = 74.58 - 7.07A + 4.61B + 0.28C - 3.88AB + 0.81AC - 2.38BC + 1.65ABC \quad (R^2 = 0.99)$$

Factors: A – α -amylase; B – xylanase; C – cellulase; AB – α -amylase*xylanase; AC – α -amylase*cellulase; BC – xylanase*cellulase; ABC – α -amylase*xylanase*cellulase.

Compared to the single enzyme, the use of a combination of enzymes reduced the water absorption of buckwheat dough to the minimum value (60.9 %) when the enzymes were added with the concentration (6, 120, 60 ppm). Table 9.3 illustrates that the interaction of α -

amylase*xylanase, xylanase*cellulase and α -amylase*xylanase*cellulase have a positive synergistic effect on the water absorption, while α -amylase*cellulase shows negative effect. Therefore, the buckwheat dough incorporated with combined enzymes required less water than the buckwheat dough with single enzyme during dough mixing. Similar to our results, Atalay et al. (2013) pointed out that the combination of transglutaminase and sodium stearyl-2-lactylate decreased the water absorption of dough incorporated with 20 % buckwheat milling product due to synergistic effect between the additives. Pescador-Piedra et al. (2009) reported that incorporation of xylanase, glucose oxidase and peroxidase to dough had lower water absorption than dough with single enzyme. This observation may be attributed to the interaction among enzymes activities (Altuna et al., 2015; W. Liu et al., 2017b).

The addition of the mixture of enzymes showed a lower development time than single enzyme when the combined enzymes added with 10, 70, 35 ppm. The interaction of α -amylase*xylanase and α -amylase*cellulase had a significantly synergistic effect on the development time. Previous research found that incorporation of xylanase and oxidative enzymes into bread formulation gave the best handling properties for dough (Hilhorst et al., 1999). According to the research of Shafisoltani et al. (2014), the combination of xylanase and glucose oxidase has an inverse effect on the development time. Additionally, Caballero et al. (2007b) indicated the interactions between transglutaminase and xylanase, and α -amylase and protease had interactive effects on viscoelastic properties of dough.

In terms of stability, combined enzymes decreased the stability of buckwheat dough from 8.7 min to 4.6 min. The interaction of α -amylase*cellulase and α -amylase*xylanase*cellulase indicated a significant positive effect on the stability. For the softening, the enzyme combination had a higher value of softening than single enzyme. Martínez - Anaya and Jiménez (1997) illustrated that combinations of α -amylase, xylanase, lipases and glucose-oxidase led to a softer dough after mixing. This result is consistent with previous research of Liu et al. (2017b), who

reported that the combination of α -amylase, xylanase and cellulase decreased the dough stability and increased dough softening.

The combination of α -amylase, xylanase and cellulase significantly influenced the resistance to extension, extensibility and stickiness. The addition of combined enzymes increased resistance to extension and reduced the extensibility of buckwheat dough. Similar observation was reported by Altuna et al. (2015) who found that the combination of transglutaminase (0–8 mg/100 g), glucose-oxidase (0–5 mg/100 g) and xylanase (0–1 mg/100 g) resulted in a decrease in extensibility and increase in resistance to extension. Primo-Martin, Valera, and Martinez-Anaya (2003) also pointed out the mixture of laccase and xylanase resulted in a lower extensibility dough. Stickiness of buckwheat dough was varied from 66.3 g to 96.0 g when combined enzymes were added with different concentrations. Table 9.2 shows that the minimum stickiness (66.3 g) was observed when the blended enzymes were added with 10, 70, 35 ppm. However, the buckwheat dough had the highest stickiness when the combined enzymes were added with the concentration (6, 120, 35 ppm). Altuna et al. (2015) also found that the combination of transglutaminase, glucose-oxidase and xylanase increased the stickiness of dough incorporated with resistant starch. Previous research also suggested that the optimum combination of stearyl-2-lactylate, transglutaminase, and xylanase could be used to minimize dough stickiness (Ribotta, Pérez, Añón, & León, 2010). These observations may be due to the degradation of cell wall components and higher water absorption of bran resulting in altered the water distribution among starch, protein and bran particles (Laurikainen et al., 1998; W. Liu et al., 2017b; Selinheimo et al., 2006).

Table 9.2 The effect of combined enzymes on the rheology of dough containing buckwheat flour

Blocks	A	B	C	WA %	DT (min)	Stability (min)	Softening (FU)	Departure time (min)	MTI (FU)	Extension (g)	Extensibility (mm)	Stickiness (g)
Buckwheat	0	0	0	65.1	7.1	8.7	53.3	12.2	31.3	17.5	34.6	77.6
1	6	70	35	61.6	6.7	5.2	98.9	9.6	59.8	40.7	16.3	68.6
2	6	70	60	61.2	6.4	5.1	104.7	9.3	63.9	39.23	16.5	75.6
3	6	120	60	60.9	6.2	5.0	112.8	9.0	61.0	25.3	18.5	86.5
4	6	120	35	61.2	6.3	5.2	107.9	9.2	58.8	52.5	17.6	96.0
5	10	70	35	62.2	6.0	5.1	101.2	8.1	69.6	36.8	18.6	66.3
6	10	120	35	62.3	6.2	4.6	126.8	8.1	75.8	21.7	20.28	66.8
7	10	120	60	60.9	6.7	5.3	109.6	8.5	63.0	21.5	19.6	67.5
8	10	70	60	60.2	6.2	5.1	105.8	9.0	63.8	22.6	20.3	69.8

All values are means (n=3). A (factor) – α -amylase; B (factor) – xylanase; C (factor) – cellulase. Buckwheat – wheat flour dough with 15 % buckwheat flour. WA %-water absorption, DT-development time, MTI-mixing tolerance index.

Table 9.3 Estimated regression coefficients of the factors of the rheological properties of buckwheat dough

Coefficients	WA%	DT (min)	Stability (min)	Softening (FU)	Departure time (min)	MTI (FU)	Extension (g)	Extensibility (mm)	Stickiness (g)
Constant	61.32	6.35	5.07	108.51	8.83	64.38	32.63	18.45	74.58
Amylase	0.10	NS	NS	2.41	-0.43	3.53	-6.82	1.24	-7.07
Xylanase	NS	NS	NS	5.92	-0.12	NS	-2.31	0.55	4.61
Cellulase	-0.53	NS	NS	NS	0.10	-1.44	-5.42	0.21	0.28
Amylase*Xylanase	0.18	0.17	NS	1.62	NS	1.11	-1.85	-0.32	-3.88
Amylase*Cellulase	-0.33	0.15	0.12	-2.85	0.24	-3.00	1.83	NS	0.81
Xylanase*Cellulase	0.09	NS	NS	-2.98	NS	-1.04	-1.55	-0.25	-2.38
Amylase*Xylanase*Cellulase	0.08	NS	0.08	-2.59	NS	NS	4.93	-0.35	1.65
R ²	95.49%	66.36%	63.11%	90.88%	86.21%	85.90%	99.25%	98.19%	99.89%

NS – no significant effect at level ($p < 0.05$); R² – adjusted square coefficient (describes the percentage of variability for which the model accounts); β_0 – global means of parameters; β_1 , β_2 and β_3 – regression coefficients corresponding to main factors; β_{12} , β_{13} , β_{23} and β_{123} – regression coefficients corresponding to interactions; ‘+’ – positive effect; ‘-’ – negative effect; WA %-water absorption, DT-development time, MTI-mixing tolerance index.

9.4 Conclusion

In this chapter, the individual and combined effect of α -amylase, xylanase and cellulase on dough incorporated with 15 % buckwheat flour were investigated. From the findings, it can be concluded that both single enzyme and blended enzymes had significant influence of buckwheat dough rheology. The individual addition of α -amylase, xylanase and cellulase into buckwheat dough reduced water absorption, development time, stability, extensibility and stickiness, whereas increased softening, MTI and resistance to extension. In comparison with single enzyme, the enzymes combination showed lower development time, water absorption and stability, and higher softening, MTI, resistance to extension and extensibility. The results obtained from 2^3 full factorial design suggested that the combined enzymes were more efficient than the single enzyme due to the synergistic effect of α -amylase, xylanase and cellulase. Therefore, the combination of enzymes revealed a better improvement of buckwheat dough rheology than single enzyme.

Chapter 10

Effect of α -amylase, xylanase and cellulase on the breadmaking properties and predicting glycaemic response of Chinese steamed bread enriched in wheat bran

This part of research has been submitted to Food Chemistry (in process)

10.1 Introduction

Currently, there is a growing demand for healthier foods with a consequent rise in interest in functional and nutritional products by the food industry. Previous research has illustrated that dietary fibre can be added to food as a functional ingredient. Brennan (2005) illustrated that dietary fibre had beneficial physiological effects, such as increasing in stool bulk, decreasing in intestinal transit time, reducing blood cholesterol levels, insulin levels and glycaemic impact. In general, the chemical nature of dietary fibre is composed of non-digestible carbohydrates, including oligosaccharides, polysaccharides and lignin, such as cellulose, hemicelluloses, β -glucan, arabinoxylan, gums, mucilage, pectin, inulin, resistant starch (Lunn & Buttriss, 2007; Sumczynski, Bubelová, & Fišera, 2015). Accordingly, dietary fibre has also been linked to disease prevention and control, such as diabetes, obesity and colon cancer (Abuajah, Ogbonna, & Osuji, 2015).

The majority of dietary fibre is present in the outer layers of cereal grains, for instance within the bran fraction, which comprises of the pericarp, testa, aleurone, germ, and part of the starchy endosperm (Hemdane, Jacobs, Dornez, Verspreet, Delcour, & Courtin, 2016). Wheat bran (WB) is a low-cost by-product from the milling industry and abundantly produced during wheat roller milling (Onipe et al., 2015). Chinese steamed bread (CSB) is a traditional fermented food and widely consumed as a staple food in China (Liu, Brennan, Serventi, & Brennan, 2016). However, the addition of wheat bran into bread generally results in negative effects on rheological properties, baking performance, texture properties of final products, such as

reducing the extensibility, increasing the dough stickiness, reducing loaf volume, darkening the crumb, and increasing the firmness (Boita, Oro, Bressiani, Santetti, Bertolin, & Gutkoski, 2016; Hemdane, Jacobs, Dornez, Verspreet, Delcour, & Courtin, 2016; Jacobs, Bogaerts, Hemdane, Delcour, & Courtin, 2016; W. Liu, M. A. Brennan, L. Serventi, & C. S. Brennan, 2017; W. J. Liu, M. Brennan, L. Serventi, & C. Brennan, 2017; Veraverbeke & Delcour, 2002).

In order to improve the quality of bread enriched in wheat bran, enzymes may be used as aids, being added to the flour during the baking process (Sanz Penella, Collar, & Haros, 2008). There are three main commercial enzymes preparations that are used in baking industry (Linko, Javanainen, & Linko, 1997). Fungal α -amylase is an enzyme derived from fungi, with widespread application in food industry. The action of amylase is to catalyse the hydrolysis of α -1, 4-glycosidic linkages into starch molecules (amylose and amylopectin), at a lower rate, maltodextrins and oligosaccharides (M. Antonia Martínez-Anaya, 1996). Xylanase is a hydrolase, which can randomly attack the arabinoxylan (AX) backbone and break the glycosidic linkages in AX, result in changing the functional and physicochemical properties of AX (Hilhorst et al., 1999). Cellulase belongs to the glycoside hydrolase family, which can catalyze the hydrolysis of (1,4)-beta-D-glucosidic linkages in cellulose and other beta-D-glucans. These enzymes are able to produce positive effects during breadmaking, such as improving the rheological behaviour of dough and hence the quality of final products (Caballero, Gómez, & Rosell, 2007b; W. Liu, M. A. Brennan, L. Serventi, & C. S. Brennan, 2017). However, there is a paucity of reports on the effects of enzymes combination, especially, the combination of cellulase, xylanase and α -amylase on the breadmaking properties and predicted glycaemic response.

Thus, this research investigates the effect of adding α -amylase, xylanase and cellulase, at different concentrations, on the quality and glycaemic response of CSB incorporated with 15 % wheat bran.

10.2 Materials and Methods

10.2.1 Ingredients

The ingredients for the Chinese steamed bread were described in 3.1.

10.2.2 Physical properties of CSB

The physical characteristics of CSB incorporated with enzymes were determined according to the method described in 3.6.1 and 3.6.3.

10.2.3 Total starch and total, soluble and insoluble dietary fibre analysis

Total starch of CSB incorporated with enzymes was performed as described in 3.7.1.

Total, soluble and insoluble dietary fibre of CSB were performed as described in 3.7.2.

10.2.4 Glycaemic response analysis

Glycaemic response of CSB incorporated with enzymes was measured using *in vitro* digestion method described by Brennan et al. (2013) as outlined in 3.8.

10.2.5 Design of experiment

Two experimental designs were performed as described in 3.9.2: the first one to evaluate the effect of single enzyme on the breadmaking properties and predicting glycaemic response, and the second one to investigate the effect of combined enzymes on the breadmaking properties and predicting glycaemic response.

10.2.6 Statistical analysis

All data were treated by ANOVA and factorial design analysis as described in 3.9.

10.3 Results and discussion

10.3.1 The effect of single enzyme on physical properties of CSB incorporated with 15 % wheat bran

The effect of the individual enzyme addition on the physical properties of CSB supplemented with 15 % of wheat bran is presented in Table 10.1. The addition of 10 ppm α -amylase increased the volume, height, moisture, cohesiveness, springiness, cell density and mean cell area of bran CSB. These samples also had lower hardness and gumminess of bread crumb compared to the control. Similar results have been reported by Caballero et al. (2007b) the addition of α -amylase can increase the bread volume. Gámbaro, Giménez, Ares, and Gilardi (2006) also pointed that the addition of amylase significantly affected the hardness, gumminess, cohesiveness of bread. However, there was no significant effect on chewiness. Additionally, Błaszczak et al. (2004) illustrated the addition of α -amylase can significantly increase the specific volume and porosity of bread crumb due to the changes in the starch-protein network.

The use of xylanase significantly ($p < 0.05$) increased specific volume and improved the texture and structure of CSB crumb incorporated with 15 % of wheat bran. This observation may be due to conversion of water-unextractable AX to enzyme-solubilized AX or water-extractable AX with high molecular weight (Courtin & Delcour, 2002; Jiang, Li, Yang, Li, & Tan, 2005). According to Courtin, Gelders, and Delcour (2001), water-unextractable AX are detrimental for breadmaking while water-extractable AX and solubilized AX with medium to high molecular weight have beneficial effects on loaf volume. Similar results were observed by Jiang, Cong, Yan, Kumar, and Du (2010) and Ghoshal et al. (2013), the addition of xylanase to bread resulted in increasing the specific volume and decreasing firmness. Schoenlechner, Szatmari, Bagdi, and Tömösközi (2013) have found that the addition of single xylanase significantly increased the mean pore area and bread volume.

The addition of cellulase to CSB supplemented with 15 % of wheat bran showed a similar performance to amylase and xylanase, such as improving the volume, increasing pore area and

softening crumb. Monica Haros et al. (2002) illustrated that the presence of cellulase in bread resulted in an improvement of specific volume and crumb texture due to the hydrolysis action on the non-starch polysaccharides. Harada, Lysenko, and Preston (2000) also pointed that the addition of cellulase led to an increase in specific volume and softer crumb.

10.3.2 The effect of single enzymes on the chemical and nutritional properties of CSB incorporated with 15 % wheat bran

Table 10.1 illustrates that the effect of single enzyme inclusion on the glycaemic response, dietary fibre and starch content of CSB. The area under the curve of glucose release (AUC) is a measurement of glycaemic response for 2 h after bread consumed (Monro et al., 2010a; Monro & Shaw, 2008). Previous research has indicated that the addition of 15 % WB could significantly increase the TDF content and reduce the predicted glycaemic response of CSB (Liu et al., 2017a; Salmenkallio-Marttila, Katina, & Autio, 2001). Table 10.1 shows that the addition of α -amylase significantly ($p < 0.05$) decreased the total starch of CSB with 15 % wheat bran from 36.67 to 34.62 %. In terms of total fibre content and AUC value, there is no significant differences between control and bran CSB supplemented with 10 ppm α -amylase. Similar results were observed by Sanz-Penella, Laparra, and Haros (2014), the addition of α -amylase to bread enriched in 10 % wheat bran significantly ($p < 0.05$) increased the total starch hydrolyzed and the values calculated for glycaemic index (GI). Compared to the control samples, the bran CSB with 70 ppm xylanase illustrated a lower total starch, total dietary fibre, soluble and insoluble dietary fibre content potentially due to the mechanism of action of xylanase.

Table 10.1 The effect of single enzyme on the physical and chemical properties of CSB with wheat bran

Bread samples	Wheat flour	15% WB (Control)	15% WB + 10ppm amylase	15% WB + 70ppm xylanase	15% WB + 35ppm cellulase
Volume (mL)	248.33 ± 2.65 ^A	176.33 ± 1.53 ^D	227.00 ± 1.03 ^B	217.33 ± 1.00 ^C	219.00 ± 2.65 ^{BC}
Specific volume (mL/g)	2.50 ± 0.03 ^A	1.69 ± 0.01 ^D	2.14 ± 0.01 ^B	2.08 ± 0.01 ^C	2.08 ± 0.01 ^C
Loaf height (mm)	62.14 ± 0.38 ^C	46.59 ± 0.15 ^C	55.66 ± 0.25 ^C	53.98 ± 0.26 ^C	56.82 ± 0.17 ^B
Moisture (%)	40.10 ± 0.01 ^E	43.35 ± 0.02 ^D	45.31 ± 0.03 ^C	48.03 ± 0.19 ^A	46.95 ± 0.32 ^B
Hardness (g)	228.24 ± 25.92 ^C	684.46 ± 43.80 ^A	443.58 ± 13.02 ^B	476.26 ± 3.04 ^B	453.33 ± 15.07 ^B
Chewiness (g)	179.83 ± 19.34 ^B	329.98 ± 39.76 ^A	365.21 ± 30.90 ^A	367.22 ± 18.51 ^A	325.03 ± 8.86 ^A
Cohesiveness (ratio)	0.88 ± 0.01 ^A	0.41 ± 0.01 ^C	0.83 ± 0.02 ^{AB}	0.82 ± 0.05 ^{AB}	0.83 ± 0.01 ^{AB}
Springiness (mm)	0.95 ± 0.01 ^A	0.79 ± 0.02 ^B	0.91 ± 0.01 ^A	0.95 ± 0.04 ^A	0.90 ± 0.01 ^A
Cell density (cells/cm ²)	53.00 ± 1.03 ^C	48.33 ± 0.86 ^D	70.33 ± 7.05 ^A	61.11 ± 1.00 ^B	60.11 ± 2.30 ^B
Cell size (mm)	0.488 ± 0.01 ^B	0.411 ± 0.02 ^{BC}	0.373 ± 0.02 ^C	0.573 ± 0.04 ^A	0.592 ± 0.08 ^A
Mean cell area (%)	21.88 ± 1.33 ^C	19.81 ± 1.34 ^D	30.65 ± 0.85 ^B	32.88 ± 0.96 ^A	32.91 ± 0.19 ^A
IDF (%)	3.48 ± 0.11 ^D	11.11 ± 0.10 ^A	10.75 ± 0.01 ^B	10.55 ± 0.07 ^B	9.78 ± 0.18 ^C
SDF (%)	0.53 ± 0.01 ^D	3.53 ± 0.05 ^B	3.66 ± 0.01 ^A	3.03 ± 0.03 ^C	3.58 ± 0.01 ^B
TDF (%)	4.01 ± 0.10 ^C	14.65 ± 0.13 ^A	14.42 ± 0.01 ^A	13.57 ± 0.05 ^B	13.37 ± 0.20 ^B
Total starch (%)	43.82 ± 1.30 ^A	36.67 ± 0.25 ^B	34.62 ± 0.58 ^{CD}	34.07 ± 0.68 ^D	35.85 ± 0.15 ^{BC}
AUC values	431.31 ± 21.4 ^A	302.12 ± 12.83 ^B	312.93 ± 8.22 ^B	308.21 ± 11.08 ^B	308.60 ± 9.02 ^B

Values are means ± standard deviation (n=3). Values in the same row with different letters differ significantly ($p < 0.05$). WB-wheat bran; AUC-

No significant difference in AUC value was observed between the control bran CSB and bran CSB with xylanase addition. According to the study of Laurikainen et al. (1998), incorporation of xylanase into rye bran bread reduced the amount of SDF and TDF due to the hydrolysis of AX. In the case of cellulase, the similar observation was found when adding 35 ppm cellulase to the bran CSB due to the mechanism of cellulase that hydrolyzes cell wall polysaccharides (Bhat, 2000; Harada et al., 2000).

10.3.3 The effect of combined enzymes on the physical properties of CSB with 15 % wheat bran

The effects of cellulase, xylanase and α -amylase at two levels each, on the physical quality of CSB supplemented with 15 % wheat bran were analyzed using 2^3 full factorial design (Table 10.2), and regression coefficients and R^2 obtained from the full factorial design in CSB quality are presented in Table 10.3. Table 10.3 shows that any enzyme and interaction of the enzymes significantly ($p < 0.05$) influences the volume, texture and crumb structure of CSB. As a result, the final empirical models for specific volume, moisture, hardness, gumminess, chewiness, cells, cell size and cell area follow:

$$W (\text{Specific volume}) = 2.39 - 0.02A + 0.02B + 0.01C - 0.06AB + 0.09AC + 0.06BC - 0.03ABC \quad (R^2 = 0.99)$$

$$W (\text{Moisture}) = 47.6 - 0.17A + 0.53B + 0.39C + 0.35AB - 0.37BC \quad (R^2 = 0.90)$$

$$W (\text{Hardness}) = 384.28 + 24.35A - 9.88C + 16.26AB - 34.62AC + 25.78BC + 8.63ABC \quad (R^2 = 0.98)$$

$$W (\text{Chewiness}) = 331.88 + 21.77A + 7.27C - 15.41AC + 9.66BC - 5.27ABC \quad (R^2 = 0.94)$$

$$W (\text{Cells}) = 45.98 + 2.38A - 3.5B - 3.26C - 1.76AC + 2.88BC + 2.93ABC \quad (R^2 = 0.95)$$

$$W (\text{Cell size}) = 0.79 - 0.03A + 0.06B + 0.04C + 0.07AC - 0.04BC - 0.05ABC \quad (R^2 = 0.97)$$

$$W (\text{Cell area}) = 33.96 - 0.43AB + 1.43AC - 0.39ABC \quad (R^2 = 0.85)$$

Factors: A – α -amylase; B – xylanase; C – cellulase; AB – α -amylase*xylanase; AC – α -amylase*cellulase; BC – xylanase*cellulase; ABC – α -amylase*xylanase*cellulase.

The addition of α -amylase to the bran enriched CSBs had a negative effect on loaf volume, whereas cellulase and xylanase had a positive effect. Furthermore, the interaction of cellulase and xylanase or α -amylase showed a significantly ($p < 0.05$) positive synergistic effects. The positive synergistic effects meant that the specific volume of bran CSB increased as an increase of α -amylase*cellulase and xylanase*cellulase. On the contrary, the interaction of α -amylase and xylanase had an antagonistic effect on the specific volume. Compared with the effect of single enzyme (Table 10.1), the combination of enzymes can improve the specific volume of bran CSB from 1.69 up to 2.50 mL/g when the enzymes were added with the level (6, 120 and 35 ppm). A similar result has been reported by Stojceska and Ainsworth (2008), when the addition of Pentopan (xylanase) and Celluclast (cellulase) significantly increased the specific volume of high-fibre bread. In the study of Laurikainen et al. (1998), the enzyme mixtures were more efficient than individual xylanase in giving a larger volume. Additionally, Katina et al. (2006) pointed out α -amylase combined with other enzymes such as xylanase can significantly improve the loaf volume and texture of breads incorporated with wheat bran. These observations could be related to the synergistic mechanism of xylanase and cellulase. In this study, the α -amylase, xylanase and cellulase combination also shows a significant synergistic effect on loaf height and moisture.

The combination of α -amylase, xylanase and cellulase led to a greater improvement of texture parameters of CSB with 15 % wheat bran than any single enzyme (Table 10.1). The interaction of α -amylase*xylanase and xylanase*cellulase had a significant positive synergistic effect on hardness, gumminess and chewiness, whereas the α -amylase*cellulase had a negative effect. Similar observations have been already reported by Caballero et al. (2007b), the combination of α -amylase, xylanase and protease significantly ($p < 0.05$) decreased the hardness, gumminess and chewiness of bread due to the modification of gluten-fibre network. Altuna et al. (2015)

illustrated that the optimum formulation of enzymes combination resulted in a lower crumb firmness of bread enriched with resistant starch than control bread due to the reverted effect of enzymes on the wheat proteins dilution. In addition, previous research illustrated that enzymes mixture had a beneficial effects on the bread enriched in high fibre owing to the changes in cell wall polysaccharides of the wheat flour (Katina et al., 2006; Laurikainen et al., 1998). In this study, the optimum results in terms of hardness, gumminess and chewiness were observed when the enzyme concentration of α -amylase, xylanase 6, 120 and 35 ppm, respectively.

The effect of α -amylase, xylanase and cellulase combination on the cell density of CSBs varied from 39 to 63.11 cells / cm². In terms of mean cell area and cell size, the effect of enzyme mixtures significantly ($p < 0.05$) increased the values from 0.503 mm and 31.85 % to 0.902 mm and 35.85 %, respectively. It can be seen from Table 4 the interaction of α -amylase and cellulase shows positive synergistic effects on mean cell area and cell size, and a negative effect on cell density. Pescador-Piedra et al. (2009) illustrated that the mixture of xylanase and glucose led to a bigger crumb cells than the sole xylanase. According to Caballero et al. (2007b), the interactive effect of laccase and transglutaminase modified significantly crumb structure, yielding bread with less cell density, but bigger cell area than those obtained by the treatment with singly transglutaminase due to a more open gluten network.

10.3.4 The effect of combined enzymes on the chemical properties of CSB

Tables 10.4 and 10.5 shows the combination of α -amylase, xylanase and cellulase reduced the dietary fibre and total starch content of CSB with 15 % wheat bran. Compared with single enzyme, the enzyme mixture was observed to decrease the levels of DF and starch content to minimum. A similar result was reported by Laurikainen et al. (1998), where mixtures of enzyme reduced the total fibre content of breads due to the enzymatic hydrolysis of fibre by the enzymes. In terms of AUC, the glycaemic response was varied when the combination of enzymes was added with different concentration. This observation probably due to the mechanism of

enzymes and interactions of α -amylase, xylanase and cellulase. Stojceska and Ainsworth (2008) reported that α -amylase can hydrolyze starch to glucose and increase the reducing sugar release. According to the study of Kumar and Wyman (2009), the interactions of cellulase-xylanase showed an incremental increase in reducing sugar release, especially cellobiose and xylose. Moreover, Song et al. (2016) illustrated synergistic combination of cellulase and xylanase can improve the reducing sugar concentrations of corncob, corn stover, and rice straw. Therefore, these observations can be suggested to explain the variation of glycaemic impact owing to the hydrolysis of enzymes resulting in varied reducing sugar release. Previous research has shown the DF can combine with proteins and form a matrix barrier surrounding the starch granules to reduce the enzyme activity (Foschia et al., 2015; Liu et al., 2016, 2017a). However, the enzymes combination can change the fibre-protein network due to the hydrolysis mechanism of α -amylase, xylanase and cellulase. The research of effect of enzyme combination on glycaemic response is limited.

Table 10.2 The effect of combined enzymes on the physical properties of CSB

Blocks	A	B	C	Volume (mL)	Specific volume (mL/g)	Loaf height (mm)	Moisture (%)	Hardness (g)	Springiness (mm)	Cohesiveness (ratio)	Chewiness (g)	Cells (cells/cm ²)	Cell size (mm)	Cell area (%)
Wheat flour	0	0	0	248.33	2.50	62.14	40.10	228.24	0.94	0.88	179.83	53.00	0.488	21.88
Wheat bran	0	0	0	176.33	1.69	46.59	43.35	686.46	0.79	0.41	329.97	48.33	0.411	19.81
1	6	70	35	264.67	2.49	59.68	46.89	370.77	0.97	0.88	308.24	48.11	0.807	35.34
2	6	70	60	228.67	2.16	56.24	48.28	385.97	0.95	0.87	323.73	45.22	0.722	31.85
3	6	120	60	266.00	2.50	60.18	47.88	383.37	0.98	0.88	341.85	39.00	0.875	34.18
4	6	120	35	266.33	2.50	60.13	48.01	299.61	0.96	0.90	266.63	41.44	0.896	35.53
5	10	70	35	247.67	2.34	56.94	45.71	473.47	0.93	0.89	366.12	63.11	0.503	32.05
6	10	120	35	230.33	2.18	55.17	48.21	432.79	0.94	0.85	357.45	43.66	0.797	32.04
7	10	120	60	261.67	2.47	56.81	48.43	412.61	0.98	0.91	349.96	45.22	0.842	34.86
8	10	70	60	270.00	2.50	60.77	47.37	315.65	0.98	0.88	341.05	41.44	0.902	35.85

All values are means (n=3). A (factor) – α -amylase; B (factor) – xylanase; C (factor) – cellulase; Wheat flour – wheat flour CSB; Wheat bran – CSB with 15 % wheat bran. (Data obtained from the 2³ full factorial design by Minitab 17. It is not one way ANOVA)

Table 10.3 The estimated regression coefficients of the factors of the physical properties of CSB incorporated with wheat bran

Coefficient estimate	Volume (mL)	Specific volume (mL/g)	Loaf height (mm)	Moisture (%)	Hardness (g)	Springiness (mm)	Cohesiveness (ratio)	Chewiness (g)	Cell density (cells/cm ²)	Cell size (mm)	Cell area (%)
Constant (β_0)	254.54	2.39	58.11	47.6	384.28	0.96	0.88	331.88	45.98	0.79	33.96
Amylase (β_1)	-1.88	-0.02	-0.93	-0.17	24.35	-0.006	NS	21.77	2.38	-0.03	NS
Xylanase (β_2)	NS	0.02	NS	0.53	NS	NS	NS	NS	-3.50	0.06	NS
Cellulase (β_3)	2.28	0.01	NS	0.39	-9.88	0.01	NS	7.27	-3.26	0.04	NS
Amylase*Xylanase(β_{12})	-8.21	-0.06	-1.14	0.35	16.26	-0.003	NS	NS	NS	NS	-0.43
Amylase*Cellulase(β_{13})	11.38	0.09	0.98	NS	-34.62	0.01	0.01	-15.41	-1.76	0.07	1.43
Xylanase*Cellulase(β_{23})	5.46	0.06	NS	-0.37	25.78	0.003	NS	9.66	2.88	-0.04	NS
Amylase*Xylanase*Cellulase	-3.46	-0.03	-0.58	NS	8.63	-0.003	0.01	-5.27	2.93	-0.05	-0.39
R^2	94.69%	99.40%	87.12%	89.85%	98.32%	90.55%	69.82%	94.15%	94.73%	96.82%	85.79%

NS – no significant effect at level ($p < 0.05$); R^2 – adjusted square coefficient (describes the percentage of variability for which the model accounts); β_0 – global means of parameters; β_1 , β_2 and β_3 – regression coefficients corresponding to main factors; β_{12} , β_{13} , β_{23} and β_{123} – regression coefficients corresponding to interactions; ‘+’ – positive effect; ‘-’ – negative effect;

Table 10.4 The effect of combined enzyme on the chemical properties of CSB containing wheat bran

Blocks	A	B	C	IDF %	SDF %	TDF %	Total starch %	AUC values
Wheat flour	0	0	0	3.48	0.52	4.01	43.82	491.3
Wheat bran	0	0	0	11.11	3.52	14.65	36.67	302.12
1	6	70	35	10.34	3.02	13.36	36.17	368.08
2	6	70	60	10.75	2.67	13.42	33.12	348.08
3	6	120	60	10.27	3.30	13.57	32.58	280.27
4	6	120	35	9.96	3.14	13.10	35.23	357.23
5	10	70	35	10.36	3.20	13.56	34.89	341.24
6	10	120	35	10.96	2.83	13.79	31.35	260.66
7	10	120	60	10.52	3.19	13.72	33.95	305.32
8	10	70	60	10.79	2.50	13.29	32.60	293.18

All values are means (n=3). A (factor) – α -amylase; B (factor) – xylanase; C (factor) – cellulase; Wheat flour – wheat flour CSB; Wheat bran – CSB with 15 % wheat bran; AUC-predicted glycaemic response. (Data obtained from the 2³ full factorial design by Minitab 17. It is not one way ANOVA)

Table 10.5 The estimated regression coefficients of factors of chemical properties of CSB containing wheat bran

Coefficient estimate	IDF %	SDF %	TDF %	Total starch %	AUC values
Constant (β_0)	10.47	2.98	13.46	33.73	319.30
Amylase (β_1)	0.15	-0.05	0.08	-0.53	-19.15
Xylanase (β_2)	-0.06	0.13	0.75	-0.45	-18.39
Cellulase (β_3)	0.07	-0.06	NS	-0.67	-12.60
Amylase*Xylanase(β_{12})	0.16	-0.05	0.11	NS	NS
Amylase*Cellulase(β_{13})	-0.11	-0.02	-0.13	0.75	11.61
Xylanase*Cellulase(β_{23})	-0.12	0.21	0.08	0.66	4.59
Amylase*Xylanase*Cellulase	-0.08	0.07	-0.02	0.56	18.81
R^2	96.25%	98.15%	97.71%	97.12%	95.15%

NS – no significant effect at level ($p < 0.05$); R^2 – adjusted square coefficient (describes the percentage of variability for which the model accounts); β_0 – global means of parameters; β_1 , β_2 and β_3 – regression coefficients corresponding to main factors; β_{12} , β_{13} , β_{23} and β_{123} – regression coefficients corresponding to interactions; ‘+’ – positive effect; ‘-’ – negative effect;

Table 10.6 Optimization of concentrations of α -amylase, xylanase and cellulase for improved quality of CSB with 15 % wheat bran

Bread samples	Wheat flour	15% Wheat bran (Control)	Optimum 1 6,120,35 ppm	Optimum 2 10,120,35 ppm
Volume (mL)	248.33 \pm 2.65 ^B	176.33 \pm 1.53 ^D	266.33 \pm 4.16 ^A	230.33 \pm 0.58 ^C
Specific volume (mL/g)	2.50 \pm 0.03 ^A	1.69 \pm 0.01 ^C	2.50 \pm 0.01 ^A	2.18 \pm 0.01 ^B
Loaf height (mm)	62.14 \pm 0.38 ^A	46.59 \pm 0.15 ^D	60.13 \pm 1.08 ^B	55.17 \pm 0.18 ^C
Moisture (%)	40.10 \pm 0.01 ^C	43.35 \pm 0.02 ^B	48.01 \pm 0.23 ^A	48.21 \pm 0.42 ^A
Hardness (g)	228.24 \pm 25.92 ^D	684.46 \pm 43.80 ^A	299.61 \pm 12.61 ^C	432.79 \pm 2.42 ^B
Chewiness (g)	179.83 \pm 19.34 ^C	329.98 \pm 39.76 ^A	266.63 \pm 11.71 ^B	357.44 \pm 2.09 ^A
Cohesiveness (ratio)	0.88 \pm 0.01 ^{AB}	0.41 \pm 0.01 ^C	0.90 \pm 0.02 ^A	0.85 \pm 0.02 ^B
Springiness (mm)	0.95 \pm 0.01 ^A	0.79 \pm 0.02 ^B	0.96 \pm 0.01 ^A	0.94 \pm 0.01 ^A
Cell density (cells/cm ²)	53.00 \pm 1.03 ^A	48.33 \pm 0.86 ^{AB}	42.11 \pm 2.77 ^C	43.67 \pm 2.08 ^{BC}
Cell size (mm)	0.488 \pm 0.01 ^C	0.411 \pm 0.02 ^C	0.89 \pm 0.06 ^A	0.80 \pm 0.01 ^B
Mean cell area (%)	21.88 \pm 1.33 ^C	19.81 \pm 1.34 ^C	35.53 \pm 0.16 ^A	32.04 \pm 0.68 ^B
IDF (%)	3.48 \pm 0.11 ^C	11.11 \pm 0.10 ^A	9.96 \pm 0.01 ^B	10.96 \pm 0.01 ^A
SDF (%)	0.53 \pm 0.01 ^D	3.53 \pm 0.05 ^A	3.12 \pm 0.01 ^B	2.82 \pm 0.01 ^C
TDF (%)	4.01 \pm 0.10 ^D	14.65 \pm 0.13 ^A	13.09 \pm 0.01 ^C	13.78 \pm 0.01 ^B
Total starch (%)	43.82 \pm 1.30 ^A	36.67 \pm 0.25 ^B	35.23 \pm 0.21 ^B	31.35 \pm 0.32 ^C
AUC values	431.31 \pm 21.4 ^A	302.12 \pm 12.83 ^C	357.26 \pm 10.25 ^B	260.53 \pm 6.59 ^D

Values are means \pm standard deviation (n=3). Values in the same row with different letters differ significantly ($p < 0.05$). AUC-predicted glycaemic response.

10.4 Conclusion

In this study, the physical – chemical properties of CSB with 15 % wheat bran were significantly affected by single enzyme addition. Compared to the single enzyme, the combination of α -amylase, xylanase and cellulase resulted in a higher volume, softer crumb, and lower AUC. The highest volume and softest crumb (optimum 1) were observed when the concentration of enzymes was 6, 120 and 35 ppm (Table 10.6). However, this concentration had the higher AUC (357.23) than the control (302.12). The lowest AUC (260.66) is shown in Table 6 when the concentration was 10, 120 and 35 ppm (optimum 2) with the lower specific volume (2.18 mL/g) and harder crumb (432.79 g). Therefore, the optimum concentrations of α -amylase, xylanase and cellulase can significantly improve the quality of bran CSB whereas reduce the nutritional value of CSB. For the baking industry, the consistent pursuit of high quality of product will also bring to the loss of nutritional value.

Chapter 11

Effect of α -amylase, xylanase and cellulase on the breadmaking properties and predicting glycaemic response of Chinese steamed bread enriched in oat bran

11.1 Introduction

Chapter 6 reported the effects of incorporating oat bran (OB) into Chinese steamed bread (CSB). As a result, the addition of 15 % oat bran significantly influenced the dough rheology and the final quality of CSB. In terms of dough rheology, the addition of oat bran significantly increased water absorption, development time and stickiness, whereas decreased extensibility of CSB dough due to the disruption of starch-gluten network by oat bran. For the physical properties of CSB, there was a significant decrease in specific volume and softening. Similar observation was reported by Campbell et al. (2008), who pointed out addition of oat bran increased water absorption of dough and reduced loaf volume. Additionally, Rieder et al. (2012) illustrated the addition of oat bran led to a significant decrease in bread volume accompanied by a significant increase in crumb firmness due to dilution of wheat gluten. However, the addition of oat bran showed a potential reduction of the sugar release, and consequently control the glycaemic response.

In order to improve the quality of CSB incorporated with 15 % oat bran, enzymes were used as individual and combination. In Chapter 8, the effects of α -amylase, xylanase and cellulase on the rheological properties of dough with 15 % oat bran were investigated. Compared to the single enzyme, the enzymes combination was more efficient on improving the dough rheology. As revealed by factorial design analysis, the mixture of α -amylase, xylanase and cellulase showed synergistic effects on the dough rheology parameters. For example, the blended enzymes can reduce the water absorption, development time and stability of oat bran dough to the minimum value when the enzyme combination were added with the high concentrations (10,

120 and 60 ppm). In particular, the combinations of α -amylase, xylanase and cellulase can increase the extensibility and stickiness of oat bran dough to higher value than the single enzyme. Therefore, the results revealed that the combination of enzymes had the potential to improve the final quality of CSB incorporated with 15 % oat bran.

Thus, this chapter investigates the effect of adding α -amylase, xylanase and cellulase, at different concentrations, on the quality and glycaemic response of CSB incorporated with 15 % oat bran.

11.2 Materials and Methods

11.2.1 Ingredients

The ingredients for the Chinese steamed bread were described in 3.1.

11.2.2 Physical properties of CSB

The physical characteristics of CSB incorporated with enzymes were determined according to the method described in 3.6.1 and 3.6.3.

11.2.3 Total starch and total, soluble and insoluble dietary fibre analysis

Total starch of CSB incorporated with enzymes was performed as described in 3.7.1.

Total, soluble and insoluble dietary fibre of CSB were performed as described in 3.7.2.

11.2.4 Glycaemic response analysis

Glycaemic response of CSB incorporated with enzymes was measured using *in vitro* digestion method described by Brennan et al. (2013) as outlined in 3.8.

11.2.5 Design of experiment

Two experimental designs were performed as described in 3.9.2: the first one to evaluate the effect of single enzyme on the breadmaking properties and predicting glycaemic response, and the second one to investigate the effect of combined enzymes on the breadmaking properties and predicting glycaemic response.

11.2.6 Statistical analysis

All data were treated by ANOVA and factorial design analysis as described in 3.9.

11.3 Results and discussion

11.3.1 Effect of single enzyme on physical and chemical properties of CSB incorporated with 15 % oat bran

Individual effects of α -amylase, xylanase and cellulase on physicochemical properties of CSB enriched with 15 % oat bran are shown in Table 11.1. With respect to α -amylase, the addition of α -amylase significantly ($p < 0.05$) improved the physical properties of CSB. For example, the volume, height, moisture, cohesiveness, springiness, cell size of oat bran CSB increased when adding 10 ppm α -amylase. Additionally, the addition of 10 ppm α -amylase resulted in a reduction of hardness, gumminess, chewiness and cell density. Similar results were observed by Renzetti, Courtin, Delcour, and Arendt (2010), who reported that enzyme addition significantly improved oat bread quality, such as increase in specific volume and decrease in crumb hardness and chewiness. Błaszczak et al. (2004) also indicated that addition of α -amylase significantly increased the specific volume and porosity of bread crumb due to the changes in the starch-protein network. However, there was no significant differences observed between oat bran CSB (control) and oat bran CSB supplemented with 10 ppm α -amylase in the chemical parameters (IDF, SDF, TDF, Total starch and AUC).

Oat bran CSB supplemented with 70 ppm xylanase had higher value of specific volume, loaf height, moisture cohesiveness, springiness and cell size compared to the control. However, the addition of xylanase significantly ($p < 0.05$) decreased hardness, gumminess, chewiness, springiness and cell density of oat bran CSB. Trogh et al. (2004) reported that the addition of xylanase to bread substituted with 40 % hull-less barley flour significantly improved the bread quality. Additionally, Jiang et al. (2010) and Ghoshal et al. (2013) illustrated that xylanase addition markedly improved the loaf volume and texture of bread. Schoenlechner et al. (2013) have found that the addition of single xylanase significantly increased the pore area and bread volume. This observation may be due to conversion of water-unextractable AX to enzyme-solubilized AX or water-extractable AX with high molecular weight (Courtin & Delcour, 2002; Jiang et al., 2005). Table 11.1 also shows that the SDF and TDF content slightly decreased when adding 70 ppm xylanase to oat bran bread due to the mechanism of xylanase. No significant differences were observed between control bread and oat bran bread treated by xylanase in total starch content and AUC value.

Table 11.1 illustrates the effect of cellulase and shows a similar trend to that observed with xylanase on the physicochemical properties of oat bran bread. According to Monica Haros et al. (2002), who illustrated that the presence of cellulase in bread resulted in an improvement of specific volume and crumb texture due to the hydrolysis action on the non-starch polysaccharides. Moreover, Harada et al. (2000) reported that the addition of cellulase led to an increase in specific volume and softer crumb. This observation maybe attributed to the mechanism of cellulase that hydrolyzes cell wall polysaccharides (Bhat, 2000; Harada et al., 2000).

These results indicate that the addition of single enzyme can improve the quality of CSB enriched with 15 % oat bran. However, compared with wheat flour bread, the single enzyme treatment can improve the bread up to the optimum standard. Therefore, the effects of combination of enzymes on CSB quality were investigated.

Table 11.1 The effect of single enzymes on the physicochemical properties of CSB containing 15 % oat bran

Bread samples	Wheat flour	15% OB (Control)	15%OB + 10ppm amylase	15%OB + 70ppm xylanase	15%OB + 35ppm cellulase
Volume (mL)	248.33 ± 2.65 ^A	194.33 ± 2.08 ^D	235.67 ± 4.04 ^C	239.33 ± 2.52 ^{BC}	246.00 ± 1.00 ^{AB}
Specific volume (mL/g)	2.50 ± 0.03 ^A	1.79 ± 0.01 ^D	2.18 ± 0.01 ^C	2.24 ± 0.01 ^B	2.27 ± 0.01 ^B
Loaf height (mm)	62.14 ± 0.38 ^A	50.62 ± 0.36 ^C	56.04 ± 0.69 ^B	55.33 ± 0.58 ^B	55.87 ± 0.17 ^B
Moisture (%)	40.10 ± 0.01 ^E	45.27 ± 0.06 ^D	45.82 ± 0.11 ^C	50.68 ± 0.29 ^A	47.37 ± 0.02 ^B
Hardness (g)	228.24 ± 25.92 ^D	519.03 ± 1.84 ^A	422.20 ± 9.80 ^B	339.59 ± 7.17 ^C	386.72 ± 8.49 ^B
Chewiness (g)	179.83 ± 19.34 ^D	419.34 ± 7.34 ^A	367.91 ± 5.82 ^B	337.58 ± 3.10 ^C	366.64 ± 4.89 ^B
Cohesiveness (ratio)	0.88 ± 0.01 ^B	0.85 ± 0.01 ^C	0.90 ± 0.01 ^{AB}	0.91 ± 0.01 ^A	0.88 ± 0.01 ^B
Springiness (mm)	0.95 ± 0.01 ^D	0.95 ± 0.01 ^D	0.98 ± 0.01 ^C	1.01 ± 0.01 ^B	1.07 ± 0.01 ^A
Cell density (cells/cm ²)	53.00 ± 1.03 ^D	80.33 ± 1.35 ^A	58.50 ± 0.86 ^C	52.17 ± 1.76 ^D	68.50 ± 1.00 ^B
Cell size (mm)	0.49 ± 0.01 ^C	0.41 ± 0.02 ^E	0.56 ± 0.01 ^B	0.61 ± 0.01 ^A	0.45 ± 0.02 ^D
Mean cell area (%)	21.88 ± 1.33 ^A	20.32 ± 0.76 ^A	21.61 ± 0.22 ^A	21.85 ± 0.58 ^A	21.38 ± 0.26 ^A
IDF (%)	3.48 ± 0.11 ^B	4.81 ± 0.14 ^A	4.76 ± 0.12 ^A	4.76 ± 0.11 ^A	4.71 ± 0.32 ^A
SDF (%)	0.53 ± 0.01 ^C	3.62 ± 0.05 ^A	3.39 ± 0.15 ^A	3.13 ± 0.07 ^B	3.00 ± 0.09 ^B
TDF (%)	4.01 ± 0.10 ^C	8.43 ± 0.13 ^A	8.15 ± 0.20 ^{AB}	7.89 ± 0.17 ^B	7.71 ± 0.25 ^B
Total starch (%)	43.82 ± 1.30 ^A	37.52 ± 0.26 ^B	36.75 ± 0.09 ^B	37.46 ± 0.08 ^B	36.65 ± 0.18 ^B
AUC values	431.31 ± 21.4 ^A	344.61 ± 2.81 ^B	336.25 ± 12.26 ^B	342.58 ± 4.20 ^B	341.77 ± 11.79 ^B

Values are means ± standard deviation (n=3). Values in the same row with different letters differ significantly ($p < 0.05$). OB-oat bran; AUC- predicted glycaemic response

11.3.2 Effect of enzymes combination on the physicochemical properties of CSB enriched in 15 % oat bran

The combined effects of cellulase, xylanase and α -amylase on the physicochemical properties of CSB enriched with 15 % oat bran were determined using full factorial design 2^3 (Table 11.2), and regression coefficients and R^2 obtained from the full factorial design are presented in Table 11.3. As a result, the final empirical models for specific volume, moisture, hardness, gumminess, chewiness, cells, cell size and cell area follow:

$$W (\text{Specific volume}) = 2.32 - 0.09A - 0.04B - 0.04C + 0.01AB + 0.01AC + 0.01BC + 0.02ABC \quad (R^2 = 0.98)$$

$$W (\text{Loaf height}) = 58.22 - 2.02A - 0.33B - 1.01C + 0.16AC \quad (R^2 = 0.90)$$

$$W (\text{Moisture}) = 46.85 + 0.53A + 0.24B + 0.66C - 1.89AB + 0.43AC + 0.36BC - 0.46ABC \quad (R^2 = 0.99)$$

$$W (\text{Hardness}) = 313.81 + 37.20A + 21.36B - 3.45AB - 9.99AC - 24.67BC - 17.25ABC \quad (R^2 = 0.98)$$

$$W (\text{Chewiness}) = 311.19 + 29.63A + 7.92B + 17.61C - 7.44AB - 11.53BC - 4.09BC - 24.21ABC \quad (R^2 = 0.98)$$

$$W (\text{Cells}) = 48.92 + 3.83A + 1.46B - 1.96C + 4.29AB - 3.66BC \quad (R^2 = 0.92)$$

$$W (\text{Cell size}) = 0.72 - 0.08A - 0.04B - 0.05AB + 0.03AC + 0.08BC - 0.03ABC \quad (R^2 = 0.97)$$

$$W (\text{Cell area}) = 21.72 - 0.58A - 0.47B - 0.33C + 0.37AC + 0.22BC \quad (R^2 = 0.85)$$

Factors: A – α -amylase; B – xylanase; C – cellulase; AB – α -amylase*xylanase; AC – α -amylase*cellulase; BC – xylanase*cellulase; ABC – α -amylase*xylanase*cellulase.

According to Table 11.2, the specific volume of CSB varied between 2.19 and 2.51 when enzymes combinations were added with different concentrations. Compared to the use of single enzyme, the use of a combination of enzymes improved the specific volume of oat bran bread up to the highest value (2.51 mL/g) when the concentration was 6, 70, 35 ppm. Table 11.2 indicates that the interaction of α -amylase, xylanase and cellulase had a positive synergistic

effect on the specific volume. Similar observation was reported by Flander, Holopainen, Kruus, and Buchert (2011), who indicated that a combination of tyrosinase, laccase, and xylanase significantly increased the specific volume and softness of oat bread. This observation may be attributed to the combined degradation of β -glucan and AX by combined enzymes. Stojceska and Ainsworth (2008) reported that combination of Pentopan (xylanase) and Celluclast (cellulase) significantly increased the specific volume of high-fibre bread. According to the study of Laurikainen et al. (1998), the enzyme mixtures were more efficient than individual xylanase in giving a larger volume.

In terms of texture of oat bran CSB, the combination of α -amylase, xylanase and cellulase significantly improved the texture of CSB. As a result, there was a significant decrease in hardness and increase in springiness, cohesiveness, gumminess and chewiness. However, the interaction of α -amylase, xylanase and cellulase showed a negative synergist effect on hardness of CSB. Similar observation was reported by Eugenia Steffolani et al. (2012), who illustrated that the combination of glucose oxidase, α -amylase and xylanase had a positive synergist effect on specific volume and crumb firmness whereas this synergistic effect was negative on crumb uniformity. Flander et al. (2011) indicated that combination of tyrosinase, laccase, and xylanase significantly increased softness of oat bread. Compared to the use of the single enzyme, the use of combined enzymes were more efficient in improving the texture due to the synergistic effects of enzymes. In this chapter, the optimum result in terms of hardness, gumminess and chewiness were observed when the enzyme concentration of α -amylase, xylanase and cellulase 6, 70 and 35 ppm, respectively.

The crumb structure of oat bran was significantly influenced when enzymes combinations were added. The addition of α -amylase, xylanase and cellulase combination decreased cell density from 80.50 to 45.33 cells / cm². However, there was an increase in cell size and cell area. The interaction of xylanase and cellulase shows a negative synergistic effect on cell density and a positive synergistic effect on cell size and cell density. According to Pescador-Piedra et al.

(2009), the mixture of xylanase and glucose resulted in larger crumb cells than just using xylanase. Moreover, Caballero et al. (2007b) illustrated that the interaction of laccase and transglutaminase significantly modified crumb structure, yielding bread with reduced cell density, but bigger cell area than those obtained by the treatment with singly transglutaminase due to a more open gluten network.

Table 11.4 illustrates that the effect of combination of enzymes on the total starch, total, soluble and insoluble dietary fibre content and predicted glycaemic impact of oat bran CSB. As a result, the enzymes combination decreased total fibre, soluble fibre, insoluble fibre and total starch content of oat bran CSB. Similar observation was reported by Laurikainen et al. (1998), who pointed that the blended enzyme reduced the total fibre content of breads owing to the enzymatic hydrolysis of enzyme. For the glycaemic impact, the AUC value was varied between 318.22 and 382.20 when the combination of enzymes was added with different concentration. This observation probably due to the mechanism of enzymes and interactions of α -amylase, xylanase and cellulase. Stojceska and Ainsworth (2008) reported that α -amylase can hydrolyze starch to glucose and increase the reducing sugar release. According to the study of Kumar and Wyman (2009), the interactions of cellulase-xylanase showed an incremental increase in reducing sugar release, especially cellobiose and xylose. Song et al. (2016) illustrated synergistic combination of cellulase and xylanase can improve the reducing sugar concentrations of corncob, corn stover, and rice straw. Therefore, these observations can be suggested to explain the variation of glycaemic impact owing to the hydrolysis of enzymes resulting in varied reducing sugar release. Previous research has found the DF can combine with proteins and form a matrix barrier surrounding the starch granules to reduce the enzyme activity (Foschia et al., 2015; Liu et al., 2016, 2017a). However, the enzymes combination can change the fibre-protein network due to the hydrolysis mechanism of α -amylase, xylanase and cellulase. Unfortunately, the research of effect of enzyme combination on glycaemic response is limited.

Table 11.2 The effect of combined enzymes on the physical properties of CSB containing 15 % oat bran

Blocks	A	B	C	Volume (mL)	Specific volume (mL/g)	Loaf height (mm)	Moisture (%)	Hardness (g)	Springiness (mm)	Cohesiveness (ratio)	Chewiness (g)	Cells (cells/cm ²)	Cell size (mm)	Cell area (%)
Wheat flour	0	0	0	248.33	2.50	62.14	40.10	228.24	0.94	0.88	179.83	53.00	0.488	21.88
Oat bran	0	0	0	194.33	1.79	50.62	45.27	519.03	0.95	0.85	419.34	80.50	0.41	20.32
1	6	70	35	266.67	2.51	60.97	44.78	233.34	1.18	0.91	257.19	46.17	0.95	23.82
2	6	70	60	265.00	2.42	60.46	43.59	270.26	1.21	0.90	275.23	49.67	0.65	21.98
3	6	120	60	252.33	2.31	59.28	49.50	305.04	1.05	0.92	346.18	38.00	0.87	21.20
4	6	120	35	260.33	2.41	60.22	47.41	297.82	1.13	0.91	247.67	46.50	0.74	22.17
5	10	70	35	251.33	2.32	58.46	47.83	300.12	1.06	0.90	305.95	45.33	0.73	21.68
6	10	120	35	242.33	2.19	57.27	44.73	419.78	1.00	0.90	363.52	65.50	0.47	20.55
7	10	120	60	246.67	2.20	54.76	46.73	318.06	1.08	0.92	319.07	51.50	0.61	21.05
8	10	70	60	239.67	2.21	54.31	50.21	366.07	0.98	0.91	374.73	48.67	0.70	21.29

All values are means (n=3). A (factor) – α -amylase; B (factor) – xylanase; C (factor) – cellulase; Wheat flour – wheat flour CSB; Oat bran – CSB with 15 % oat bran.

Table 11.3 The estimated regression coefficients of the factors of the physical properties of oat bran CSB

Coefficient estimate	Volume (mL)	Specific volume (mL/g)	Loaf height (mm)	Moisture (%)	Hardness (g)	Springiness (mm)	Cohesiveness (ratio)	Gumminess (g)	Chewiness (g)	Cell density (cells/cm ²)	Cell size (mm)	Cell area (%)
Constant (β_0)	253.09	2.32	58.22	46.85	313.81	1.08	0.91	288.56	311.19	48.92	0.72	21.72
Amylase (β_1)	-8.08	-0.09	-2.02	0.53	37.20	-0.06	NS	31.55	29.63	3.83	-0.08	-0.58
Xylanase (β_2)	-2.66	-0.04	-0.33	0.24	21.36	-0.02	NS	20.90	7.92	1.46	-0.04	-0.47
Cellulase (β_3)	-2.17	-0.04	-1.01	0.66	NS	NS	NS	5.95	17.61	-1.96	NS	-0.33
Amylase*Xylanase(β_{12})	2.18	0.01	NS	-1.89	-3.45	0.03	NS	-13.91	-7.44	4.29	-0.05	NS
Amylase*Cellulase(β_{13})	NS	0.01	0.16	0.43	-9.99	NS	NS	-12.93	-11.53	NS	0.03	0.37
Xylanase*Cellulase(β_{23})	NS	0.01	NS	0.36	-24.67	NS	NS	-10.01	-4.09	-3.66	0.08	0.22
Amylase*Xylanase*Cellulase	2.74	0.02	NS	-0.46	-17.25	0.03	NS	-17.83	-24.21	NS	-0.03	NS
R ²	86.68%	98.45%	90.85%	99.58%	98.96%	90.44%	39.82%	98.35%	98.19%	91.89%	96.87%	85.11%

NS – no significant effect at level ($p < 0.05$); R² – adjusted square coefficient (describes the percentage of variability for which the model accounts); β_0 – global means of parameters; β_1 , β_2 and β_3 – regression coefficients corresponding to main factors; β_{12} , β_{13} , β_{23} and β_{123} – regression coefficients corresponding to interactions; ‘+’ – positive effect; ‘-’ – negative effect;

Table 11.4 The effect of enzymes combination on the chemical properties of oat bran CSB

Blocks	A	B	C	IDF %	SDF %	TDF %	Total starch %	AUC values
Wheat flour	0	0	0	3.48	0.52	4.01	43.82	491.3
Oat bran	0	0	0	4.81	3.62	8.43	37.52	344.61
1	6	70	35	4.66	3.09	7.75	34.11	371.65
2	6	70	60	4.76	2.76	7.52	35.92	346.74
3	6	120	60	4.32	3.24	7.56	33.13	336.24
4	6	120	35	4.41	3.36	7.77	34.63	355.67
5	10	70	35	4.63	3.08	7.71	35.32	351.06
6	10	120	35	4.09	3.15	7.24	36.39	375.73
7	10	120	60	4.41	2.68	7.09	33.08	318.22
8	10	70	60	4.73	2.90	7.63	34.44	382.20

All values are means (n=3). A (factor) – α -amylase; B (factor) – xylanase; C (factor) – cellulase; Wheat flour – wheat flour CSB; Oat bran – CSB with 15 % oat bran. AUC-predicted glycaemic response.

Table 11.5 The estimated regression coefficients of factors of the chemical properties of CSB containing oat bran

Coefficient estimate	IDF %	SDF %	TDF %	Total starch %	AUC
Constant (β_0)	4.51	3.01	7.52	34.63	394.73
Amylase (β_1)	-0.04	-0.06	-0.10	0.18	NS
Xylanase (β_2)	-0.19	0.07	-0.12	-0.32	-8.18
Cellulase (β_3)	0.05	-0.14	-0.09	-0.48	-8.88
Amylase*Xylanase(β_{12})	NS	-0.11	-0.14	0.25	NS
Amylase*Cellulase(β_{13})	0.06	-0.04	NS	-0.56	NS
Xylanase*Cellulase(β_{23})	NS	NS	NS	-0.71	-10.44
Amylase*Xylanase*Cellulase	0.05	-0.07	NS	NS	-11.72
R^2	89.61%	89.36%	97.71%	96.76%	90.23%

NS – no significant effect at level ($p < 0.05$); R^2 – adjusted square coefficient (describes the percentage of variability for which the model accounts); β_0 – global means of parameters; β_1 , β_2 and β_3 – regression coefficients corresponding to main factors; β_{12} , β_{13} , β_{23} and β_{123} – regression coefficients corresponding to interactions; ‘+’ – positive effect; ‘-’ – negative effect;

Table 11.6 Optimization of concentrations of α -amylase, xylanase and cellulase for improved quality of CSB with 15 % oat bran

Bread samples	Wheat flour	15% Oat bran (Control)	Optimum 1 6,70,35 ppm	Optimum 2 10,120,60 ppm
Volume (mL)	248.33 \pm 2.65B	194.33 \pm 2.08C	266.67 \pm 5.18A	246.67 \pm 3.58B
Specific volume (mL/g)	2.50 \pm 0.03A	1.79 \pm 0.01C	2.51 \pm 0.02A	2.20 \pm 0.01B
Loaf height (mm)	62.14 \pm 0.38A	50.62 \pm 0.36C	60.97 \pm 1.18A	54.76 \pm 0.68B
Moisture (%)	40.10 \pm 0.01C	45.27 \pm 0.06AB	44.78 \pm 0.53B	46.73 \pm 0.62A
Hardness (g)	228.24 \pm 25.92C	519.03 \pm 1.84A	233.61 \pm 6.61C	318.06 \pm 3.62B
Gumminess (g)	191.75 \pm 19.15C	438.80 \pm 4.29A	211.14 \pm 12.02C	292.26 \pm 3.88B
Chewiness (g)	179.83 \pm 19.34D	419.34 \pm 7.34A	257.19 \pm 8.71C	319.07 \pm 3.11B
Cohesiveness (ratio)	0.88 \pm 0.02AB	0.85 \pm 0.01B	0.91 \pm 0.02A	0.92 \pm 0.02A
Springiness (mm)	0.95 \pm 0.01C	0.95 \pm 0.01C	1.18 \pm 0.03A	1.08 \pm 0.02B
Cell density (cells/cm ²)	53.00 \pm 1.03B	80.33 \pm 1.35A	46.17 \pm 3.97C	51.50 \pm 3.21B
Cell size (mm)	0.488 \pm 0.01C	0.41 \pm 0.02D	0.95 \pm 0.07A	0.61 \pm 0.01B
Mean cell area (%)	21.88 \pm 1.33B	20.32 \pm 0.76B	23.82 \pm 0.26A	21.05 \pm 0.68B
IDF (%)	3.48 \pm 0.11C	4.81 \pm 0.14A	4.66 \pm 0.01A	4.41 \pm 0.03B
SDF (%)	0.53 \pm 0.01D	3.62 \pm 0.05A	3.09 \pm 0.01B	2.68 \pm 0.01C
TDF (%)	4.01 \pm 0.10D	8.43 \pm 0.13A	7.75 \pm 0.01C	7.09 \pm 0.03B
Total starch (%)	43.82 \pm 1.30A	37.52 \pm 0.26B	34.11 \pm 0.23C	33.08 \pm 0.41C
AUC values	431.31 \pm 21.4A	344.61 \pm 2.81C	371.26 \pm 8.25B	318.22 \pm 6.59D

Values are means \pm standard deviation (n=3). Values in the same row with different letters differ significantly ($p < 0.05$). AUC-predicted glycaemic response.

11.4 Conclusion

This chapter investigated the effects of using α -amylase, xylanase and cellulase either used individually or in combination on the physicochemical properties of CSB substituted with 15 % oat bran. In terms of physical properties of oat bran CSB, the single enzyme can significantly improve the specific volume and texture of CSB. Compared to the single enzyme, the use of enzyme combinations increased the specific volume and cell size to higher value and decreased hardness to lower value due to the synergistic effect of enzymes. With respect to chemical properties, no significant effect was observed on TDF, SDF, IDF, total starch and AUC value when adding single enzymes. In contrast, the enzymes combination decreased fibre and starch content and valid the AUC value. Table 11.6 indicates that the two optimal concentrations of enzymes combination. The first one shows the highest volume and lowest hardness, whereas the higher AUC than oat bran CSB without enzymes. In contrast, the second solution has the lower AUC value than oat bran CSB accompanied by lower volume and higher hardness.

In conclusion, the optimum concentrations of α -amylase, xylanase and cellulase can significantly improve the quality of oat bran CSB whereas reduce the nutritional value of oat bran CSB. For the baking industry, the consistent pursuit of high quality of product will also bring to the loss of nutritional value.

Chapter 12

Effect of α -amylase, xylanase and cellulase on the breadmaking properties and predicting glycaemic response of Chinese steamed bread enriched in buckwheat flour

12.1 Introduction

In earlier research (Chapter 9), it was found that both individual and combined addition of α -amylase, xylanase and cellulase had a significant improvement of rheological properties of dough incorporated with 15 % buckwheat. For example, the addition of single enzyme into buckwheat flour dough reduced water absorption, development time, stability, departure time, extensibility and stickiness, whereas raised the softening, MTI and resistance to extension. Compared to single enzyme, the combined enzymes were more effective in improving the rheological properties of dough incorporated with 15 % bran and buckwheat flour due to the synergistic effect of α -amylase, xylanase and cellulase. The buckwheat dough incorporated with combined enzymes need less water and mixing time than buckwheat dough with single enzyme during dough handling. According to the study of Atalay et al. (2013), who illustrated that the combination of transglutaminase and sodium stearoyl-2-lactylate decreased the water absorption of dough incorporated with 20 % buckwheat milling product owing to synergistic effect between the additives. Additionally, Altuna et al. (2015) reported that the combination of transglutaminase, glucose-oxidase and xylanase improved the quality of dough incorporated with resistant starch to the regular dough (wheat flour dough produced without resistant starch or enzymes) level. In consequence, the combination of enzymes revealed a potential to improve the final quality of CSB incorporated with bran and buckwheat flour.

In order to prove the hypothesis, this chapter investigates the effect of adding α -amylase, xylanase and cellulase, at different concentrations, on the quality and glycaemic response of CSB incorporated with 15 % buckwheat flour.

12.2 Materials and Methods

12.2.1 Ingredients

The ingredients for the Chinese steamed bread were described in 3.1.

12.2.2 Physical properties of CSB

The physical characteristics of CSB incorporated with enzymes were determined according to the method described in 3.6.1 and 3.6.3.

12.2.3 Starch and fibre content analysis

Total starch of CSB incorporated with enzymes was performed as described in 3.7.1.

Total, soluble and insoluble dietary fibre of CSB were performed as described in 3.7.2.

12.2.4 Glycaemic response analysis

Glycaemic response of CSB incorporated with enzymes was measured using *in vitro* digestion method described by Brennan et al. (2013) as outlined in 3.8.

12.2.5 Design of experiment

Two experimental designs were performed as described in 3.9.2: the first one to evaluate the effect of single enzyme on the breadmaking properties and predicting glycaemic response, and the second one to investigate the effect of combined enzymes on the breadmaking properties and predicting glycaemic response.

12.2.6 Statistical analysis

All data were treated by ANOVA and factorial design analysis as described in 3.9.

12.3 Results and discussion

12.3.1 The effect of single enzymes on the physical and chemical properties of CSB incorporated with 15 % buckwheat flour

The use of single enzyme on the physicochemical properties of CSB supplemented with 15 % buckwheat flour is shown in Table 12.1. The addition of 10 ppm α -amylase did not significantly affect the volume and texture of buckwheat CSB, whereas increased the porosity of crumb. In terms of xylanase and cellulase, no significant differences could be observed between control CSB and CSB supplemented with 15 % buckwheat flour. Similar results were observed by Stefano Renzetti and Arendt (2009), who reported that the addition of glucose oxidase had no effects on the volume and texture of buckwheat bread. In addition, Atalay et al. (2013) found that the addition of transglutaminase to buckwheat milling products did not significantly improve the volume and texture of bread. According to Hamada, Suzuki, Aoki, and Suzuki (2013), the α -amylase activity decreased from 2.44 U/mL to 0.46 U/mL as fermentation progressed due to the lower thermal stability of the enzyme during fermentation of gluten-free dough. This research also suggested that the activities of cellulase and xylanase were significantly low and decreased during fermentation. Therefore, this observation may be due to the low activity of enzymes during the fermentation of buckwheat dough. The rheological properties of buckwheat dough were investigated in Chapter 9.

Table 12.1 Effect of single enzyme on the physical and chemical properties of CSB with buckwheat flour

Bread samples	Wheat flour	15% BW (Control)	15%BW + 10ppm amylase	15%BW + 70ppm xylanase	15%BW + 35ppm cellulase
Specific volume (mL/g)	2.50 ± 0.03 ^A	2.19 ± 0.01 ^B	2.20 ± 0.01 ^B	2.20 ± 0.01 ^B	2.22 ± 0.01 ^B
Loaf height (mm)	62.14 ± 0.38 ^A	57.65 ± 0.30 ^B	57.31 ± 0.31 ^B	56.46 ± 0.68 ^B	56.91 ± 0.88 ^B
Moisture (%)	40.10 ± 0.01 ^D	41.15 ± 0.10 ^C	45.93 ± 0.21 ^A	42.68 ± 0.29 ^B	45.91 ± 0.12 ^A
Hardness (g)	228.24 ± 25.92 ^B	401.31 ± 61.49 ^A	441.42 ± 10.80 ^A	459.16 ± 18.17 ^A	450.56 ± 15.89 ^A
Chewiness (g)	179.83 ± 19.34 ^C	307.93 ± 49.46 ^B	398.93 ± 9.35 ^A	401.35 ± 5.11 ^A	383.68 ± 13.75 ^A
Cohesiveness (ratio)	0.88 ± 0.01 ^A	0.85 ± 0.02 ^A	0.87 ± 0.02 ^A	0.87 ± 0.01 ^A	0.88 ± 0.02 ^A
Springiness (mm)	0.95 ± 0.01 ^A	0.89 ± 0.01 ^B	0.96 ± 0.02 ^A	0.95 ± 0.02 ^A	0.96 ± 0.02 ^A
Cell density (cells/cm ²)	53.00 ± 1.03 ^B	51.35 ± 2.05 ^B	56.85 ± 0.66 ^A	55.17 ± 1.86 ^{AB}	58.60 ± 1.20 ^A
Cell size (mm)	0.49 ± 0.01 ^A	0.39 ± 0.02 ^C	0.46 ± 0.01 ^B	0.51 ± 0.01 ^A	0.47 ± 0.02 ^{AB}
Mean cell area (%)	21.88 ± 1.33 ^A	20.12 ± 0.78 ^B	22.36 ± 0.12 ^A	23.15 ± 0.51 ^A	21.68 ± 0.21 ^B
IDF (%)	3.48 ± 0.11 ^B	4.76 ± 0.15 ^A	4.66 ± 0.21 ^A	4.72 ± 0.15 ^A	4.69 ± 0.26 ^A
SDF (%)	0.53 ± 0.01 ^B	2.50 ± 0.05 ^A	2.39 ± 0.15 ^A	2.43 ± 0.05 ^A	2.37 ± 0.06 ^A
TDF (%)	4.01 ± 0.10 ^C	7.26 ± 0.12 ^A	7.05 ± 0.25 ^A	7.15 ± 0.13 ^A	7.06 ± 0.25 ^A
Total starch (%)	43.82 ± 1.30 ^A	42.07 ± 0.21 ^B	40.86 ± 0.08 ^C	42.02 ± 0.06 ^B	41.76 ± 0.18 ^B
AUC values	431.31 ± 21.4 ^A	337.27 ± 2.81 ^B	326.25 ± 12.26 ^B	332.58 ± 12.20 ^B	350.17 ± 11.79 ^B

Values are means ± standard deviation (n=3). Values in the same row with different letters differ significantly ($p < 0.05$)

12.3.2 Effect of enzymes combination on the physicochemical properties of CSB

The combined effects of cellulase, xylanase and α -amylase on the physicochemical properties of CSB supplemented with 15 % buckwheat flour were determined using full factorial design 2^3 (Table 12.2 and 12.4), and regression coefficients and R^2 obtained from the full factorial design are presented in Table 12.3 and 12.5. The combination of cellulase, xylanase and α -amylase resulted in a greater improvement of loaf volume, texture and crumb structure of buckwheat CSB, compared to the single enzyme. The specific volume of buckwheat CSB was increased from 2.19 to 2.50 when the concentration was 6, 70, 60 ppm. A similar observation was reported by Atalay et al. (2013), who illustrated that the combination of transglutaminase and sodium stearoyl-2-lactylate improved the volume of bread containing buckwheat flour. Altuna et al. (2015) illustrated that the optimum formulation of enzymes combination resulted in a lower crumb firmness of bread enriched with resistant starch than control bread due to the reverted effect of enzymes on the wheat proteins dilution. The combination of enzymes also reduced the fibre and starch content of CSB containing 15 % buckwheat flour. In terms of glycaemic response, the values for AUC was varied when the combination of enzymes. Laurikainen et al. (1998) suggested that the combination of enzymes reduced the total fibre content of bread owing to the hydrolysis of fibre by enzymes. As discussed in previous chapters (Chapter 9, 10 and 11), this observation could be due to the synergistic and antagonistic effects of cellulase, xylanase and α -amylase.

In addition, Table 12.3 and 12.5 show the significant interactions of cellulase, xylanase and α -amylase. For example, the interaction of amylase*xylanase, amylase*cellulase and xylanase*cellulase shows a positive synergistic effects on the specific volume of buckwheat CSB. Table 12.5 illustrates that the interaction of amylase*cellulase and xylanase*cellulase had a positive effect on the predicted glycaemic response. This observation may be supported by Kumar and Wyman (2009) who illustrated that the interactions of cellulase-xylanase had an

incremental increase in reducing sugar release, especially cellobiose and xylose. Additionally, Song et al. (2016) illustrated synergistic combination of cellulase and xylanase can improve the reducing sugar concentrations of corncob, corn stover, and rice straw. The optimum results in terms of physical properties of buckwheat CSB were observed when the concentration of enzymes was 6, 70 and 60 ppm.

12.4 Conclusion

In this chapter, the individual addition of cellulase, xylanase and α -amylase did not significantly affect physicochemical properties of CSB incorporated with 15 % buckwheat flour. In comparison, the combination enzymes significantly improved loaf volume, texture and crumb structure of CSB enriched in 15 % buckwheat flour. The optimum physical properties of CSB were observed when the concentration of enzymes was 6, 70 and 60 ppm. However, the CSB added with optimum concentration enzymes showed a higher AUC value (341.74) than the control (337.27). In general, the optimum concentrations of cellulase, xylanase and α -amylase can significantly improve the quality of buckwheat CSB whereas reduce the nutritional value.

Table 12.2 Effect of enzymes combination on the physical properties of CSB incorporated with 15 % buckwheat flour

Blocks	A	B	C	Specific volume (mL/g)	Loaf height (mm)	Moisture (%)	Hardness (g)	Springiness (mm)	Cohesiveness (ratio)	Chewiness (g)	Cells (cells/cm ²)	Cell size (mm)	Cell area (%)
Wheat flour	0	0	0	2.50	62.14	40.10	228.24	0.94	0.88	179.83	53.00	0.488	21.88
Buckwheat	0	0	0	2.19	57.65	41.15	401.31	0.89	0.85	307.97	51.35	0.39	20.12
1	6	70	35	2.25	57.18	45.18	398.42	0.92	0.87	334.43	49.18	0.55	23.82
2	6	70	60	2.50	60.88	47.69	271.46	0.95	0.88	230.43	46.68	0.70	26.98
3	6	120	60	2.31	61.28	46.31	391.76	0.97	0.89	349.32	50.10	0.65	23.90
4	6	120	35	2.30	59.68	44.25	364.13	0.98	0.88	314.25	51.50	0.55	24.13
5	10	70	35	2.28	54.79	46.31	390.35	0.97	0.97	343.27	49.63	0.53	23.18
6	10	120	35	2.27	57.51	43.63	356.43	0.96	0.90	327.09	52.50	0.49	23.85
7	10	120	60	2.22	57.88	44.33	379.78	0.95	0.90	359.09	51.50	0.51	22.05
8	10	70	60	2.29	54.91	46.28	346.86	0.98	0.91	305.13	49.67	0.60	22.29

All values are means (n=3). A (factor) – α -amylase; B (factor) – xylanase; C (factor) – cellulase; Wheat flour – wheat flour CSB; Buckwheat – CSB with 15 % buckwheat flour.

Table 12.3 Estimated regression coefficients of the factors of the physical properties of CSB containing buckwheat flour

Coefficient estimate	Specific volume (mL/g)	Loaf height (mm)	Moisture (%)	Hardness (g)	Springiness (mm)	Cohesiveness (ratio)	Chewiness (g)	Cell density (cells/cm ²)	Cell size (mm)	Cell area (%)
Constant (β_0)	2.26	56.88	45.65	331.68	0.96	0.90	308.18	49.52	0.58	22.88
Amylase (β_1)	-0.02	-3.01	0.33	18.20	-0.01	NS	23.53	1.86	-0.11	-0.68
Xylanase (β_2)	-0.05	-0.03	0.47	11.33	-0.02	NS	8.52	1.26	-0.06	-0.56
Cellulase (β_3)	-0.07	-1.46	0.56	NS	-0.01	NS	11.31	-1.81	NS	-0.33
Amylase*Xylanase(β_{12})	0.02	0.12	-1.96	-2.14	0.02	NS	-3.21	3.66	-0.03	0.88
Amylase*Cellulase(β_{13})	0.03	0.11	0.66	-6.69	NS	NS	-12.11	NS	0.03	0.27
Xylanase*Cellulase(β_{23})	0.01	NS	0.26	-11.74	NS	NS	-3.08	-1.66	0.07	0.33
Amylase*Xylanase*Cellulase	0.02	NS	-0.44	-13.21	0.01	NS	-18.22	NS	-0.03	NS
R^2	98.45%	91.85%	99.81%	98.18%	89.98%	40.82%	98.69%	91.19%	96.89%	89.85%

NS – no significant effect at level ($p < 0.05$); R^2 – adjusted square coefficient (describes the percentage of variability for which the model accounts); β_0 – global means of parameters; β_1 , β_2 and β_3 – regression coefficients corresponding to main factors; β_{12} , β_{13} , β_{23} and β_{123} – regression coefficients corresponding to interactions; ‘+’ – positive effect; ‘-’ – negative effect;

Table 12.4 The effect of combination of enzymes on the chemical properties of CSB containing buckwheat flour

Blocks	A	B	C	IDF %	SDF %	TDF %	Total starch %	AUC values
Wheat flour	0	0	0	3.48	0.52	4.01	43.82	491.3
Buckwheat flour	0	0	0	4.76	2.50	7.26	42.07	337.27
1	6	70	35	4.46	2.39	7.05	41.26	351.65
2	6	70	60	4.09	2.33	6.42	40.89	341.74
3	6	120	60	4.12	2.38	6.50	40.02	346.24
4	6	120	35	4.11	2.35	6.46	40.59	344.67
5	10	70	35	4.23	2.30	6.53	40.26	351.06
6	10	120	35	4.09	2.45	6.54	42.15	358.83
7	10	120	60	4.11	2.38	6.49	41.36	338.22
8	10	70	60	4.23	2.40	6.63	40.78	312.20

All values are means (n=3). A (factor) – α -amylase; B (factor) – xylanase; C (factor) – cellulase; Wheat flour – wheat flour CSB; Buckwheat – CSB with 15 % buckwheat flour.

Table 12.5 Estimated regression coefficients of factors of the chemical properties of CSB containing buckwheat flour

Coefficient estimate	IDF %	SDF %	TDF %	Total starch %	AUC
Constant (β_0)	4.32	2.02	6.56	40.13	377.93
Amylase (β_1)	-0.02	-0.01	-0.08	-0.01	-7.18
Xylanase (β_2)	-0.13	0.03	-0.21	-0.02	-8.08
Cellulase (β_3)	0.07	-0.11	-0.10	-0.08	-9.22
Amylase*Xylanase(β_{12})	NS	-0.08	-0.12	0.15	NS
Amylase*Cellulase(β_{13})	0.02	-0.02	-0.08	-0.26	8.19
Xylanase*Cellulase(β_{23})	0.01	NS	NS	-0.52	8.44
Amylase*Xylanase*Cellulase	0.05	-0.03	NS	NS	1.72
R^2	91.11%	90.12%	96.58%	96.96%	95.23%

NS – no significant effect at level ($p < 0.05$); R^2 – adjusted square coefficient (describes the percentage of variability for which the model accounts); β_0 – global means of parameters; β_1 , β_2 and β_3 – regression coefficients corresponding to main factors; β_{12} , β_{13} , β_{23} and β_{123} – regression coefficients corresponding to interactions; ‘+’ – positive effect; ‘-’ – negative effect;

Chapter 13

General discussions and conclusions

13.1 General discussions

The first aspect of this study investigated the effect of the addition of wheat bran, oat bran and buckwheat flour on dough rheology and the quality of CSB. There was a significant ($p < 0.05$) increase in water absorption as the addition of wheat bran, oat bran and buckwheat flour levels increased from 0 to 15 %. The addition of wheat bran, oat bran and buckwheat flour to wheat flour dough significantly increased the development time and MTI. For the extension and stickiness test, there was a significant increase in dough stickiness and a decrease in extension and extensibility with increasing additions. Previous studies have found that the incorporation of dietary fibre into flour could disrupt the starch-gluten network structure, thus affecting dough viscoelastic behaviour and constraining dough machinability (Bonnand-Ducasse et al. 2010; Gómez et al. 2011). These observations were attributed to the dilution of gluten in wheat flour-based dough as well as disruption of the gluten hydration, which leads to the reduction of gas retention capacity (Elleuch et al., 2011; Foschia et al., 2015; Gómez et al., 2011; Rieder et al., 2012; Sudha et al., 2012). The final quality of CSB can be predicted from the results of dough rheology. In order to confirm the hypothesis, the physical properties of CSB incorporated with wheat bran, oat bran and buckwheat flour were investigated. The addition of bran and buckwheat flour were detrimental to the specific volume and texture of CSB, such as decrease in loaf volume, loaf height and softness. Foschia et al. (2015) indicated that the addition of dietary fibre led to inferior quality of baking products due to the disruption of gluten-starch network. However, the study reported in this thesis also showed that the addition of cereal bran and buckwheat flour significantly reduced the predicted glycaemic response. In particular, the CSB added with 15 % of bran and buckwheat had the lower AUC value than wheat flour CSB which may confer significant health benefits to the consumer. According to previous research,

the fibre enriched bread showed a significant reduction of sugar release. Foschia (2015) have also found that dietary fibre can combine with proteins and form a matrix barrier surrounding the starch granules to reduce the enzymes activity. Compare to the CSB incorporated cereal bran, CSB enriched with buckwheat flour shows better physical properties than bran CSB. For example, the specific volume of CSB with 15 % buckwheat flour (2.2 mL/g) was much higher than CSB with 15 % wheat bran (1.7 mL/g) and CSB with 15 % oat bran (1.8 mL/g). However, the addition of 15 % wheat bran had the most effective control of sugar release. This observation may be attributed to the different function of AX, β -glucan and resistant starch. Previous chapters indicated that the major dietary fibre of wheat bran, oat bran and buckwheat are AX, β -glucan and resistant starch, respectively.

In order to overcome the negative effects of the addition of wheat bran and oat bran, the second aspect of research investigated the individual and combined effect of α -amylase, xylanase and cellulase on the rheological properties of CSB. The addition of xylanase to oat and wheat bran dough did not significantly affect the water absorption, development time, stability, softening and departure time, whereas the addition of xylanase significantly ($p < 0.05$) decreased MTI and increased extensibility and stickiness. However, xylanase addition decreased the water absorption, development time, stability, departure time, extensibility and stickiness, but increased softening, MTI and resistance of buckwheat flour dough. There was no significant effect on water absorption, development time, stability, departure time and MTI of oat and wheat bran dough with added α -amylase. The incorporation of α -amylase to buckwheat flour dough reduced water absorption, development time, stability, departure time, extensibility and stickiness, whereas raised the softening, MTI and resistance to extension. The addition of cellulase to wheat bran dough increased the development time, whereas decreased water absorption and stability. For oat bran dough, cellulase addition reduced water absorption, softening and MTI, whereas increased stability, resistance to extension and extensibility of oat bran dough. The effects of cellulase on the buckwheat flour dough showed the same trend with

xylanase inclusion in buckwheat dough. In comparison with single enzyme, the enzymes combinations are more effective in the rheological properties of dough incorporated with 15 % bran and buckwheat flour due to the synergistic effect of α -amylase, xylanase and cellulase. The addition of enzymes combination decreased water absorption, development time, stability, whereas increased softening, MTI and extensibility. The results obtained from 2^3 full factorial design illustrated that synergistic effect of α -amylase, xylanase and cellulase significantly impact dough rheology. Similar observation was reported by Pescador-Piedra et al. (2009), the addition of glucose oxidase, peroxidase and xylanase combination had lower water absorption and mixing time than the addition of single enzyme. According to Liu et al. (2017b), who suggested that the combination of α -amylase, xylanase and cellulase had a synergetic effect on the dough rheology due to the interactions among enzyme activities and their coupled reactions. Additionally, Eugenia Steffolani et al. (2012) found that dough added with enzyme mixture (α -amylase, xylanase and glucose oxidase) had the intermediate stickiness. Therefore, the combination of enzymes revealed a better improvement of CSB dough handling, in consequence might improve the final quality of CSB incorporated with bran and buckwheat flour.

Thus, the third aspect of this study investigated the effect of α -amylase, xylanase and cellulase on the breadmaking properties and predicting glycaemic response of Chinese steamed bread enriched in bran and buckwheat flour. The results show that addition of single enzyme improved the loaf volume, texture and crumb structure of CSB. No significant change to total fibre and starch content was observed. The combination of enzymes led to a higher volume and softer crumb compared to the samples produced using a single enzyme inclusion. Moreover, the combination of enzymes reduced the fibre and starch content. However, the optimum concentrations of α -amylase, xylanase and cellulase showed highest loaf volume and softest crumb accompanied by higher AUC value. A similar observation was reported by Flander et al. (2011), who indicated that combination of tyrosinase, laccase, and xylanase significantly

increased the specific volume and softness of oat bread. This observation maybe attributed to the combined degradation of β -glucan and AX by combined enzymes. Additionally, Stojceska and Ainsworth (2008) reported that a combination of Pentopan (xylanase) and Celluclast (cellulase) significantly increased the specific volume of high-fibre bread. According to the study of Laurikainen et al. (1998), the enzyme mixtures were more efficient than individual xylanase in giving a larger volume. Stojceska and Ainsworth (2008) reported that α -amylase can hydrolyze starch to glucose and increase the reducing sugar release. According to the study of Kumar and Wyman (2009), the interactions of cellulase-xylanase showed an incremental increase in reducing sugar release, especially cellobiose and xylose. Song et al. (2016) illustrated that synergistic combinations of cellulase and xylanase could improve the reducing sugar concentrations of corncob, corn stover, and rice straw. These observations may help to explain the variation of glycaemic impact owing to the hydrolysis of enzymes resulting in varied reducing sugar release. Previous research has found the dietary fibre can combine with proteins and form a matrix barrier surrounding the starch granules to reduce the enzyme activity (Foschia et al., 2015; Liu et al., 2016, 2017a). However, combination of enzymes can change the fibre-protein network due to the hydrolysis mechanism of α -amylase, xylanase and cellulase. The research of effect of enzyme combination on glycaemic response is limited.

13.2 Conclusions

13.2.1 The effect of wheat bran, oat bran and buckwheat flour on the dough rheology and quality of Chinese steamed bread

The addition of wheat bran, oat bran and buckwheat flour into the CSB showed detrimental effects on the rheological properties of dough and physical properties of CSB due to the high hydration property of dietary fibre and the disruption of gluten network. In terms of dough rheology, the addition of bran and buckwheat flour significantly ($p < 0.05$) increased water absorption and development time. That meant the dough formed with bran and buckwheat flour were added more water and a longer mixing time than the control dough. Additionally, the

extensibility of dough was decreased with the levels of bran and buckwheat flour increasing from 5 % to 15 %. In consequence, the specific volume and softness were reduced significantly as the addition of buckwheat flour and bran increased. However, as revealed by *in vitro* method, the addition of bran and buckwheat flour has the potential reduction of the sugar release, and consequently control the glycaemic response, especially the 15 % level.

Specific Volume (mL/g):

Control (2.50) > 15 % Buckwheat (2.19) > 15 % Oat bran (1.79) > 15 % Wheat bran (1.69)

Hardness (g):

Control (228) < 15 % Buckwheat (401) < 15 % Oat bran (519) < 15 % Wheat bran (685)

AUC

Control (431) > 15 % Oat bran (344) ≥ 15 % Buckwheat (337) > 15 % Wheat bran (302)

This research indicates that wheat bran, oat bran and buckwheat flour can be used as a functional ingredient in food industry which may confer significant health benefits to the consumer.

13.2.2 The effect of α -amylase, xylanase and cellulase on the rheological properties of CSB dough enriched with wheat bran, oat bran and buckwheat flour

The addition of α -amylase, xylanase and cellulase created different effects on the rheology of regular dough and dough when incorporated into CSBs containing 15 % of bran and buckwheat flour. The addition of single enzyme did not significantly affect the water absorption, development time and stability, whereas such an inclusion increased extensibility and stickiness of bran dough. For the buckwheat flour dough, the single enzyme reduced water absorption, development time, stability, extensibility and stickiness, whereas increased softening, MTI and resistance to extension. In comparison with single enzyme, the enzymes combination significantly decreased the water absorption and development time to the minimum value, meanwhile increased the extensibility, softening and MTI of dough. The results obtained from factorial design suggested that the combination of α -amylase, xylanase and cellulase had a synergistic effect on the dough rheology due to the interactions among enzymes and their

coupled reactions. Therefore, the combination enzymes can be used to improve the dough handling in baking industry.

13.2.3 Effect of α -amylase, xylanase and cellulase on the breadmaking properities and predicting glycaemic response of CSB enriched in bran and buckwheat flour

In this study, both single enzyme and combined enzymes can overcome the negative effects of bran and buckwheat on the physical properties of CSB. Compared to the single enzyme, the combined enzymes increased the specific volume and cell size to higher value and decreased hardness to lower value due to the synergistic effect of enzymes. With respect to chemical properties, no significant effect was observed on TDF, SDF, IDF, total starch and AUC value when adding single enzymes. In contrast, the enzymes combination decreased fibre and starch content and valid the AUC value. The optimum concentrations of α -amylase, xylanase and cellulase were observed to improve the quality of CSB enriched in bran and buckwheat to the level of CSB without additives. However, this optimum solution was associated with higher AUC value than the bran and buckwheat CSB without enzymes.

13.2.4 Summary

This research found that wheat bran, oat bran and buckwheat flour can be good sources of dietary fibre added to Chinese steamed bread which may confer significant health benefits to the consumer. However, the addition of bran and buckwheat altered the rheological properties of the dough, and consequently the physical properties of CSB due to the high hydration property of dietary fibre and the disruption of gluten network. In order to overcome the negative effects of bran and buckwheat, the individual and combined effect of α -amylase, xylanase and cellulase on the dough rheology and physical-chemical properties of CSB were investigated. It was found that the combination of enzymes revealed a better improvement of CSB dough handling and breadmaking. However, the optimum solution was associated with higher AUC value than the bran and buckwheat CSB without enzymes. In addition, the results obtained from

factorial design illustrated that the combination of α -amylase, xylanase and cellulase had interactions among enzymes and their coupled reactions. Therefore, my personal view is that the combination of enzymes is more effective than single enzyme due to the synergistic effects of α -amylase, xylanase and cellulase. For baking industry, the consistent pursuit of high quality of product will also bring to the loss of nutritional value.

13.3 Recommendations for the future work

13.3.1 Investigation of Molecular weight distribution of Arabinoxylans and β -glucan

The present study found that the combination enzymes significantly reduced the TDF, SDF and IDF contents of CSB enriched in bran. Previous research indicated that the major dietary fibre component of wheat bran and oat bran is arabinoxylan (AX) and β -glucan respectively (Hemdane et al., 2016; Sudha et al., 2012). Additionally, xylanase and cellulase are hydrolases, which can hydrolyse the non-starch polysaccharides, resulting in a change in the of molecular weight distribution of the AX and β -glucan (Hilhorst et al., 1999). This change also influences the dough rheology and breadmaking properties of CSB (Pavlovich-Abril et al., 2016). Therefore, two possible future investigations should include:

1. Effect of α -amylase, xylanase and cellulase on the molecular weight distribution of AX and β -glucan in dough during fermentation.
2. Effect of α -amylase, xylanase and cellulase on the molecular weight distribution of AX and β -glucan in CSB during breadmaking.

13.3.2 Scanning electron microscopic (SEM) observations of dough and bread crumb

In this study, the image analysis was used to investigate the effects of addition of α -amylase, xylanase and cellulase on the crumb structure. As a result, the addition of bran and enzymes significantly influenced the porosity of CSB crumb. According to the previous studies, the

addition of additives can deform the starch-protein matrix, and consequently affect the dough rheology and crumb structure (Indrani et al., 2003; Kim, Morita, Lee, & Moon, 2003).

Therefore, another two possible future investigations should be done:

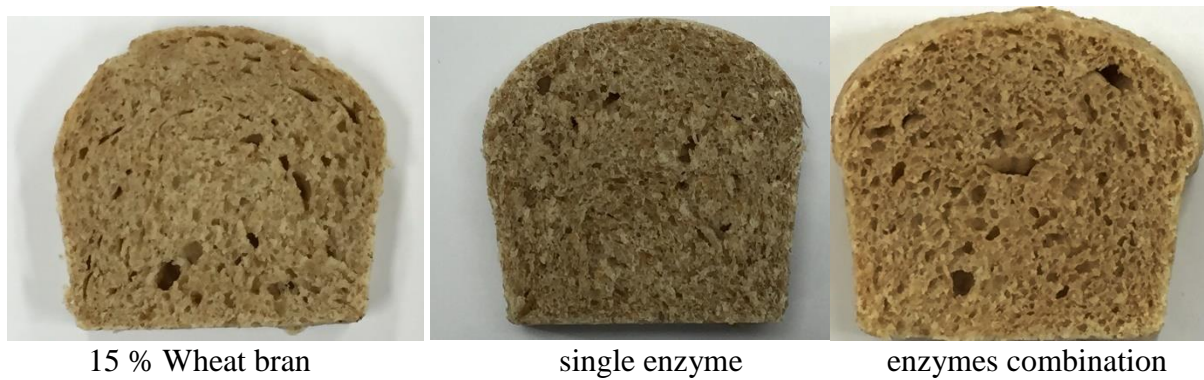
1. Scanning electron microscopic (SEM) observations of dough treated by bran and enzymes.
2. Scanning electron microscopic (SEM) observations of bread treated by bran and enzymes.

13.3.3 Sensory evaluation of CSB incorporated with additives

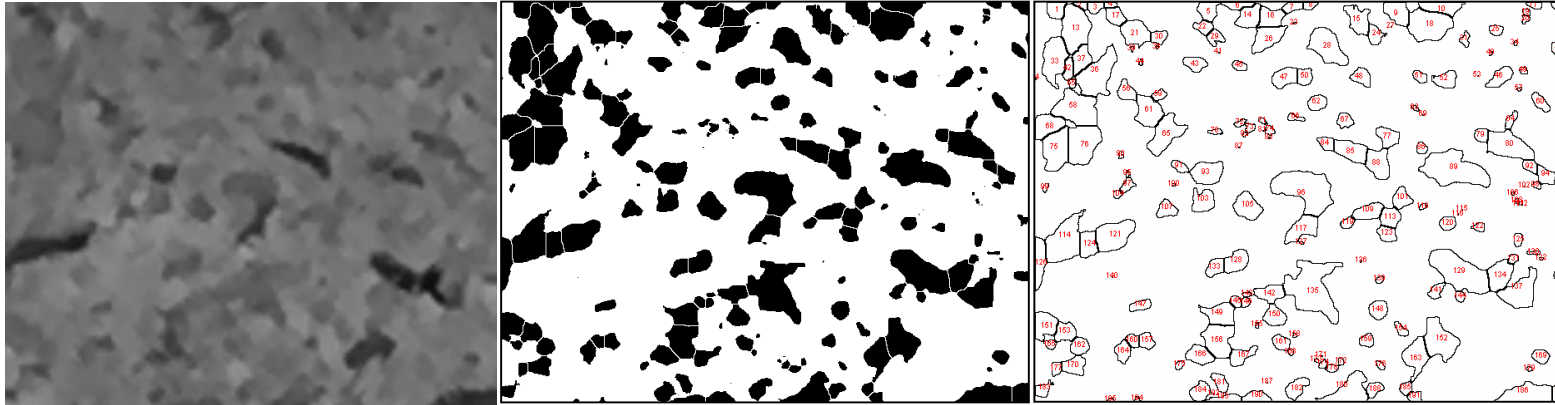
Previous research pointed out addition of bran, buckwheat flour and enzymes can influence the colour and flavour of the CSB (Gómez et al., 2003; Shah et al., 2006). Therefore, after the microbiological profiles of CSB are tested to ensure their hygiene safety for consumer, a sensory evaluation would be conducted.

Appendix

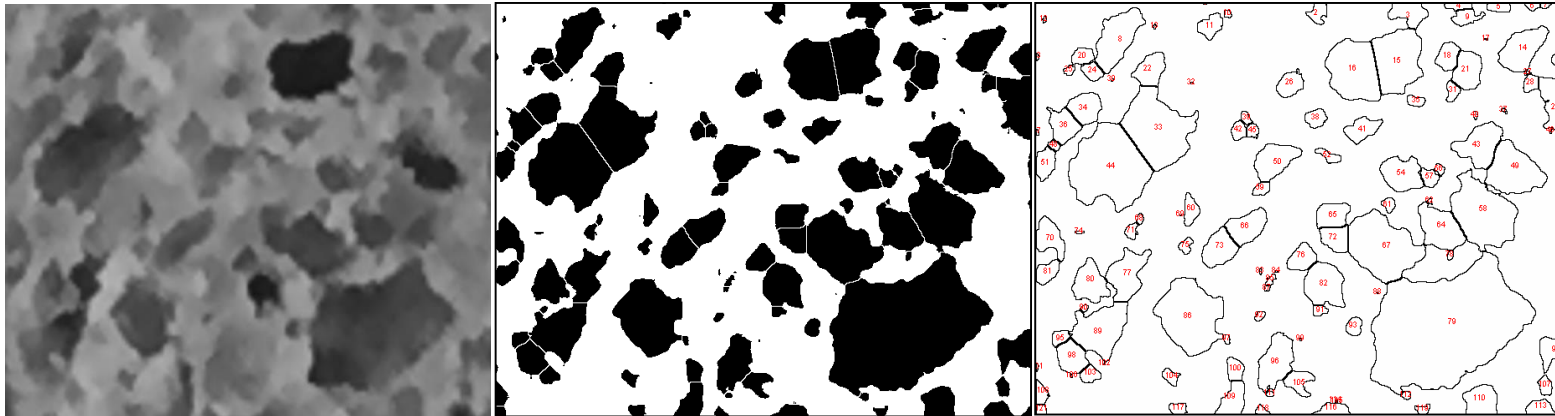
A.1 Photograph of Chinese steamed bread with 15 % wheat bran



A.2 Image J analysis



1. 15 % wheat bran CSB



2. 15 % wheat bran CSB + enzymes

A.3 DougLab report

18/11/2015

3:14:34p.m.

Test Details

Test file name Test wheat flour3.dwp
 Sample Details
 Test date & time November 18, 2015 2:52 pm
 DLW Software version My Version: 1.4.1.234
 Test run by User brennac2

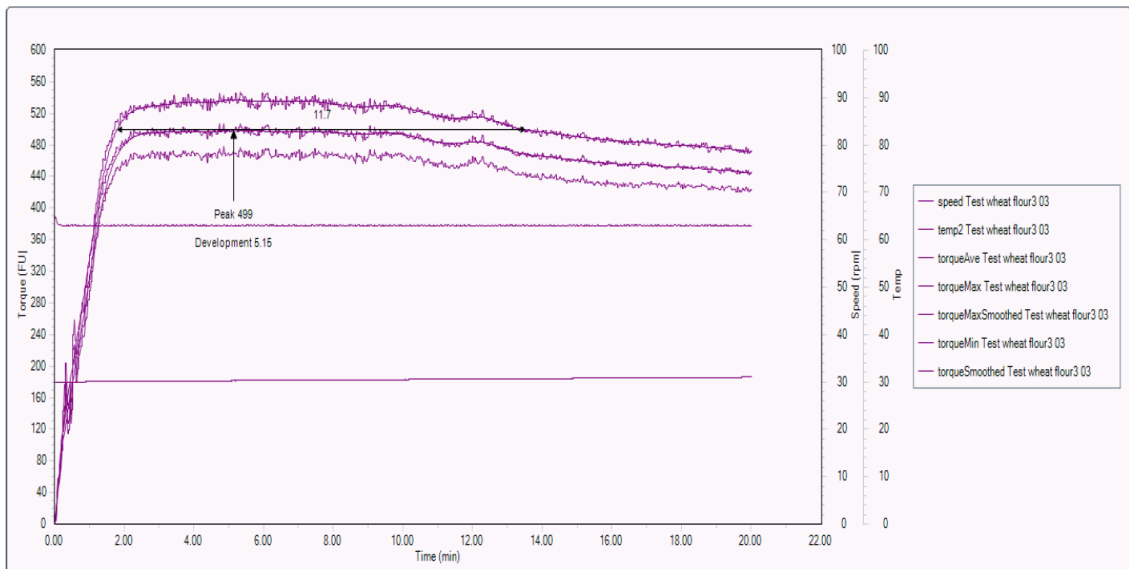
Sample Details

Bowl size	300 g	Expected absorption	63.80 %
Base flour amount	300.00 g	Flour weight	293.95 g
Added material solid	0.00 g	Water target	197.45 ml
Total added material solid	300.00 g	Water used	197.45 ml
Added material liquid	0.00 ml	Target peak resistance	500 FU
Moisture basis	14.00 %		
Sample moisture	12.23 %		

Analysis Formula Results

Peak	499.0 FU	WA as is	65.8 %
WA at target corrected	63.8 %	Development time	5.2 min
Stability	11.7 min	Softening	45.0 FU
MTI	11.4 FU	Peak Energy	4.7 Wh/kg
Bandwidth at Peak	68.1 FU	Quality Number	74.9

Graph



References

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