ผลของกากจากการผลิตน้ำนมถั่วเหลือง (โอการะ) หรือแป้งถั่วเหลืองต่อความไม่สดและคุณภาพการเก็บของขนมปัง

นางสาวศิริพรรณ รุ่งทองศรี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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ปีการศึกษา 2551

EFFECTS OF SOYMILK RESIDUE (OKARA) OR SOY FLOUR ON STALING AND KEEPING QUALITY OF BREAD

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Food Technology
Department of Food Technology
Faculty of Science
Chulalongkorn University
Academic Year 2008

Thesis Title	EFFECTS OF SOYMILK RESIDUE (OKARA) OR SOY FLOUR ON STALING AND KEEPING QUALITY OF BREAD
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ศิริพรรณ รุ่งทองศรี : ผลของกากจากการผลิตน้ำนมถั่วเหลือง (โอการะ) หรือแป้งถั่วเหลืองต่อความไม่สดและคุณภาพการเก็บของขนมปัง. (EFFECTS OF SOYMILK RESIDUE (OKARA) OR SOY FLOUR ON STALING AND KEEPING QUALITY OF BREAD) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : อาจารย์ ดร. ธนจันทร์ มหาวนิช, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: อาจารย์ ดร. เกียรติศักดิ์ ดวงมาลย์, 112 หน้า.

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของการทดแทนแป้งสาลีบางส่วนด้วยแป้งถั่วเหลืองไขมันเต็มและกากจากการผลิตน้ำนมถั่วเหลือง (โอการะ) ที่มีต่อความไม่สดและคุณภาพการเก็บของขนมปังขาว โดยทดแทนแป้งสาลีในสูตรขนมปังด้วยแป้งถั่วเหลืองหรือโอการะที่ระดับ 5 10 และ 15% โดยน้ำหนัก โดยทั่วไปพบว่าเมื่อทดแทนด้วยแป้งถั่วเหลืองและโอการะ ตัวอย่างขนมปังมีปริมาณโปรตีนหยาบ ไขมันหยาบ เส้นใยหยาบ และเถ้าสูงขึ้น ในขณะที่คาร์โบไฮเดรตที่ร่างกายใช้ได้มีปริมาณลดต่ำลง ขนมปังที่เติมแป้งถั่วเหลืองและโอการะที่ผลิตเสร็จใหม่ๆ มีปริมาณความชื้นและน้ำที่แช่แข็งได้สูงกว่าขนมปังแป้งสาลีควบคุมอย่างมีนัยสำคัญ ($p \leq 0.05$) ปริมาณความชื้นและน้ำที่แช่แข็งได้มีค่าลดต่ำลงเมื่อเวลาการเก็บเพิ่มขึ้น โดยตัวอย่างควบคุมมีการลดลงที่เร็วกว่า ขนมปังทุกตัวอย่างมีปริมาณน้ำที่แช่แข็งไม่ได้ไม่แตกต่างกันอย่างมีนัยสำคัญ ($p > 0.05$) และปริมาณน้ำที่แช่แข็งไม่ได้นี้มีค่าคงที่ตลอดการเก็บเป็นเวลา 7 วัน การเกิดเป็นผลึกใหม่ของอะมิโลเพกตินเกิดขึ้นมากที่สุดในตัวอย่างควบคุมที่เก็บไว้ ขนมปังที่เติมโอการะมีการเกิดเป็นผลึกใหม่ของอะมิโลเพกตินน้อยกว่าขนมปังที่เติมแป้งถั่วเหลืองที่ระดับการทดแทนเดียวกันเล็กน้อย การเพิ่มขึ้นของการเกิดเป็นผลึกใหม่ของอะมิโลเพกตินให้ผลที่สอดคล้องกับการลดลงของปริมาณสตาร์ชที่ละลายน้ำได้ การเปลี่ยนแปลงเหล่านี้เกิดขึ้นสูงสุดในช่วงสองวันแรกของการเก็บ ขนมปังที่เติมโอการะมีการลดลงของปริมาตร ปริมาตรจำเพาะ และความสูงของก้อนขนมปัง ในขณะที่น้ำหนักของก้อนขนมปังมีค่าเพิ่มขึ้นอย่างมีนัยสำคัญ การเพิ่มขึ้นของค่าความแข็งและการลดลงของค่าการเกาะตัวกันของเนื้อขนมปังและค่าความยืดหยุ่นสามารถเห็นได้ชัดเจนที่สุดในตัวอย่างที่เติมโอการะในระดับสูง อย่างไรก็ตามตัวอย่างควบคุมมีการเปลี่ยนแปลงค่าพารามิเตอร์ด้านเนื้อสัมผัสเหล่านี้มากที่สุดในระหว่างการเก็บ จากการศึกษาโดยใช้กล้องจุลทรรศน์แบบสเตอริโอพบว่าเซลล์อากาศของขนมปังที่มีส่วนผสมของถั่วเหลืองมีขนาดเล็กกว่าและมีรูปร่างและขนาดแตกต่างกันภายในตัวอย่างเดียวกัน เนื้อในขนมปังทุกตัวอย่างมีค่ามุมสีประมาณ 90° ซึ่งแสดงถึงสีเหลือง ความเข้มของสีเหลืองเพิ่มขึ้นเมื่อแทนที่ด้วยส่วนผสมของถั่วเหลือง ซึ่งมีการเพิ่มขึ้นสูงกว่าในตัวอย่างที่เติมแป้งถั่วเหลือง เปลือกนอกขนมปังทุกตัวอย่างมีค่ามุมสีอยู่ในช่วง 60° ซึ่งแสดงถึงสีเหลืองส้ม สำหรับการเปลี่ยนแปลงความเข้มสีของเปลือกนอกขนมปังที่มีส่วนผสมของถั่วเหลืองพบว่าไม่มีทิศทางที่แน่นอน จากการประเมินเชิงประสาทสัมผัสพบว่าตัวอย่างที่เติมแป้งถั่วเหลืองและโอการะมีกลิ่นถั่วเพิ่มขึ้นอย่างมีนัยสำคัญ จากการประเมินความชอบต่อขนมปังที่ผลิตเสร็จใหม่ๆ ผู้ชิมระบุว่าชอบตัวอย่างควบคุมมากกว่าตัวอย่างที่มีส่วนผสมของถั่วเหลือง อย่างไรก็ตามเมื่อพิจารณาจากคะแนนความชอบโดยรวมพบว่าตัวอย่างที่ทดแทนด้วยแป้งถั่วเหลืองที่ระดับ 5 10 และ 15% และตัวอย่างที่ทดแทนด้วยโอการะที่ระดับ 5% ยังได้คะแนนอยู่ในช่วงที่ผู้ชิมยอมรับได้

ภาควิชา เทคโนโลยีทางอาหาร	ลายมือชื่อนิสิต ศิริพรรณ รุ่งทองศรี
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4873603323 : MAJOR FOOD TECHNOLOGY

KEYWORDS : SOYMILK RESIDUE/OKARA / SOY FLOUR / STALING / BREAD

SIRIPHAN ROONGTHONGSRI : EFFECTS OF SOYMILK RESIDUE (OKARA) OR SOY FLOUR ON STALING AND KEEPING QUALITY OF BREAD. ADVISOR : THANACHAN MAHAWANICH, Ph.D., CO-ADVISOR: KIATTISAK DUANGMAL, Ph.D., 112 pp.

This research aimed to investigate the effects of partial substitution of wheat flour by full-fat soy flour or soymilk residue (okara) on staling and keeping quality of white bread. Wheat flour in the bread recipe was substituted by soy flour or okara at 5, 10 and 15% by weight. Upon substitution of soy flour and okara, bread samples generally exhibited an increase in crude protein, crude fat, crude fiber and ash contents with a decrease in available carbohydrate content. Moisture and freezable water contents of freshly baked soy flour- and okara-containing breads were significantly higher than those of the control wheat bread ($p \leq 0.05$). Both moisture and freezable water contents became decreasing with increasing storage time, with a sharper reduction in the control. Meanwhile, unfreezable water content was not significantly different among the samples ($p > 0.05$) and remained relatively constant throughout 7-day storage. Amylopectin recrystallization was observed to be most pronounced in the stored control bread. The okara-containing breads possessed slightly lower amylopectin recrystallization as compared to the soy flour-containing sample of the same substitution level. The increase in amylopectin recrystallization was consistent with the decrease in water soluble starch content. These changes were found to occur rapidly in the first two days of storage. Significant decreases in loaf volume, specific loaf volume and loaf height, and a significant increase in loaf weight, were observed in those breads containing okara. An increase in hardness and a decrease in cohesiveness and springiness were found to be especially prominent in the samples with higher levels of okara substitution. In spite of this, the control exhibited the greatest changes in those textural parameters during storage. As revealed under a stereomicroscope, crumb alveoli became smaller and varying in shape and size upon substitution of soy ingredients. Hue angle of all crumb samples was approximately 90°, representing yellow color. The intensity of yellowness increased upon soy substitution which was more pronounced in the soy flour-containing crumbs. Bread crusts exhibited hue angle in the 60° range, representing orange-yellow color. There appeared to be no definite trend in crust chroma changes upon soy substitution. From sensory evaluation, it was indicated that beany flavor significantly increased in soy flour- and okara-containing samples. From hedonic evaluation of freshly baked breads, the panel preferred the control over the soy-containing samples. However, as shown through overall preference score, the 5, 10 and 15% soy flour-substituted and 5% okara-substituted samples were still considered acceptable.

Department : Food Technology	Student's Signature Siriphan Roongthongsri
Field of Study : Food Technology	Advisor's Signature Thanachan Mahawanich
Academic Year : 2008	Co-Advisor's Signature Kiattisak Duangmal

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my thesis advisor and co-advisor, Dr. Thanachan Mahawanich and Dr. Kiattisak Duangmal, for their guidance, devotion and encouragement through the development of this thesis. I also wish to express appreciation to the thesis committee members, Asst. Prof. Dr. Romanee Sanguandeekul, Asst. Prof. Dr. Varapha Kongpensook and Asst. Prof. Dr. Kullaya Limroongreungrat, for their constructive comments and contributions to the improvement of this work. The financial support of Chulalongkorn University Graduate School is also gratefully acknowledged.

I am indebted to my colleagues and the staffs of the Department of Food Technology, Chulalongkorn University for their valuable help and suggestion. Finally, I would like to give my special thanks to my family whose loving support enabled me to complete this work.

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CHAPTER 1

INTRODUCTION

A sizable economic loss of bread industry is resulted from bread staling which characterizes by crumb firming, development of crumbliness and flavor change (Bárcenas and Rosell, 2006; Ribotta and Le Bail, 2007). Zobel and Kulp (1996) estimated that approximately 3% of the bread produced in the U. S. A. was returned as "unsalable," causing an economic burden on both producer and consumer. Over the years, food scientists have been trying to elucidate bread staling mechanisms as well as searching for a means to reduce bread staling. The majority of bread staling studies have been focusing on starch retrogradation. However, it has been shown that moisture migration, gluten changes and glassy-rubbery transition of the continuous amorphous domain also play a role in bread staling (Vodovotz, Hallberg and Chinachoti, 1996; Baik and Chinachoti, 2000; Hallberg and Chinachoti, 2002).

Soy flour is a good source of high quality protein. It contains about 40% protein on a dry basis. Soy protein provides not only nutritional value but also various functional properties including solubility, emulsification, foaming, gelation, and water-holding capacity (Kinsella, 1979). Soy protein, as well as soy fiber, helps enhance moisture retention in baked goods, thereby prolonging their freshness (Wolf, 1970).

Soymilk residue or okara is a by-product of soymilk and tofu manufacture. It contains high amounts of fiber (56%, db) and protein (27%, db) (Taruna and Jindal, 2002; Wachiraphansakul and Devahastin, 2005). Even though okara was known to provide many desirable nutritional and functional properties, it is currently underutilized in human diets. At present, okara from soymilk and tofu plants is mainly used as animal feed. Due to its large amounts of fiber and high-quality protein, there has been an attempt to use okara to fortify various food products. The protein fraction in okara possesses excellent water holding and emulsifying properties (O' Toole, 1999) which could be advantageous to the keeping quality of bakery products. Okara had lower solubility than commercial soy protein isolate at both acidic and alkaline pH, which is probably due to protein aggregation. However, their functional properties (i. e., emulsifying, water and fat binding, and foaming properties) were comparable to commercial soy protein isolate (Ma *et al.,* 1997).

The objective of this study was to investigate the substitution of soy flour or okara to wheat flour in white bread and its effect on the freshly baked bread quality, as well as on staling and keeping quality of the stored breads.

CHAPTER 2

LITERATURE REVIEW

2.1 Wheat flour

2.1.1 Wheat flour composition

Chemical composition of wheat flour is summarized in Table 2.1. Starch is the main component, constituting approximately 60% of the flour (Belitz and Grosch, 1986). Wheat flour contains very small amount of sugars which is less than 1% by weight (USDA, 2008). The sugars include fructose, sucrose, maltose and other oligosaccharides. Despite their minute amount, sugars play an important role in bread making. Besides serving as carbon source for yeast fermentation, they also provide the desirable golden brown crust color due to non-enzymatic browning during baking.

Table 2.1 Chemical composition of wheat flour

Composition	% (wb)
Moisture	13.2
Protein (N x 6.25)	11.7
Lipid	2.2
Starch	59.2
Other carbohydrates	10.1
Crude fiber	2.0
Minerals	1.5

(Source: Adapted from Belitz and Grosch, 1986)

Wheat flour contains trace amount of cellulose and hemicelluloses (Naivikul, 1997). Both polysaccharides are not digested in the human upper digestive system and hence considered dietary fibers. Pentosan, a water-soluble polysaccharide consisted of 5-carbon sugars (such as arabinose and xylose), is also found in wheat flour. With its good water holding capacity (fifteen times of its weight) and viscosity-providing property, it increases water absorption of the flour and therefore reduces dough mixing time, resulting in bread with higher loaf volume and better texture.

Small amount of lipids is present in wheat flour, including triglycerides, free fatty acids and sterols. Linoleic is the major fatty acid of wheat lipid, followed by palmitic and oleic acids. The lipids affect keeping quality, nutritional value and properties of the bread.

Wheat flour for bread-making contains 12-13% protein, which is higher than all-purpose (10-11% protein) and cake flour (8-9% protein). Wheat protein is divided into four different fractions based on their solubility (Table 2.2) being the water-soluble (albumins), salt-soluble (globulins), alcohol-soluble (prolamins, in wheat also known as gliadin) and acid-soluble (glutelins, in wheat also known as glutenin). Gliadin is preferentially responsible for viscosity while glutenin is responsible for elasticity (Belitz and Grosch, 1986). These gluten proteins (glutenin and gliadin), together with the wheat glycoprotein, provide viscoelasticity and gas-retaining capacity of the dough. Other proteins play a role in non-enzymatic browning during baking.

Table 2.2 Wheat protein distribution in Osborne-fractions

Fraction	Designation in wheat	%
Albumins	-	14.7
Globulins	-	7.0
Prolamins	Gliadin	32.6
Glutelins	Glutenin	45.7

(Source: Adapted from Belitz and Grosch, 1986)

Owing to the gluten ability to provide elasticity and retain gas, gluten protein quality and gluten content are the major factors affecting dough handling and bread quality. Gluten proteins interact and associate with one another to form elastic and coherent dough. Chemical interactions among proteins include hydrogen bonds (providing dough strength), disulfide linkages (providing dough cohesion), hydrophobic bonds (providing dough expansion with increasing temperature and therefore important in the early stage of baking) and ionic bonds (providing minor roles in dough consistency).

Cysteine side chain plays an important role in maintaining tertiary structure in polypeptide chains by forming intra- and inter-chain disulfide bridges between pairs of cysteine residues. The inter-chain disulfide linkages are of great importance in providing cohesion and elasticity. This is especially so for glutenin, which owes its high molecular weight to the disulfide crosslinking of smaller protein chains.

Proline, with its unusual ring structure, is known to disrupt the regular helical structure of polypeptide chain and thus lower the α-helix content of gluten protein (Bushuk, and Wrigley, 1974). This results in chain irregularities which contribute to chain entanglement and elasticity of the gluten.

2.1.2 Starch molecules and starch granule

Starch consists of two different types of polysaccharides, amylose and amylopectin. Amylose is a linear polymer, consisted of glucose units linked by α-(1⟶4) glucosidic linkages (Figure 2.1). Wheat amylose is composed of 1,000-6,000 monomeric glucose units (Sriroth and Piyachomkwan, 2000). Amylose molecule may contain a few α-(1⟶6) branches. However, the branch points are separated by several linearly-linked glucose units so that properties of amylose are essentially governed by the linear portion. Wheat starch contains about 28% amylose (BeMiller and Whistler, 1996).

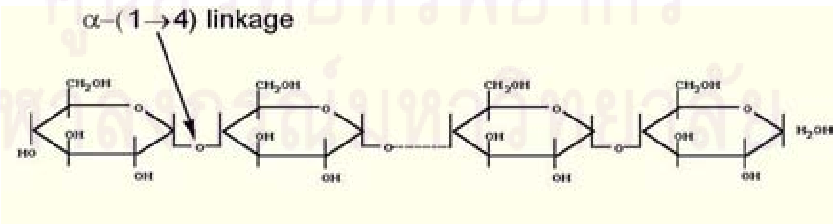


Figure 2.1 Amylose molecule

Amylopectin is a branched structure composed of glucose units linked together by α-(1⟶4) glucosidic linkages in the main chain and by α-(1⟶6) glucosidic linkages at the branch point (Figure 2.2). About 5% of all glucose units are α-(1⟶6)-linked.

Wheat amylopectin is composed of approximately $2x10^6$ glucose units (Sriroth and Piyachomkwan, 2000).

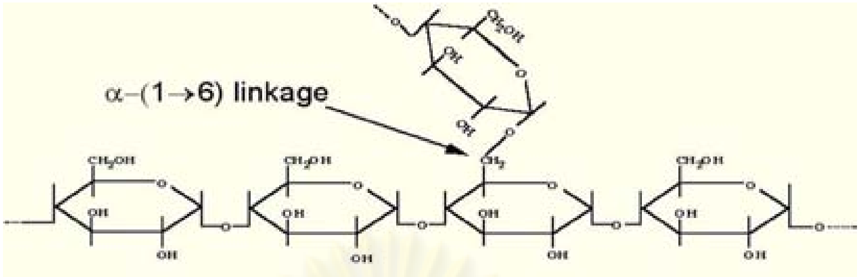


Figure 2.2 Amylopectin molecule

The branched structure of amylopectin consists of three types of chains, namely A-chain, B-chain and C-chain (Figure 2.3). The chain containing the only reducing end-group is called a C-chain. Therefore, a single amylopectin molecule has only one C-chain. C-chain has numerous branches, termed B-chains, to which A-chains are attached. A-chain is an unbranched structure which attached to another chain at only one position. Amylopectin structure consists of A-chain and B-chain in the ratio of 0.8-0.9:1. The branches of amylopectin are clustered and occur as double helices.

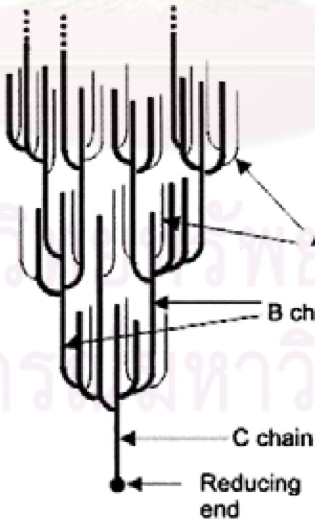


Figure 2.3 Branched structure of amylopectin molecule (Source: Chaplin, 2008)

Starch granules of wheat vary in sizes (Figure 2.4), ranging from 2-55 μm (BeMiller and Whister, 1996). Starch granule consists of amylose and/or amlopectin molecules which radially arrange, resulting in alternating layers of crystalline and amorphous regions. The linear amylose molecules, together with the clustered double-helix branches of amylopectin molecules, form highly ordered crystalline structure (Figure 2.5). Meanwhile, ordered arrangement could hardly form in the branching areas of amylopectin molecules due to steric hindrance, resulting in less dense amorphous layers. The radial, ordered arrangement of starch molecules in a granule as observed under a microscope reveals alternating layers of amorphous and crystalline regions, resembling growth rings in wood or layers of an onion (Figure 2.6). The ordered arrangement of starch molecules is evident when observed under a polarizing microscope, showing a "Maltese cross" pattern or birefringence (Figure 2.7).

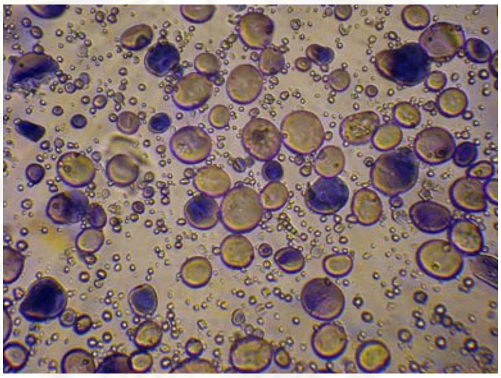


Figure 2.4 Wheat starch granules (Source: Kiselov, 2006)

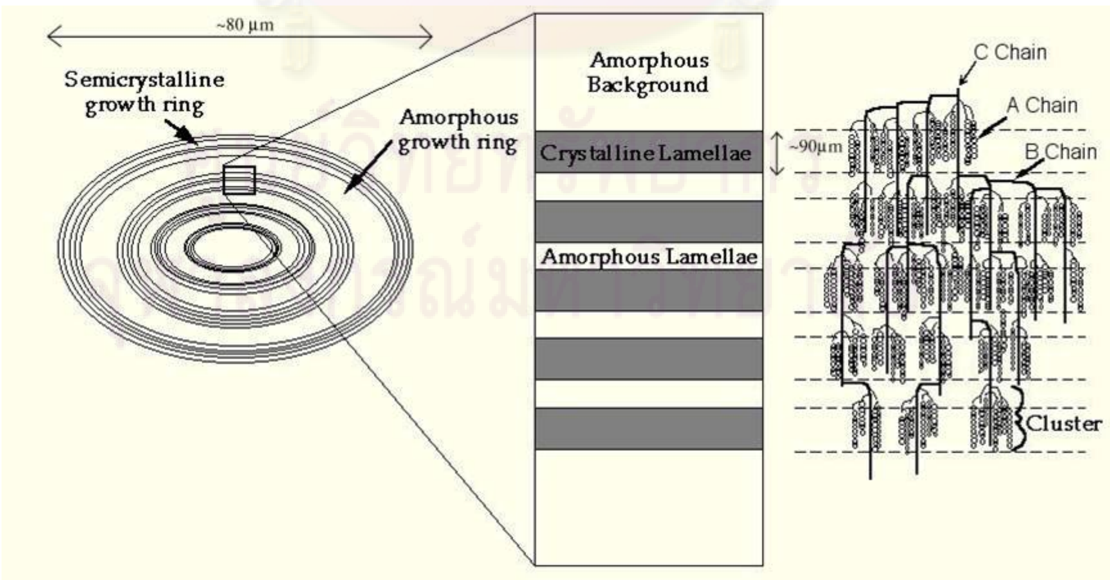


Figure 2.5 Radially arranged structure of alternating amorphous and crystalline regions in starch granule (Source: Nowjee, 2004)

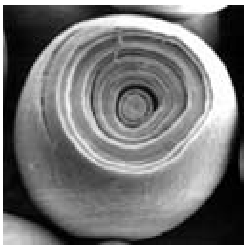


Figure 2.6 “Growth rings” or “onion-like structure” of starch granule (Source: Nowjee, 2004)

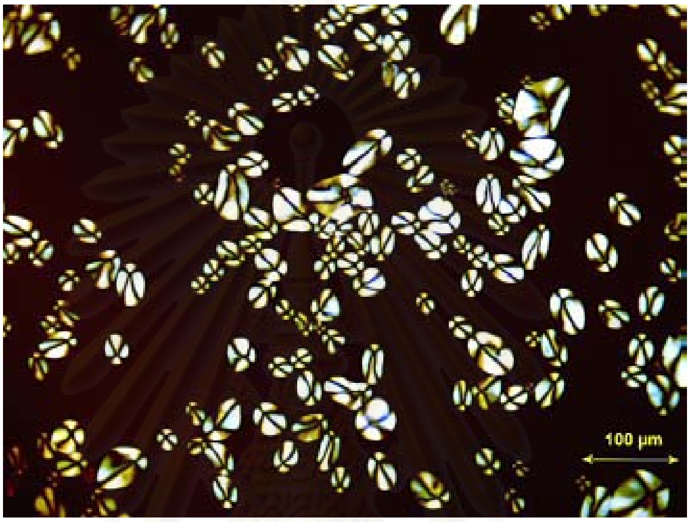


Figure 2.7 “Maltese cross” pattern or birefringence of starch granules as viewed under polarized light (Source: Matthew, 2004)

2.1.3 Granule gelatinization and pasting

Undamaged starch granules swell slightly in cold water and form a suspension. Cold water can penetrate into the amorphous areas, but not the crystalline regions due to their ordered and dense structure. By applying heat to a starch suspension, starch granules suddenly absorb water and swell beyond the reversible point. Water molecules enter between starch chains, break inter-chain hydrogen bonds, and establish hydration layers around the separated molecules. This leads to the loss of crystalline structure with the concomitant swelling of starch granule. The immediate swelling of starch granule is termed gelatinization. Wheat starch has a gelatinization temperature in the range of 52-63 ^{o}C (BeMiller and Whistler, 1996). Viscosity of the starch suspension starts to increase due to the friction among the swollen granules until reaching its maximum or peak viscosity. Upon continued heating, the fragile granules become disrupted, causing a decrease in viscosity (Figure 2.8).

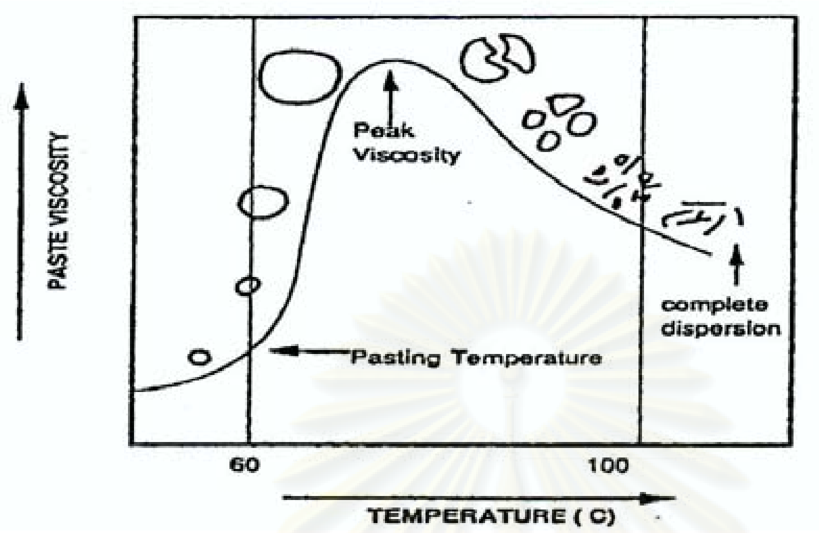


Figure 2.8 Viscosity changes during heating of starch suspension (Source: E-KMUTT, 2005)

2.1.4 Starch retrogradation

As starch paste or gel cools, dehydration and insolubilization of starch take place. Starch molecules reassociate via hydrogen bonding. The collective process of dissolved starch becoming less soluble is called retrogradation. Slow retrogradation can cause the starch molecules to reassociate and precipitate while a gel can form in the case of rapid retrogradation (Figure 2.9). Amylose undergoes retrogradation at a much more rapid rate than amylopectin. Retrogradation rate depends on several factors, such as ratio of amylose to amylopectin, temperature, starch concentration and other molecules present in the system. The effect of starch retrogradation could be demonstrated in various food systems including staling of bread and loss of viscosity and precipitation in soups and sauces.

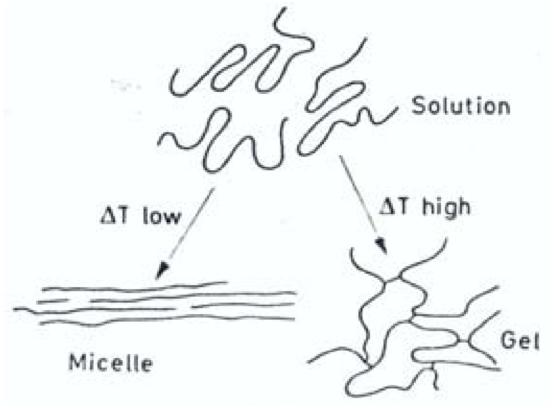


Figure 2.9 Retrogradation of amylose (Source: Belitz and Grosch, 1986)

2.2 Soybeans and soy products

2.2.1 Soybeans

Soybeans (*Glycine max* (L.) Merr.) have been cultivated since 2800 B. C. It is believed that the place of origin was in East Asia (Watanabe and Kishi, 1984). Soy foods have been widely consumed in the Orient since the ancient time. Early interests in the Western world, especially in the American soy industry, were in oil processing. However, food shortages have brought about the growing interest in soybeans as a source of food protein. Health benefits pertaining to their high isoflavone content also stimulate the soy product market.

Stored mature soybeans contain about 13% moisture to ensure stability. Soybeans have 35% protein, 17% oil, 31% carbohydrate and 4.4% ash on a wet basis. Based on solubility patterns, soy proteins are divided into two classes, albumins and globulins. Albumins are water-soluble while globulins are salt-soluble. The majority of soy protein is globulins, accounted for about 90% of the total proteins. Globulins of legume species are divided into legumins and vicilins. In soybeans, legumins and vicilins are commonly known as glycinins and conglycinins, respectively, after the soy genus name Glycine.

About 10% of the total soy carbohydrates are soluble carbohydrates. This soluble carbohydrate fraction consists of monosaccharides (such as glucose and arabinose) in very low concentration. Most of the soluble carbohydrates are the disaccharides and oligosaccharides, with 2.5-8.2% sucrose, 0.1-0.9% raffinose, and 1.4-4.1% stachyose. Raffinose and stachyose are known to cause flatulence and abdominal discomfort, thus limiting the use of soybeans and soy products. However, recent studies demonstrated some beneficial effects of dietary oligosaccharides, such as reducing toxic metabolites, reducing blood pressure and providing anticarcinogenic effects (Slavin, 1999; Arslanoglu, Moro and Boehm, 2007).

Insoluble carbohydrates in soybeans include cellulose, hemicelluloses and pectin. These insoluble carbohydrates are found mainly in cell walls. Kikuchi *et al.* (1971) reported that soybean cell walls contain about 20% cellulose, 50% hemicelluloses and 30% pectin. Soybeans contain negligible amount of starch.

2.2.2 Soy flour

Soy flour is available as full-fat and defatted products. Full-fat soy flour is made from dehulled whole soybeans. The dehulled soybeans are steam treated to destroy anti-nutritional factors (such as trypsin inhibitor, hemagglutinins, goitrogens and urease) and to inactivate lipoxygenase, an enzyme endogenous to soybeans. Full-fat soy flour is similar to whole soybeans in composition (Figure 2.10). Defatted soy flour is obtained after fat removal, either by mechanical means or by solvent extraction. Defatted soy flour contained about 5% fat as compared to that of 20% in full-fat soy flour (Mangino and Harper, 2007).

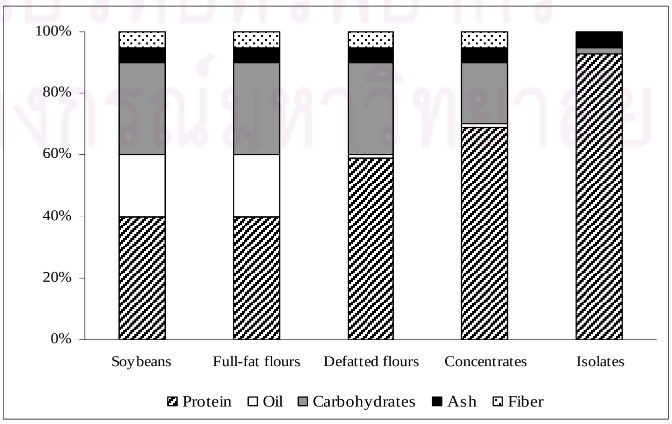


Figure 2.10 Composition (dry basis) of soybeans and soy protein products shown as approximated values (Source: De Meester, Kempener and Mollee, 2000)

2.2.3 Soymilk and soymilk residue (okara)

2.2.3.1 Soymilk and okara production and composition

Traditionally, soymilk is made by grinding soaked soybeans with water, filtering to remove the residue and then heating the extract. Composition of soymilk, and therefore soymilk residue, varies depending on variety and quality of soybeans, ratio of soybeans to water, type of equipment and processing conditions. Chemical compositions of soymilk at various ratios of dry soybeans to water as reported by Tanteeratarm, Nelson and Wei (1993) were summarized in Table 2.3.

Table 2.3 Composition of soymilk at various ratios of dry soybeans[a] to water

Ratio of dry soybeans to water	Total solids (%, wb)	Protein (%, wb)	Oil (%, wb)	Carbohydrates (%, wb)	Ash (%, wb)
1:5	9.2	4.5	2.4	1.9	0.48
1:6	8.7	4.2	2.2	1.9	0.44
1:7	7.9	3.8	1.9	1.8	0.39
1:8	7.2	3.4	1.7	1.7	0.35
1:9	6.3	2.9	1.5	1.6	0.30
1:10	5.6	2.6	1.4	1.3	0.27

[a] Sherman variety

(Source: Tanteeratarm *et al*., 1993)

The residue left from soymilk production is called okara or soy pulp. It consists of insoluble parts of the soybeans. About 1.1 kg of fresh okara is produced from every kilogram of soybeans processed for soymilk (Khare, Jha and Gandhi, 1995; O'Toole, 1999). Fresh okara contains about 80% moisture. Préstamo *et al.* (2007) reported that okara contains 49.0% dietary fiber, 33.4% protein, 19.8% oil and 3.5% ash on a dry basis. Roughly 18% of the proteins present in soybeans are recovered in okara.

Ma *et al.* (1997) reported that the protein isolated from okara had essential amino acid profiles similar to the FAO scoring pattern, with methionine and cysteine as the limiting amino acids. Their solubility was lower than commercial soy protein isolate which might be due to protein aggregation. However, other functional properties, including emulsifying, water and fat binding, and foaming properties, were comparable to the commercial soy protein isolate. O'Toole (1999) reported that okara had a protein efficiency ratio (PER) of 2.71, compared to a PER of 2.11 for soymilk, while the maximum PER of about 4.4 is reported for egg.

Redondo-Cuenca, Villanueva-Suárez and Mateos-Aparicio (2007) reported okara dietary fiber content of 55.48% on a dry basis. The insoluble fiber-to-soluble fiber ratio was 11:1. The insoluble fiber comprises of cellulose and hemicelluloses while pectic substances represent the majority of soluble fiber.

2.2.3.2 Okara utilization

Okara is generally used as animal feed for livestock producers (Noguchi, 1987; O'Toole, 1999). The use of okara as human food is very limited. A very small proportion of the okara produced is used in some traditional East-Asian and Southeast-Asian dishes such as *zha doufu* (a type of Chinese tofu), *biji-jjigae* (a Korean stew), *Unohana* (a Japanese side dish) and *tempe gembus* (an Indonesian okara tempeh).

At room temperature, okara spoils quickly due to its high moisture content (Noguchi, 1987; O'Toole, 1999). Fermentation by lactic acid bacteria is one method that can be used to preserve okara (O'Toole, 1999). As lactic fermentation occurs, pH value is lowered to less than 4.2, which inhibits the growth of spoilage microorganisms. The fermented okara can be stored at room temperature for 4 days or longer. Another method used to prolong the shelf-life of okara is drying. This must be done soon after the okara production. O'Toole (1999) reported that dried okara containing 7.4% moisture had a storage life of 8 months at 5 °C.

Because of its abundant dietary fiber, okara is suitable for use as a source of dietary fiber and to reduce caloric intake. It also contains high-quality protein which possesses beneficial functionalities such as water holding and emulsifying properties which could be incorporated in many food recipes. Due to its high nutritional value, researchers have been trying to increase the use of okara as human food. However, in some products, its used is limited by the high fiber content (O'Toole, 1999).

Khare, Jha and Sinha (1995) fortified biscuits with okara at the levels of 20-100%. It was reported that biscuit with 60% okara supplementation was the most acceptable to consumers. Protein and dietary fiber contents of the biscuit were 8.72 and 5.98%, respectively, and the biscuit can be stored for at least one month.

2.3 Bread

Bread is a staple food prepared by baking a dough of flour and water. Primarily, the flour used for bread making is wheat flour. The bread could be leavened or unleavened and could be added with various ingredients such as yeast, salt, fat, sugar, milk, malt, other cereals, fruit and vegetable purees and spices. Additives such as antimicrobial agent and dough enhancer could also be added in order to improve dough handling and increase the product shelf-life.

2.3.1 Bread ingredients and their functionalities

Basic white bread ingredients comprise of wheat flour, yeast, salt, water, fat and sugar. Wheat flour provides the bulk and gives structure to the bread. Wheat flour for bread making must have sufficient protein content (usually 12-13%) in order to provide elasticity, strength and stability to the dough.

The yeast used in bakery products is *Saccharomyces cerevisiae* or Baker's yeast. Upon fermenting sugars, the yeast produces carbon dioxide along with ethanol and water. Carbon dioxide is essential for dough expansion and this gives the baked product desirable soft and porous texture. The ethanol produced also gives characteristic flavor to the bread.

In most bread recipes, salt is required in small quantity. Apart from bringing out the flavor in bread, salt helps strengthen the dough and thus increase the loaf volume. Salt also slow down the yeast fermentation rate which results in bread with finer grain and evenly distributed air cells. Water in a bread recipe acts as a plasticizer and a solvent. With proper water content, a homogeneous mixture could be obtained and gluten could be formed. Water also activates enzymes and induces starch gelatinization.

In bread making, various types of fat could be used. Examples are butter, shortening, vegetable oil and lard. Fat helps provide bread with higher loaf volume, softer crumb, thinner crumb cell wall and improved flavor. Sugar provides sweetness as well as serving as a substrate for yeast fermentation. It also retains moisture and gives the crust a golden brown color.

2.3.2 Bread-making

2.3.2.1 Dough preparation

Generally, there are four methods of dough preparation, the “sponge and dough,” “straight dough,” “brew process,” and “continuous mixing” methods. In the sponge and dough method, there are two mixing periods and two fermentation periods. First, the sponge is prepared by mixing part of the ingredients which generally consists of 60-70% of the flour, yeast, yeast food and 65% of the water in the recipe. Then, the mixture is allowed to ferment for 3-5 hours. The fermented sponge is then mixed with the remaining ingredients to form a dough. After gluten has fully developed, the dough is fermented for the second time for a shorter period of 15-20 minutes.

In the straight dough method, ingredients are mixed all at once and the dough is prepared using a single fermentation step. Generally, fermentation time for the straight dough method lasts about 1-3 hours. At about 80% of the total fermentation time, the dough is punched to release the gas and the dough is let to ferment for the remaining time. The bread produced using this straight dough method has coarser grain and the crumb is not as soft as those produced by other methods. The loaf volume will be lower than that made with the sponge and dough method.

For the brew process, the yeast is mixed with small amount of sugar and a portion or all of the water in the recipe. The mixture is allowed to ferment for 10-15 minutes. This yeast mixture is then mixed with the remaining ingredients to form a dough which is then allowed to rise for 1-2 hours. This brew process is also used commercially with continuous, computerized process called continuous mixing method.

2.3.2.2 Changes during bread making

Physical properties of bread dough vary according to the process being used. In general, bread making process includes hydration of raw materials, mixing and other mechanical handling steps, which modify the physical form of the ingredients in the dough that determines initial rheological property of the system.

In the initial stages of dough preparation, wheat flour is mixed with water to form viscoelastic dough. This occurs in two separate stages, the rapid hydration of flour components and the changes of protein phase caused by intensive mixing of the dough. In the first stage, proteins in the flour hydrate to their full capacity while starch granules hydrate within their amorphous regions. The water was distributed between the continuous phase of protein gel, which also contains the hydrocolloid pentosan, and the dispersed phase of native starch granules and those starch granules which have been damaged during milling. Damaged starch granules lose their crystallinity during milling (Beckett, 1995; Guy, 1995) and the starch polymers can be fully plasticized by the available water, so that they swell and form a viscoelastic gel. Within the hydrated flour, native starch granules remain almost intact. Upon continued mixing, wheat proteins transform into gluten, which has the ability to flow under low stress (i. e., dough expansion) as well as to retain air bubbles in the structure.

Dough fermentation occurs in two different stages. The first stage involves the action of enzymes of wheat flour (α- and β-amylases) and those in yeast (maltase and invertase). These enzymes digest carbohydrates from damaged starch and converted them into maltose and dextrins. The small carbohydrates produced by enzyme action, as well as free sugars of the flour, are further digested by the yeast zymase enzyme complex in the second stage, with the production of carbon dioxide which causes the dough to rise. Ethanol, carbonyl compounds, esters and acids which are also produced in this second-stage fermentation contribute to aroma and flavor of the bread.

Upon baking, various physicochemical changes occur, including water evaporation, moisture migration, gas formation, volume expansion, protein denaturation, starch gelatinization and non-enzymatic browning (Maillard reactions and caramelization) (Mondal and Datta, 2008). The quality and shelf life of the product are mostly governed by the time and temperature of the baking process (Mondal and Datta, 2008).

During heating, dough temperature begins to rise as the heat transfers from the outside towards the center. Water vaporizes from the dough surface and a portion of water condenses in the cooler inner part of dough. Therefore, final moisture content at the center of the freshly baked bread may be as high as the initial dough. The dough expands due to the inside pressure which continues to increase. The temperature of the loaf keeps increasing until reaching the melting temperature of the starch granules, which is about 65-70 °C. At this point, the viscosity of the bread cell wall increases abruptly and the cell ruptures, releasing the gas pressure. This event occurs from the outer surface of the loaf towards the center. Eventually, the center of the bread is set so that porous structure is formed throughout the loaf. Confocal scanning laser microscopy of the bread revealed a continuous protein phase and a discontinuous non-gelatinized starch phase in the crust while a gelatinized starch network associated with a protein network was observed in the crumb (Mondal and Datta, 2008).

2.3.3 Bread staling

Freshly baked bread can be characterized by its crisp crust, soft crumb and appealing aroma. Bread staling refers to all undesirable changes that take place after baking, as the crust and crumb age, which are caused by physicochemical reactions but not by the action of spoilage microorganisms. Such undesirable changes could be those changes in sensory characteristics (such as loss of aroma, changes in mouthfeel) or physical properties (such as increase in hardness and crumbliness). Because it leads to decreased consumer acceptance as well as important economic losses, bread staling has been studied extensively during the last decades. In spite of this, its complex mechanism is still not fully elucidated.

2.3.3.1 Roles of starch on bread staling

During earliest studies, moisture loss was believed to be the sole factor affecting bread staling. However, it was later proven that bread can stale without any loss of moisture (Chen *et al*., 1997). Presently, it is generally accepted that changes occurred in the starch gel component and in the crumb are the major causes of bread staling. Colwell *et al.* (1969) proposed that the basic mechanism of bread staling involves changes that are analogous to crystallization of the starch fraction of the crumb. Hug *et al*. (1999) later confirmed this statement by reporting that during aging, bread crumb regained birefringence from reordering of amylose and amylopectin fractions. This increase in crystallinity is concomitantly observed with the increase in crumb firmness, for which average consumer has been using a “squeeze test” as an index of bread freshness. Axford *et al.* (1968) reported a high correlation between firmness level and taste panel assessment of staling. Changes in starch properties were identified to correlate with bread staling. Enzyme susceptibility, swelling power, solubility and iodine absorption were reported to decrease as the starch molecules become reassociate. On the other hand, crystallinity, firmness and crumb rigidity were found to increase with aging of bread (Zobel and Kulp, 1996).

Amylopectin is believed to have a major role in bread staling (Zobel and Kulp, 1996). Kim and D’Appolonia (1977a, b) reported its decreased solubility upon aging. Ghiasi *et al.* (1984) demonstrated the role of amylopectin in bread staling using “refreshing” experiment. Aged bread could be “refreshed” and became more acceptable by using moist heat at 85-90 ^{o}C. This reflects the ability of retrograded amylopectin to become redispersed upon heating. Development of crystalline order of amylopectin is readily monitored using differential thermal analysis (DTA) and differential scanning calorimetry (DSC) (Zobel and Kulp, 1996). Several researchers have been using these techniques to investigate bread staling (Eliasson, 1983; Russell, 1983; Zeleznak and Hoseney, 1986; Rogers *et al*., 1988; Vittadini and Vodovotz, 2003; Gavilighi *et al*., 2006; Ribotta and Le Bail, 2007).

While amylopectin plays such a major role, amylose was not as important regarding bread staling because it becomes reassociate and retrograde rapidly in the first 24 hours after baking and shows little change after that (Zobel and Kulp, 1996). Biliaderis and Zawistowski (1990) reported that amylose molecules quickly crystallize as observed by x-ray diffraction in 2.4-7.0% starch gels. This retrogradation of amylose, however, is important pertaining to the setting of fresh bread structure as demonstrated by the work of Stampfli and Nersten (1995).

2.3.3.2 Roles of non-starch constituents on bread staling

Although starch has been proven to play a dominant role, other components may also exert an effect on bread staling (Rogers *et al*., 1988; Chen *et al*., 1997).

2.3.3.2.1 Gluten

Even though many studies have been conducted to investigate the effect of gluten on bread staling, there is still no significant evidence that wheat proteins or gluten contribute to crumb firming of bread. Katz (1934; cited from Zobel and Kulp, 1996) reported that, while solubility of carbohydrates decreased during bread staling, protein solubility remained unchanged. Thus, the author concluded that starch retrogradation, rather than gluten changes, was associated with bread staling.

With modern techniques like x-ray diffraction and DSC, Hoseney, Zelesnak and Lai (1986) reported that upon drying, gluten gave no indication of crystalline order or, in other words, it actually is a glassy polymer. Kim and D'Appolonia (1977a) explained that breads showed similar crystallization process regardless of the flour protein content. Gerrard *et al.* (2001) replaced gluten in bread with other proteins (soy and milk proteins) and reported no significant difference in staling rate as compared to the gluten-containing bread.

2.3.3.2.2 Pentosans

Bread flour typically contains about 3% pentosans. Approximately half of the pentosans present are soluble in water while the other half is associated with starch fraction (Zobel and Kulp, 1996). Wheat pentosans consist of a linear backbone chain of anhydro-D-xylopyranosyl units linked by β-(1⟶4) glycosidic bonds and possibly arabinose branching point at the C2 or C3 position of anhydroxylose units (Figure 2.11).

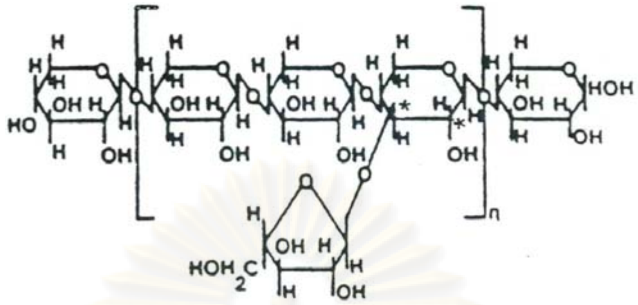


Figure 2.11 Structure of wheat pentosans with arabinose branching point at C2 or C3 position of anhydroxylose unit (designated by *) (Source: Zobel and Kulp, 1996)

Pentosans are believed to have anti-staling effect. Michniewicz, Biliaderis and Bushuk (1992) reported that with added pentosans, the firming rate of wheat bread decreased significantly. Denli and Ercan (2001) later confirmed this finding. The water-insoluble pentosans were reported to be more effective in retarding bread staling than the water-soluble fraction (Kim and D'Appolonia, 1977c). Fessas and Schiraldi (1998) reported that pentosans had no effect on starch retrogradation. Later, these authors proposed that the anti-staling effect of pentosans could be due to their excellent water holding capacity. The pentosans act as moisture sinks in the crumb. The water which is slowly released during the course of bread storage would pose a plasticizing effect and the crumb stays moist and soft for a longer time (Schiraldi and Fessas, 2001).

2.3.3.2.3 Lipids

Russell (1983) reported that lipids have a significant anti-staling effect. Earlier, Willhoft (1973) suggested that monoglycerides, especially unsaturated monoglycerides, could retard bread staling by interacting with gluten. By adding native lipids to defatted flour, Rogers *et al.* (1988) found that the firming rate of bread decreased.

2.3.3.3 Bread staling mechanism

Zobel and Kulp (1996) proposed a bread staling model which is shown in Figure 2.12. This model explains the changes in bread from the dough stage, to freshly baked bread, to staled bread and also to bread that is refreshened from staled bread by heating.

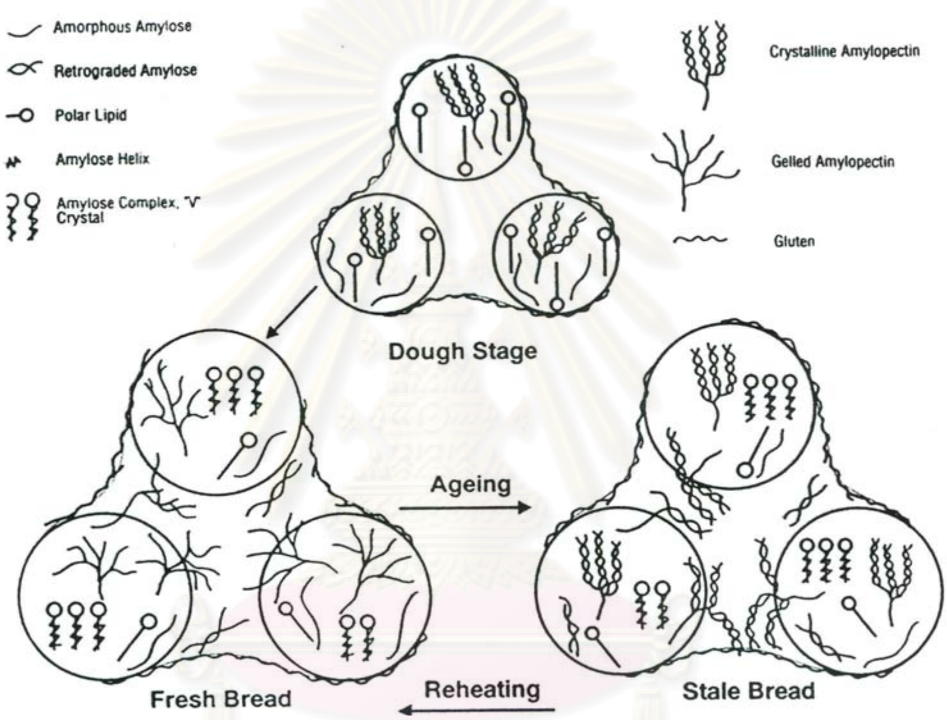


Figure 2.12 A schematic model of bread crumb staling (Source: Zobel and Kulp, 1996)

In the dough stage, smaller starch granules are depicted to indicate their unswollen state. Gluten covers the granule surfaces as well as bridging between granules, forming a continuous phase. The amylopectin branches with their helical structure aggregate and form a crystalline region.

During baking, the crystalline region of amylopectin is disrupted. Starch gelatinizes and the granules become swollen. Part of the amylopectin molecules, as well as amylose, leaches from the swollen granules into intergranular gel phases. Upon cooling, amylose becomes retrograde and form junction points. This initiates starch gelation and provides initial loaf firmness. In fresh bread, some of the amylose interacts with polar lipids and forms a complex. This complex formation would reduce the amount of amylose available to retrograde.

Upon aging, helical structure of amylopectin reappears. Aggregation of amylopectin leads to the formation of crystalline region. In the intergranular phases, helical structures are formed and ultimately crystallize, interlock adjacent granule and cause an increase in crumb firmness. Upon reheating the staled bread, reverse changes occur.

2.4 Soy uses in breads and related products

Increasing acceptance of soy products might stem from the recognized health benefits of the isoflavones which have been linked to lowering cholesterol, reducing risk of heart disease, breast and prostate cancers, and bone deterioration. A study of Dalais *et al.* (2004) suggested that a daily diet containing bread fortified with soy grits could reduce the risk of prostate cancer development and progression. Scientific researches have been extensively conducted to investigate numerous arrays of soy bread properties, from nutritional values to organoleptic, chemical and physical properties of freshly baked bread and changes in those properties upon storage of the bread.

2.4.1 Effect of soy addition on nutritional quality of breads

Dhingra and Jood (2001) studied the effect of soy flour substitution on nutritional composition of wheat bread. Wheat flour in the bread recipe was substituted by either full fat (SF) or defatted soy flour (DSF). The control (wheat) bread contained 11.5% protein while the 10% SF and DSF samples contained 13.7 and 13.8% protein, respectively. Protein content was found to increase with increasing level of soy flour substitution. The increase in lysine content also followed similar trend as the protein content. The 10% SF and DSF samples contained 3.02 and 3.05 mg lysine/100 g protein, respectively, compared to that of 2.36 g/100 g protein in the control. The SF samples exhibited increased fat content (6.32 and 6.83% in 5 and 10% substitution samples) as compared to the control (5.44%). On the other hand, fat content of the DSF samples was not significantly different from that of the control. The control bread was found to contain 8.90, 3.95 and 4.95% of total, soluble and insoluble dietary fiber, respectively. Interestingly, the total and insoluble dietary fiber contents of SF and DSF samples decreased with increasing substitution level. Meanwhile, soluble dietary fiber content of the soy-containing samples was found to increase slightly as compared to the control. The authors explained that the decrease in dietary fiber content in soy breads may be due to the fact that soybeans used in the study were dehulled. The content of insoluble fibers, which are mainly present in the hulls, therefore decreased while that of soluble fibers, which are in the cotyledons, was found to increase. Despite the increase in protein content in soy breads, Dhingra and Jood (2001) reported that *in vitro* protein digestibility decreased with increasing soy flour substitution. The 15% SF and DSF samples exhibited a protein digestibility of 69.1 and 70.7% as compared to 74.0% of the control. The authors suggested that this could be attributable to the increased concentration of trypsin inhibitors, polyphenols and phytic acid in soy-supplemented samples. Trypsin inhibitors are responsible for inhibiting the activity of proteolytic enzymes whereas polyphenols and phytic acid could become associated with proteins and form insoluble complexes, resulting in the decreased *in vitro* protein digestibility.

Similar results were also reported later by Olaoye, Onilude and Idowu (2006). Wheat flour in the bread recipe was substituted by full fat soy flour at 5-15% levels. Protein content of the bread increased with increasing soy flour substitution from 7.01% in control (wheat) sample to 8.39% in the 15% substitution sample. However, the increase in crude fiber content with soy flour substitution was reported. The control contained 0.03% crude fiber whereas the 15% substitution sample contained 0.14% crude fiber. Crude fat and ash contents also increased, while carbohydrate content decreased, with increasing level of soy flour substitution.

Lysine and tryptophan are the first and second limiting amino acids of corn while soybean has been known as a rich source of lysine. Waliszewski, Pardio and Carreon (2002) supplemented corn tortilla with okara and reported that upon fortification with 10% okara, lysine and tryptophan contents of the tortilla increased from 56 and 70% to 93 and 92% of the FAO profile, respectively. The authors suggested the fortification of okara to tortilla to help alleviate lysine and tryptophan deficiency in the poorest sectors of Latin American society where tortilla is the main protein source.

Soy isoflavones have been recognized for their numerous health benefits. Zhang *et al.* (2003) investigated isoflavone profile and biological activity of soy-containing bread. The ability of isoflavone extracts from whole soy bread and two soy bread fractions, which are crumb and crust, to modulate the proliferation of human prostate cancer PC-3 cells was monitored. It was reported that total isoflavone content in the crumb and crust were similar (3.17 μmol/g dry basis). However, conjugate patterns of the isoflavones found in these two fractions were different. Both fractions of soy bread contained similar concentration of isoflavone aglycones. Wheat bread extracts were found to increase cell proliferation at all concentrations tested. Low concentrations of soy bread extracts increased PC-3 cell proliferation as compared to untreated control. However, at higher extract concentration the PC-3 cell proliferation was reduced. This indicated the potential health benefit of soy-containing bread in reducing risk of cancer.

2.4.2 Effect of soy addition on physical, chemical and sensory characteristics of breads

Dhingra and Jood (2001) evaluated sensory characteristics of soy-substituted bread using a 9-point hedonic scale. It was reported that bread samples substituted with up to 10% full fat or defatted soy flour received scores in crust color, appearance, flavor, crust texture, taste and overall acceptability similar to those of the control (wheat) sample. With higher soy flour substitution level (> 10%), the breads became organoleptically unacceptable. The authors also confirmed this finding in another study (Dhingra and Jood, 2004). Olaoye *et al.* (2006) reported that bread samples supplemented with up to 5% with full fat soy flour were similar to control (wheat) bread in aroma, crumb texture, taste and general acceptability. However, crust texture, shape and appearance of soy-substituted breads were different from the control.

Waliszewski *et al.* (2002) studied the effects of okara supplementation on properties of corn tortilla. It was demonstrated that up to 10% okara substitution, the tortilla still possessed aroma, flavor, after taste, appearance, manual texture and oral texture similar to the control. However, okara substitution of over 10% would result in an organoleptically unacceptable product.

Roccia *et al.* (2009) studied the effect of soy protein addition to rheological properties and water retention capacity of gluten protein. They found that the substitution of wheat protein by soy protein negatively affected the dough rheological properties due to weakening of protein network. This resulted from both the dilution of gluten protein and the interference of soy protein in gluten network. This study supported the work of Mohamed *et al.* (2006) which reported that dough mixing time became increased with increasing substitution of soy protein isolate. The authors concluded that soy protein present in the bread formula delayed gluten formation and thus lengthened the dough mixing time.

2.4.3 Effect of soy addition on keeping quality of breads

Vittadini and Vodovotz (2003) substituted wheat flour in white bread recipe with 20, 30 and 40% defatted soy flour. Changes in physical properties of breads were investigated during 7-day storage at room temperature (25 ^{o}C). By using a DSC technique, it was found that, for freshly baked bread, soy flour substitution caused a slight increase in freezable water content as compared to the control (wheat) bread. This was due to the fact that the soy-containing formulas had higher moisture content. As storage time increased, freezable water content of all samples decreased. At Day 7, the control showed a higher degree of freezable water reduction (5.1%) than the soy-containing samples (2.0-3.8%). This indicated greater stability of the soy-containing breads. The authors also monitored recrystallization of amylopectin using DSC. Amylopectin recrystallization was observed in all samples during 7-day storage. However, the increase in amylopectin recrystallization was significantly lower in soy-containing samples. The staling retardation effect could be due to the strong water-holding capacity of soy flour. This could be explained using the work of Ryan *et al.* (2002) which proposed that the hydrated soy fractions could interact very strongly with starch. This soy-starch interaction interfered with interactions among starch molecules and thus hindering amylopectin recrystallization.

Lodi, Abduljalil and Vodovotz (2007) studied water distribution in breads containing 19.92% soy flour by weight. Using a magnetic resonance imaging (MRI) technique, homogeneous water distribution was reported for soy-containing bread while inhomogeneous water distribution was found in control (wheat) bread. The authors proposed that this homogeneous water distribution help retarding the bread-staling rate during prolonged storage.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Bread ingredients

The following bread ingredients were used in this study:

- Bread flour, White Swan™ (United Flour Mill, Bangkok, Thailand)
- Calcium propionate, food-grade (UFM Food Center, Bangkok, Thailand)
- Dehulled soybean, Raitip™ (Thai Cereals World, Bangkok, Thailand)
- Full-fat soy flour, Doi Kham™ (Doi Kham, Bangkok, Thailand)
- Instant baker's yeast, European™ (Akmaya Yeast Industry, Istanbul, Turkey)
- Iodized salt, Tipp™ (Saha Pathanapibul, Bangkok, Thailand)
- Refined sugar, Mitr Phol™ (Mitr Phol Sugar, Bangkok, Thailand)
- Shortening, Olympic Kream™ (Katevanich Industry, Bangkok, Thailand)

3.1.2 Chemical reagents

The following chemical reagents were used in the study:

- Boric acid, AR grade (Ajax Finechem, Taren Point, Australia)
- Ethyl alcohol, 95%, AR grade (Mallinckrodt Chemicals, Phillipsburg, NJ)
- Hydrochloric acid, 37%, AR grade (J. T. Baker, Phillipsburg, NJ)
- Iodine, AR grade (Carlo Erba, Rodano, Italy)
- Methyl red, AR grade (Merck, Bangkok, Thailand)
- Methylene blue, AR grade (Riedel-de Haën, Seelze, Germany)
- Petroleum ether, AR grade (Fisher Scientific, Loughborough, U. K.)
- Potassium iodide, AR grade (Ajax Finechem, Taren Point, Australia)
- Selenium reagent mixture, AR grade (Merck, Bangkok, Thailand)
- Sodium hydroxide, AR grade (Carlo Erba, Rodano, Italy)

- Sulfuric acid, 96.5%, AR grade (J. T. Baker, Phillipsburg, NJ)

3.2 Instruments

The following instruments were used in the study:

- Centrifuge, IEC Multi RF™ (Thermo Fisher Scientific, Waltham, MA)
- Color meter system, ColorFlex® (Hunter Associates Laboratory, Reston, VA)
- Convection kitchen oven, Teba® Model TFL1231 (Avanti Products, Miami, FL)
- Differential scanning calorimeter (DSC), Diamond DSC® (Perkin Elmer, Waltham, MA)
- Food blender, Model HR1791 (Philips, Jakarta, Indonesia)
- Food mixer, Model KM230 (Kenwood, Havant, U. K.)
- Food processor, Model 2102240 (Mara, Taipei, Taiwan)
- Hot air oven, Model 600 (Memmert, Schwabach, Germany)
- Kjeldahl digestion unit, Model K-424 (Buchi, Flawil, Switzerland)
- Kjeldahl distillation unit, Model B-324 (Buchi, Flawil, Switzerland)
- Muffle furnace, Model CWF1200 (Carbolite, Hope Valley, U. K.)
- Rotary evaporator, Eyela® Model N-N (Tokyo Rikakikai, Tokyo, Japan)
- Stereomicroscope, Model SMZ-1000, with Plan Apo 1x WD-70 objective lens (Nikon Instruments, Melville, NY)
- Tray dryer, Model HA-100S (Yeo Heng, Bangkok, Thailand)
- Universal materials testing machine, Instron® Model 5565 (Instron, Norwood, MA)
- UV/Vis spectrophotometer, Model V-530 (Jasco, Easton, MD)
- Vacuum packaging machine, Webomatic® Easypack Model (Webomatic, Bochum, Germany)
- Water activity meter, AquaLab® Series 3TE (Decagon Devices, Pullman, WA)

3.3 Methods

3.3.1 Flour preparation

Commercial wheat flour (United Flour Mill, Bangkok, Thailand) and soy flour (Doi Kham, Bangkok, Thailand) were passed through a US70 mesh sieve (0.210-mm opening). This was also done for okara (Section 3.3.2) so that the three ingredients had similar particle size.

3.3.2 Okara preparation

To prepare okara, a hot water blanching method adapted from that described by Wilkens, Mattick and Hand (1967), was used in this study. Dehulled soybean (Thai Cereals World, Bangkok, Thailand) was soaked in tap water (soybean:water = 1:10) at room temperature (25 ^{o}C) for 14 hours to soften the cotyledons. The soaked bean was drained, rinsed with tap water and ground with hot water (90±5 ^{o}C) using soaked bean-to-water ratio of 1:8. The hot water treatment was used in order to inactivate lipoxygenase, an enzyme endogenous to soybean which is responsible for the development of beany flavor in okara. The slurry was then filtered through several layers of cheesecloth and pressed to remove excess liquid using a hydraulic press. The wet okara was steamed at 100 °C for 30 minutes to ensure a complete inactivation of antinutritional factors present in raw soybean. The steamed okara was pressed again to remove excess liquid. The okara was then transferred to a stainless steel tray and dried in a tray dryer (Model HA-100S, Yeo Heng, Bangkok, Thailand) at 60 ^{o}C until moisture content similar to that of wheat flour and soy flour was obtained (approximately 10%, wb). The drying time lasted about 14 hours. Food processor (Model 2102240, Mara, Taipei, Taiwan) was used to disintegrate any lumps present. The dried okara was then passed through a US70 mesh sieve and stored under vacuum in a sealed aluminum laminated bag at 4 ^{o}C until needed. Figure 3.1 summarized the okara preparation procedure.

3.3.3 Bread making

Ingredients of basic white bread are listed in Table 3.1.

Dehulled soybean

↓

Soaking in tap water (soybean:water = 1:10) at room temperature for 14 hours

↓

Draining and rinsing with tap water

↓

Grinding with hot water (90±5 °C) (soaked bean:water = 1:8)

↓

Filtering and pressing ⟶ Soymilk

↓

Wet okara

↓

Steaming at 100 °C for 30 minutes

↓

Pressing

↓

Steamed okara

↓

Drying at 60 °C until obtaining approximately 10% (wb) moisture content

↓

Grinding

↓

Passing through US70 mesh sieve

↓

Dried okara

↓

Packing and storing at 4 °C

Figure 3.1 Okara preparation

Table 3.1 Ingredients of basic white bread (adapted from Woodall, 2007)

Ingredient	Amount (g)	Baker percentage
Wheat flour (bread flour)	334.0	100.0
Instant yeast	4.0	1.2
Shortening	16.0	4.8
Sugar	16.0	4.8
Salt	6.0	1.8
Water	200.0	59.9
Calcium propionate*	1.0	0.3

*Calcium propionate is a mold inhibitor. The maximum level permitted to be used in bread is 2000 mg/kg (Ministry of Public Health, 1984).

The bread containing all wheat flour was a control. For the soy-substituted breads, wheat flour in the recipe was substituted by either soy flour or okara at 5, 10 and 15% by weight.

To prepare bread samples, a method adapted from that described by Woodall (2007) was followed. Dry ingredients (i. e., wheat flour, soy flour, okara, instant yeast and calcium propionate) were mixed and sifted. The dry mixture was then transferred to a Kenwood food mixer (Model KM230, Kenwood, Havant, U. K.). Salt and sugar was dissolved in the water specified in the recipe. The solution was then added to the dry mixture in the mixer bowl. Using dough hook and speed level 3, the mixture was mixed for 3 minutes. Shortening was added to the flour mixture and the mixing speed was increased to level 6. The dough was obtained after mixing for 10 minutes. The properly mixed dough will appear smooth and can be stretched into a thin sheet. The dough was then formed into a ball, transferred to a greased bowl and covered with moistened cheesecloth. The dough was let to rise at 32 ^{o}C for 60 minutes. After that, the risen dough was punched to release gas, hand kneaded and cut into pieces of 140 g each. Each dough piece was spread into a sheet, rolled into a loaf and placed in a greased loaf pan (3 ½ x 5 ½ x 2 ½ inch). Then the dough was let to rise for another 60 minutes at 32 ^{o}C.

The dough was baked in a Teba® convection kitchen oven (Model TFL1231, Avanti Products, Miami, FL) at 180 °C for 25 minutes and cooled at room temperature (25 °C) for 1 hour. The bread was then placed in a low-density polyethylene bag, hot-sealed and stored at room temperature (25 °C) for a period of 7 days.

3.3.4 Proximate compositions of raw materials and freshly baked breads

Proximate compositions (moisture, crude protein, crude fat, crude fiber and ash) of wheat flour, soy flour, okara and freshly baked bread crumbs were determined using the standard methods outlined by AOAC (2000) (Appendices A1-A5). Available carbohydrate content was obtained by difference using Equation 3.1 (James, 1995):

%Available carbohydrates (wb) = 100-(%moisture+%crude protein+%crude fat+%crude fiber+%ash) (3.1)

3.3.5 Evaluation of bread properties

3.3.5.1 Moisture content

Freshly baked and stored bread crumbs were determined for their moisture content using the standard method outline by AOAC (2000) (Appendix A1).

3.3.5.2 Water activity

Freshly baked and stored bread crumbs were determined for their water activity using an AquaLab® water activity meter (Series 3TE, Decagon Devices, Pullman, WA). Measurement was done at 25 °C.

3.3.5.3 Freezable water content, unfreezable water content and amylopectin recrystallization

Freshly baked and stored bread crumbs were monitored for their freezable and unfreezable water, as well as amylopectin recrystallization, using a Diamond DSC® (Perkin Elmer, Waltham, MA) according to the method described by Vittadini and Vodovotz (2003). Bread crumb (10-15 mg) was accurately weighed into an aluminum DSC pan (Perkin Elmer, Waltham, MA) and hermetically sealed. An empty pan was used as a reference. The sample was cooled to -40 °C in the DSC furnace and then scanned from -40 to 85 °C with an increment of 5 °C/minute. An endothermic transition around 0 °C is attributed to ice melting (Vodovotz *et al.*, 1996; Baik and Chinachoti, 2001). Freezable and unfreezable water contents were calculated using Equations 3.2 and 3.3 (Vittadini and Vodovotz, 2003):

$$\%\text{Freezable water} = \left[h \times \frac{1}{\Delta H_{fus}} \times \frac{1}{M} \right] \times 100 \qquad (3.2)$$

Where h is peak enthalpy (J/g).

ΔH_{fus} is latent heat of fusion of ice (334 J/g).

M is g water per g sample.

%Unfreezable water = %moisture-%freezable water (3.3)

To investigate amylopectin recrystallization, an endothermic peak around 40-70 °C corresponding to the melting of amylopectin crystallite (Vittadini and Vodovotz, 2003) was monitored. Peak enthalpy per g sample was recorded.

3.3.5.4 Water soluble starch content

Freshly baked and stored bread crumbs were monitored for their water soluble starch content using the method of Shaikh, Ghodke and Ananthanarayan (2007). Fifteen ml of distilled water were added to 200 mg of bread crumb. The crumb-water mixture was placed in a shaking water bath at 25 °C for 20 minutes. The slurry was then centrifuged at 5000 rpm for 5 minutes. Ten ml of the supernatant was later treated with 2 ml of standard iodine solution (2 mg of iodine and 20 mg of potassium iodide in 100 ml water). Optical density (OD) was measured using a UV/Vis spectrophotometer (Model V-530, Jasco, Easton, MD) at 680 nm. A standard curve of OD at 680 nm (OD_{680}) versus starch concentration is shown in Appendix B1.

3.3.5.5 Baking characteristics

Freshly baked and stored bread samples were determined for loaf volume, loaf weight, specific loaf volume and loaf height. Loaf volume was determined using sesame seed displacement method. A 800-ml beaker was first slightly overfilled by pouring sesame seeds at a constant rate. Excess seeds were then scraped off using a spatula. The seeds in the beaker were then transferred to a 1000-ml graduated cylinder and the volume was recorded. Bread loaf was then placed in the beaker. The beaker was overfilled by pouring in sesame seeds. After scraping off excess seeds, the seeds in the beaker were transferred to the graduated cylinder and the volume was recorded. Loaf volume was obtained by difference. Specific volume was calculated by dividing loaf volume by loaf weight. Loaf height was measured from the bottom to the highest point of the loaf.

3.3.5.6 Textural characteristics

Texture profile analysis of freshly baked and stored bread samples was carried out using a modified method of Guarda *et al.* (2004). Hardness, cohesiveness and springiness were measured using a universal materials testing machine (Model 5565, Instron, Norwood, MA) equipped with 5-kg load cell. A 2x2x2 cm^3 sample cube was compressed using a 6 cm-diameter cylindrical probe with a cross-head speed of 1.0 mm/second until 70% deformation was obtained.

3.3.5.7 Crumb grain structure

Freshly baked and stored bread crumbs were investigated for their structure using an SMZ-1000 stereomicroscope equipped with Plan Apo 1x WD-70 objective lens (Nikon Instruments, Melville, NY).

3.3.5.8 Color

Color of freshly baked and stored bread crumbs and crusts were measured using a ColorFlex® color meter system (Hunter Associates Laboratory, Reston, VA). The measurement was done in the CIELAB color system (L^* a^* b^*), using D65 light source. L^* is the lightness coordinate. a^* is the red/green coordinate, with $+a^*$ indicating red, and $-a^*$ indicating green. b^* is the yellow/blue coordinate, with $+b^*$ indicating yellow, and $-b^*$ indicating blue. Eight measurements at eight different positions were done for each sample piece (5x5x4 cm^3). Hue angle and chroma were obtained from a^* and b^* according to Equations 3.4 and 3.5. Whiteness index was also calculated for bread crumb samples (Equation 3.6).

$$\text{Hue angle} = \arctan (b^*/a^*) \tag{3.4}$$

$$\text{Chroma} = (a^{*2}+b^{*2})^{1/2} \tag{3.5}$$

$$\text{Whiteness index} = 100-[(100-L^*)^2 + a^{*2} + b^{*2}]^{1/2} \tag{3.6}$$

3.3.5.9 Sensory attributes

3.3.5.9.1 Descriptive analysis (DA)

Descriptive analysis was carried out for freshly baked and stored bread samples. The sensory panel consisted of 14 individuals. The experiment was divided into two phases: training and sample testing.

For the training phase, the panelists were first trained to familiarize with each sensory attribute. Then, the panelists were trained to rate the intensity of sensory attributes on a 15-cm line scale (Appendix B2). Panelists' performance was monitored and additional training was provided to improve accuracy. For sample testing, panelists individually evaluated samples which were served at room temperature (25 ^{o}C) in odor-free white plastic plates. The experiment was carried out under fluorescent light in an air-conditioned room (25 ^{o}C). Bottled drinking water was used to clear the palate.

3.3.5.9.2 Acceptance test

A 9-point hedonic scale was used to evaluate the degree of liking of the freshly baked bread samples. The sensory panel consisted of 50 individuals. The scale ranged from "dislike extremely (1)" to "like extremely (9)" (Appendix B3). Panelists individually evaluated samples which were served at room temperature (25 ^{o}C) in odor-free white plastic plates. The experiment was carried out under fluorescent light in an air-conditioned room (25 ^{o}C). Bottled drinking water was used to clear the palate.

3.3.6 Statistical analysis

Experiments were done in triplicate. A completely randomized design (CRD) was used for all experiments, except sensory evaluation part which was carried out in a randomized complete block design (RCBD). Data were analyzed using Analysis of Variance (ANOVA). A Duncan's New Multiple Range Test was used to determine the difference among sample means at p=0.05.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Proximate compositions of raw materials

Proximate compositions of wheat flour, soy flour and okara are shown in Table 4.1. Moisture content of commercial wheat flour and soy flour used in this study was in the range of 8.89-9.98% (wb). Dried okara was prepared in the laboratory by air-drying wet okara from soymilk extraction until the similar moisture content was obtained (9.35%, wb). Moisture content of the three ingredients was therefore not significantly different (p>0.05).

Table 4.1 Proximate compositions of wheat flour, soy flour and okara

Composition (%, wb)	Wheat flour	Soy flour	Okara
Moisture^{ns}	9.98±0.50	8.89±0.32	9.35±0.87
Crude protein*	12.28^{c}±1.50	39.84^{a}±3.26	32.67^{b}±2.24
Crude fat	1.34^{c}±0.13	20.50^{a}±0.94	6.59^{b}±1.45
Crude fiber	2.50^{c}±0.23	4.84^{b}±0.48	14.28^{a}±1.69
Ash	0.56^{c}±0.09	5.18^{a}±0.57	2.59^{b}±0.31
Available carbohydrates	73.34^{a}±1.69	20.25^{c}±3.65	34.52^{b}±3.42

*To calculate crude protein content, conversion factors of 5.70 and 5.71 were used for wheat and soy ingredients, respectively (FAO, 2003).

Values are means ± SD of three different determinations.

Sample means within a row which do not share a common superscript letter differ significantly at p=0.05.

“ns” indicates no significant difference among sample means at p=0.05.

Soy flour had the highest crude protein content (39.84%, wb). Even though a portion of soy protein was extracted into soymilk, okara was still high in crude protein content (32.67%, wb or 36.06%, db). This was similar to that of 33.4% (db) reported earlier by Préstamo *et al.* (2007). Wheat flour contained 12.28% crude protein on a wet basis which is common for bread flour. Crude fat and ash contents were also highest in soy flour, followed by okara and wheat flour. Okara contained the highest crude fiber content (14.28%, wb), which was about three and six folds higher than that of soy flour and wheat flour, respectively. Available carbohydrate content was highest in wheat flour (73.34%, wb). Starch is the major component in wheat, constituting approximately 60% of the flour (Belitz and Grosch, 1986). This starch makes up the major fraction of available carbohydrates in wheat flour. The proximate compositions of wheat flour and soy flour obtained in this study were comparable to those reported in the USDA National Nutrient Database (USDA, 2008). Okara compositions reported previously (Van der Riet *et al*., 1989; Taruna and Jindal, 2002; Anonymous, 2005; Wachiraphansakul and Devahastin, 2005; Préstamo *et al*., 2007; Redondo-Cuenca *et al*., 2007) were found to vary within some extent. This discrepancy is due to the differences in soybean and production method used (Tanteeratarm *et al*., 1993). In this study, okara was produced using a hot water blanching method, which was adapted from that described by Wilkens *et al.* (1967). Soaked dehulled soybeans was ground with hot water (90±5 ^{o}C) using a bean-to-water ratio of 1:8.

4.2 Proximate compositions of freshly baked breads

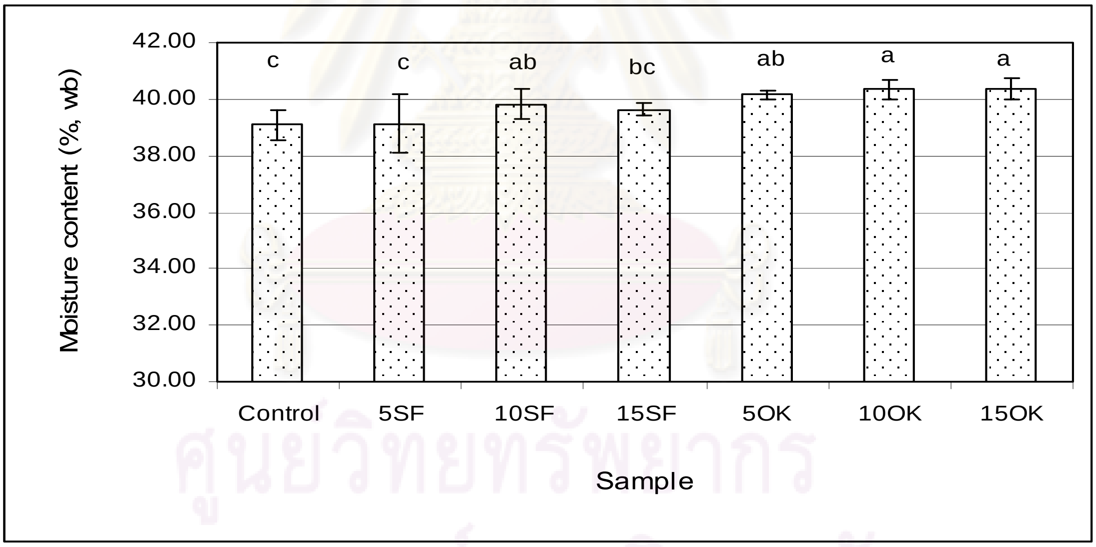
For convenience, the following term and abbreviations will be used throughout this manuscript to represent different bread samples: “control” for wheat bread, “5SF” for 5% soy flour-substituted bread, “10SF” for 10% soy flour-substituted bread, “15SF” for 15% soy flour-substituted bread, “5OK” for 5% okara-substituted bread, “10OK” for 10% okara-substituted bread, and “15OK” for 15% okara-substituted bread. Proximate compositions of freshly baked breads are illustrated in Figure 4.1.

Moisture content of the bread samples differed significantly ($p \leq 0.05$) (Figure 4.1a). Soy-containing samples, in general, possessed higher moisture content than the control. Similarly, Sidhu, Al-Hooti and Al-Saqer (1999) reported an increase in moisture content with 10-30% wheat bran substitution in bread. The increased moisture content upon substitution of high-fiber ingredient has been attributable to the high water-holding capacity of fiber (Sidhu, Al-Saqer and Al-Zenki, 1997).

Crude protein content (Figure 4.1b) was highest in 15SF (12.04%, wb) which was attributable to the high protein content of soy flour and high level of substitution.

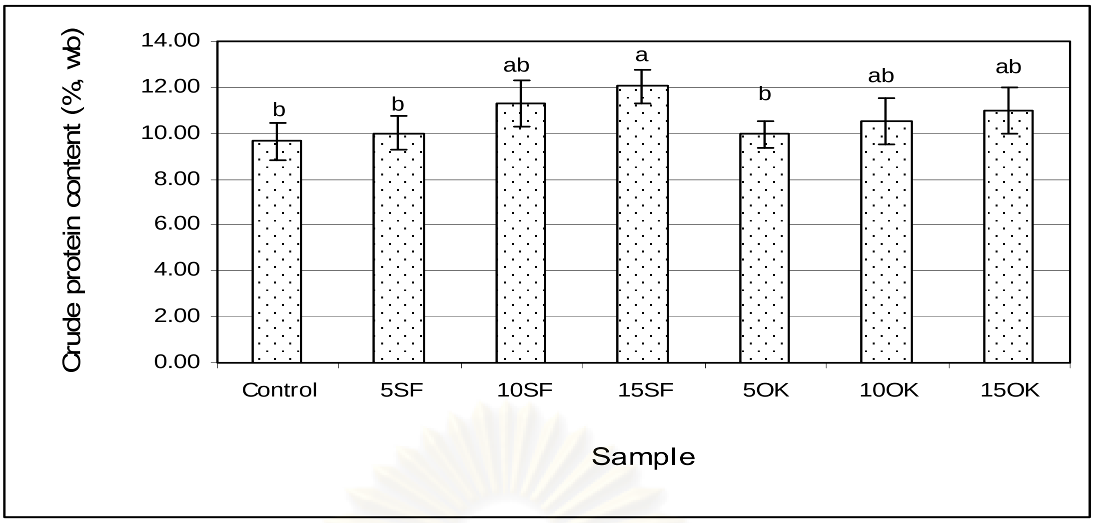
However, this was not significantly different from that of 10SF, 10OK and 15OK. The control had lowest protein content (9.63%, wb) but did not differ significantly from that of SF and OK samples, except 15SF. Crude fat content (Figure 4.1c) was highest in SF samples. Okara, with some fat still left after soymilk extraction, resulted in bread samples moderately high in crude fat content. The control and 5OK samples contained the lowest crude fat content. Soy-containing samples (SF and OK) had increasing crude fat content with increasing level of substitution.

Because okara contained large amount of crude fiber (14.28%, wb), the OK breads had the highest crude fiber content (Figure 4.1d). However, different levels of okara substitution did not significantly affect crude fiber content of the bread. As compared to the control, soy flour with 4.84% crude fiber which was only slightly higher than wheat flour (2.50% crude fiber), yielded breads with higher crude fiber content only at higher level of substitution (15SF).

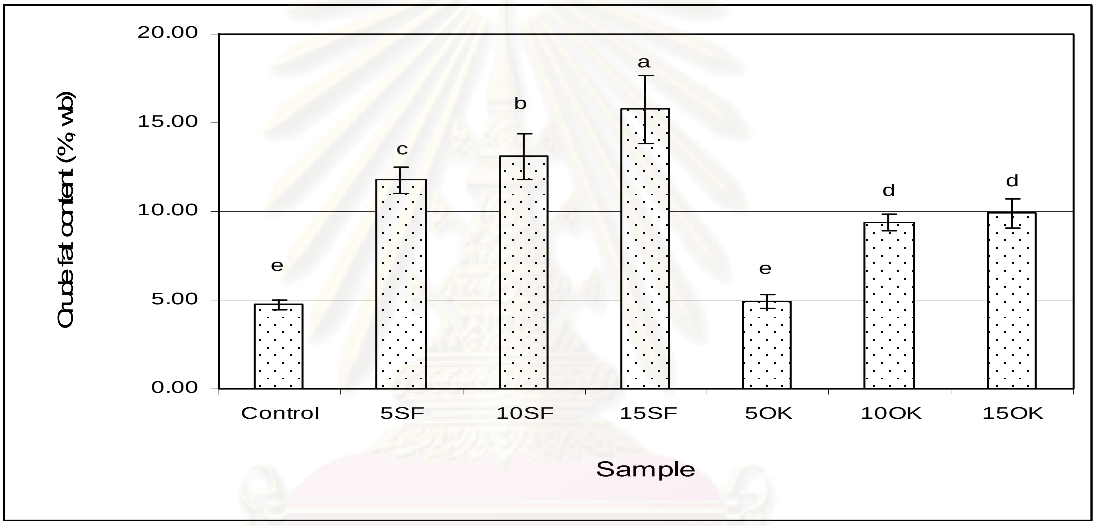


(a) Moisture content

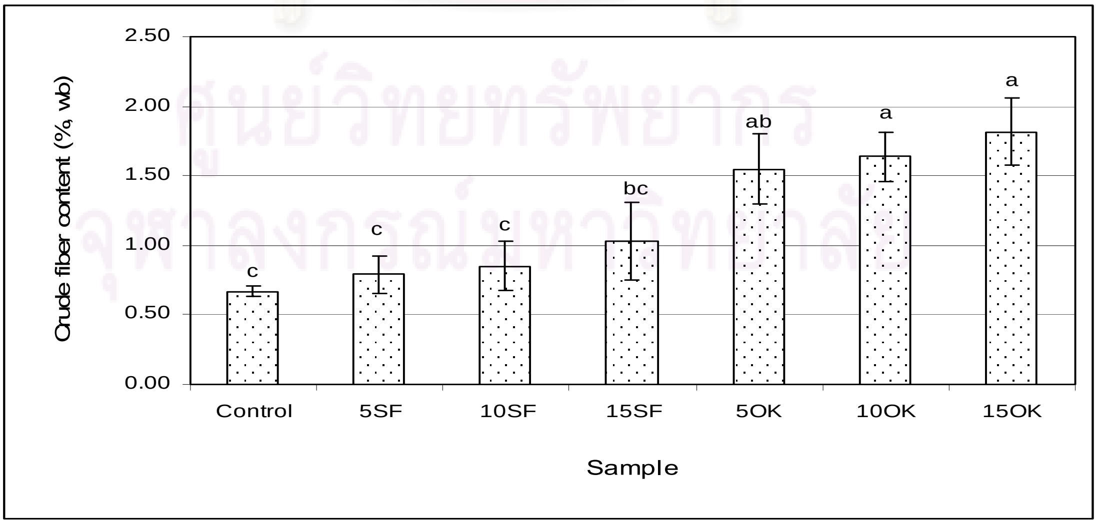
Figure 4.1 Proximate compositions of wheat, soy flour-substituted and okara-substituted breads. Sample means within each composition which do not share a common letter differ significantly at p=0.05.



(b) Crude protein (N x 6.25) content

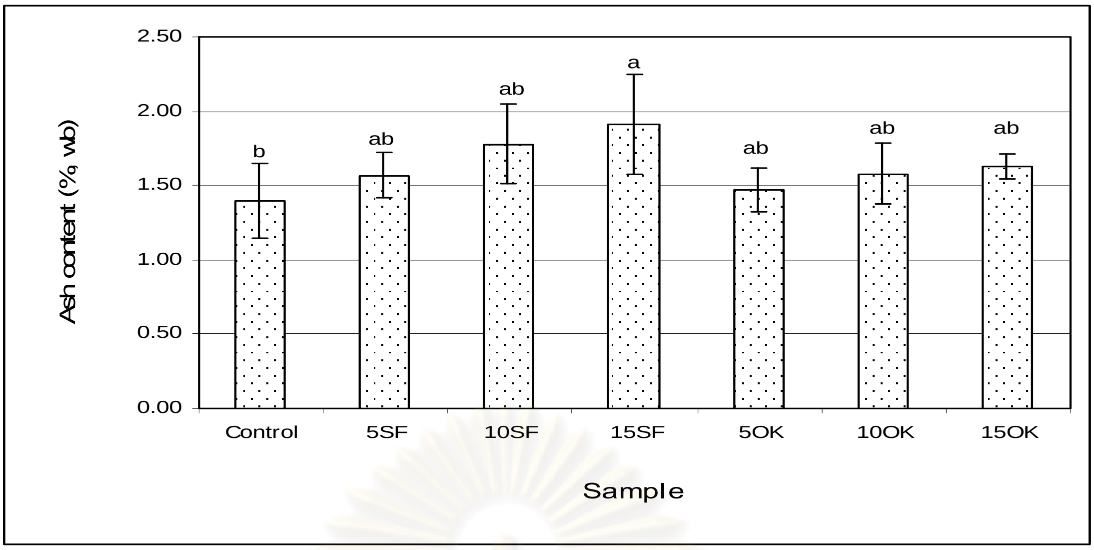


(c) Crude fat content

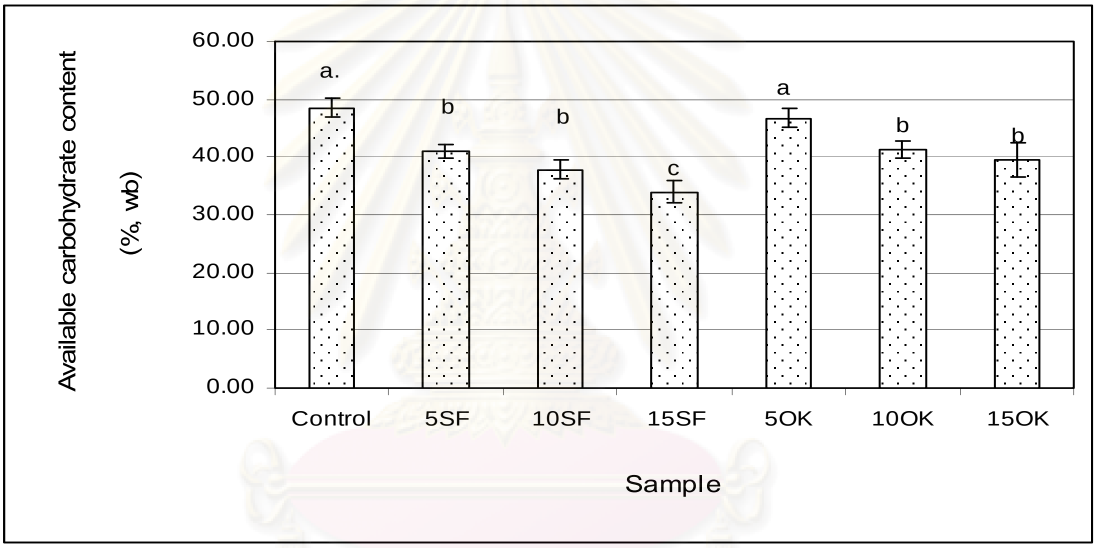


(d) Crude fiber content

Figure 4.1 (Continued)



(e) Ash content



(f) Available carbohydrate content

Figure 4.1 (Continued)

Even though, ash content was significantly different (p≤0.05) among the samples (Figure 4.1e). This difference was not quite large in values. Ash content of the samples was in the range of 1.40-1.78% (wb), with that of 15SF and control being the highest and lowest, respectively.

Due to high available carbohydrate content of wheat flour, the control bread possessed highest available carbohydrate content (Figure 4.1f). With increasing level of soy ingredient substitution, available carbohydrate content became decreasing due to lower concentration of available carbohydrates in soy flour and okara.

The result of this study was similar to those reported earlier. Dhingra and Jood (2001) reported that upon fortification of full-fat or defatted soy flour at the levels of 5 and 10%, bread exhibited increased protein content from 11.5% (wb) in control (wheat) bread to 13.7 and 13.8% (wb) in 10% full-fat and defatted soy flour-substituted samples, respectively. Fat content also increased from 5.44% (wb) in the control to 6.83% (wb) in 10% full-fat soy flour-substituted bread. On the other hand, the 10% defatted soy flour-substituted bread possessed similar fat content (5.38%, wb) to the control.

Olaoye *et al.* (2006) substituted full-fat soy flour to wheat flour in bread at 5, 10 and 15%. It was reported that the contents of crude protein, crude fiber, crude fat and ash increased with increasing level of substitution. The 15% substituted sample contained 8.39% protein (wb) as compared to that of 7.01% (wb) for control (wheat) bread. Meanwhile, available carbohydrate content became decreasing upon increasing level of substitution, from 60.58% (wb) in the control to 52.25% (wb) in the 15% substituted sample.

In summary, proximate compositions of bread varied according to the concentration of those compositions in raw materials and the level of substitution. In general, upon substitution of soy flour or okara, crude protein, crude fat, crude fiber and ash contents increased while available carbohydrate content decreased.

4.3 Effect of partial substitution of wheat flour by soy flour or okara on properties of freshly baked and stored breads

4.3.1 Moisture content

Moisture content of foods is one of the common indicators for food quality. It is therefore important to monitor moisture content of bread because of its potential impact on sensory and physical properties, as well as chemical, biochemical and microbiological stability of the bread (Hathorn *et al.*, 2008). Moisture content of freshly baked bread samples was in the range of 39.08-40.38% (wb) or 64.15-67.73% (db) as already discussed in Section 4.2.

Upon storage for a period of 7 days, moisture content of all bread samples decreased in a similar fashion, but slightly more pronounced in the control (Figure 4.2). Moisture reduction of bread during storage has been attributed to moisture migration from wetter crumb to drier crust and also from bread to surrounding atmosphere (Baik and Chinachoti, 2000). The result of this study was agreeable with the work of Vittadini and Vodovotz (2003) which explained that the presence of soy fiber greatly increased water-holding capacity, resulting in lower moisture loss in soy-containing bread. Similar results were also reported by Fleming and Sosulski (1977), Porter and Skarra (1999) and Doxastakis *et al.* (2002).

4.3.2 Water activity

Water activity (a_w) indicates the intensity of water association with nonaqueous constituents. Strongly bound water supports degradative activities and reactions to a lesser extent than weakly bound water (Fennema, 1996). It was found that even though a_w values of freshly baked SF and OK breads (0.92-0.94) were slightly higher than that of the control (0.92), this difference was not statistically significant (p>0.05) (Figure 4.3). Sidhu *et al.* (1997) substituted wheat flour in Arabic bread (*khaboos*) with 10-20% wheat bran and reported higher a_w values in those substituted breads (0.78-0.80) as compared to the control (wheat) bread (0.74).

The a_w of all bread samples remained relatively constant during 7-day storage (Figure 4.3). This might be because the bread samples were kept in air-tight and moisture-tight package. Similar to this study, Hathorn *et al.* (2008) substituted wheat flour in bread with sweet potato flour and reported no significant changes in a_w during 8-day storage. On the other hand, Sidhu *et al.* (1997) observed slight a_w reduction in wheat bran-substituted and control (wheat) bread upon storing for 4 days. This reduction in a_w over time could be attributable to moisture loss during storage.

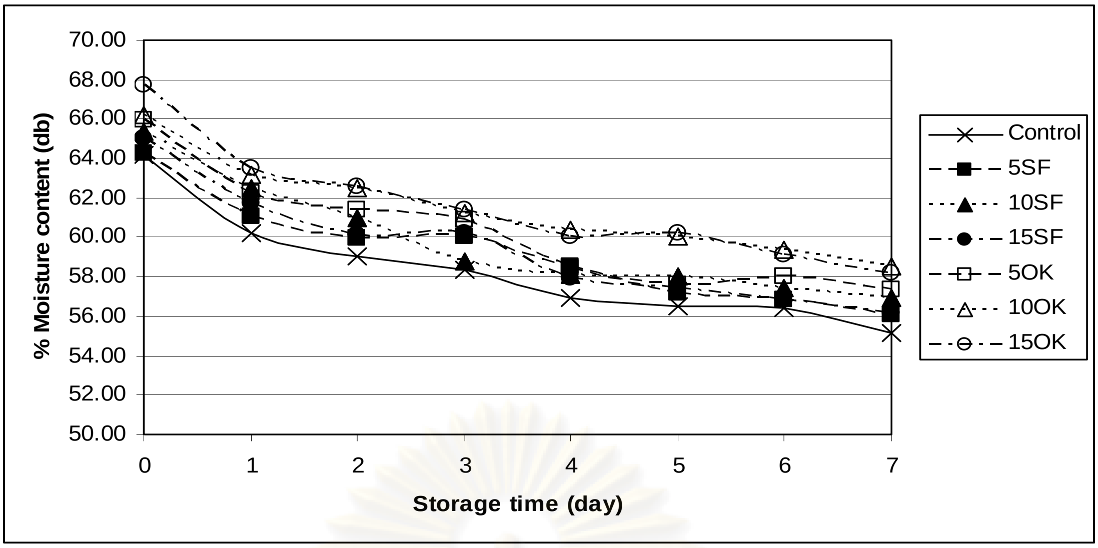


Figure 4.2 Moisture content of wheat, soy flour-substituted and okara-substituted breads during 7-day storage.

4.3.3 Freezable and unfreezable water contents

Freezable water can be readily monitored using a differential scanning calorimetry (DSC) technique (Vittadini and Vodovotz, 2003). An endothermic transition around 0 °C is attributed mainly to ice melting (Vodovotz *et al.*, 1996; Baik and Chinachoti, 2001). Typical curve of ice melting as investigated using DSC is shown in Figure 4.4. In the current study, it was observed that the onset temperature for ice melting in bread samples was in the range of -14.35 to -12.45 °C, as compared to that of -15.2 to -13.5 °C reported earlier by Vittadini and Vodovotz (2003).

Freezable water content can be calculated from the peak enthalpy and unfreezable water content is obtained from the difference between moisture content and freezable water content. Freezable and unfreezable water contents of the bread samples are depicted in Figures 4.5 and 4.6.

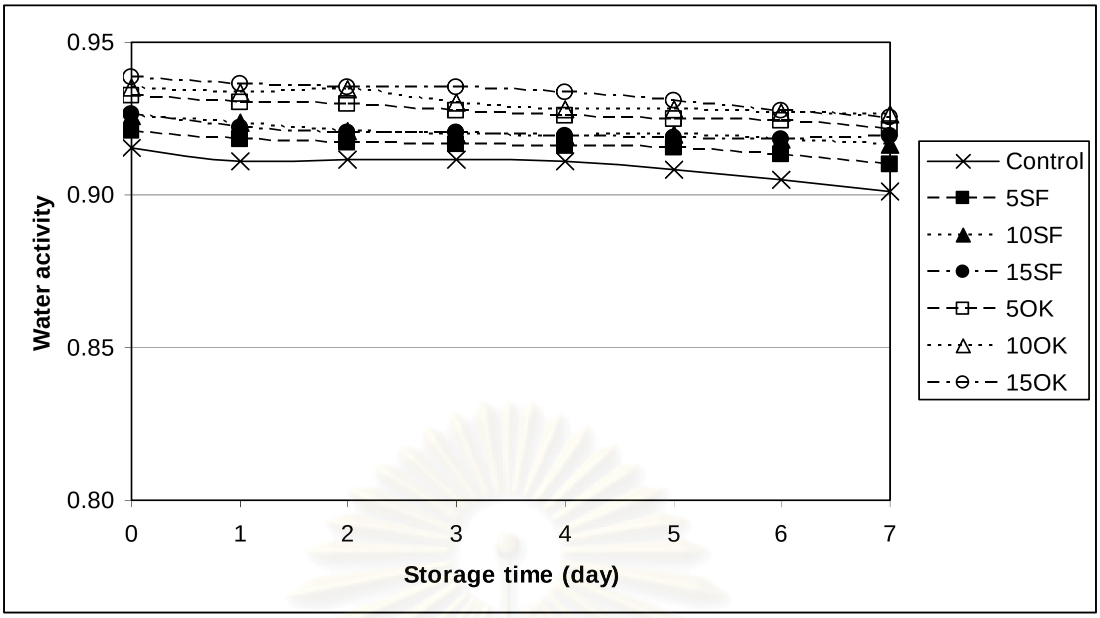


Figure 4.3 Water activity of wheat, soy flour-substituted and okara-substituted breads during 7-day storage.

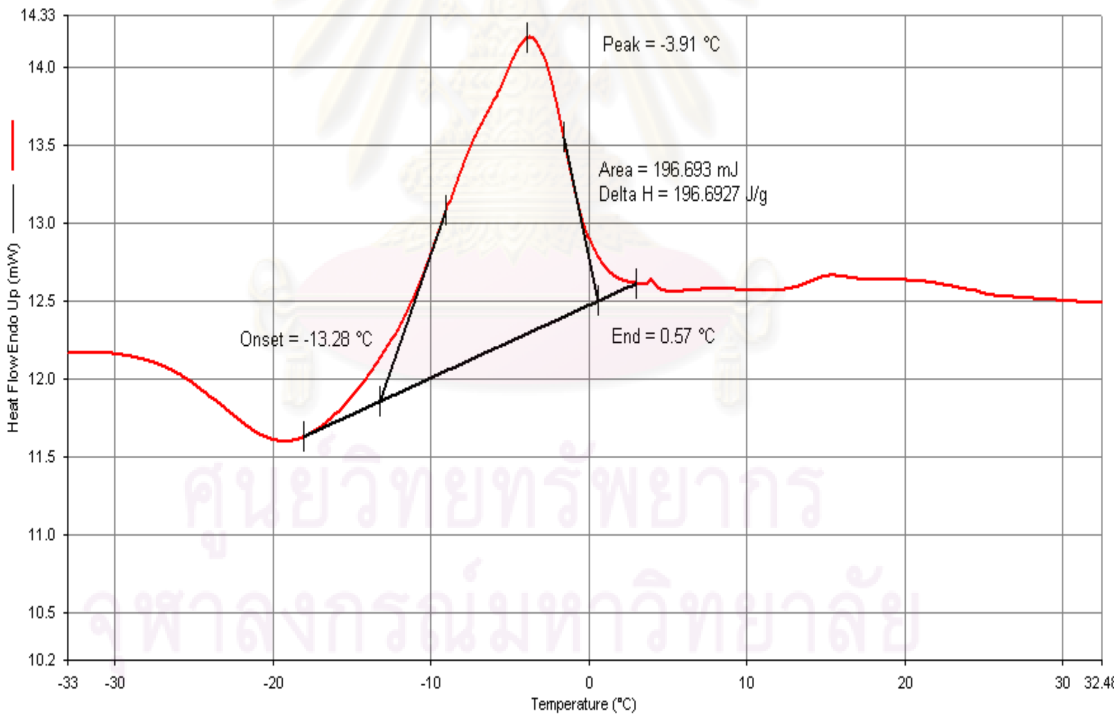


Figure 4.4 Typical DSC curve of ice melting showing an endothermic transition around 0 ^{o}C.

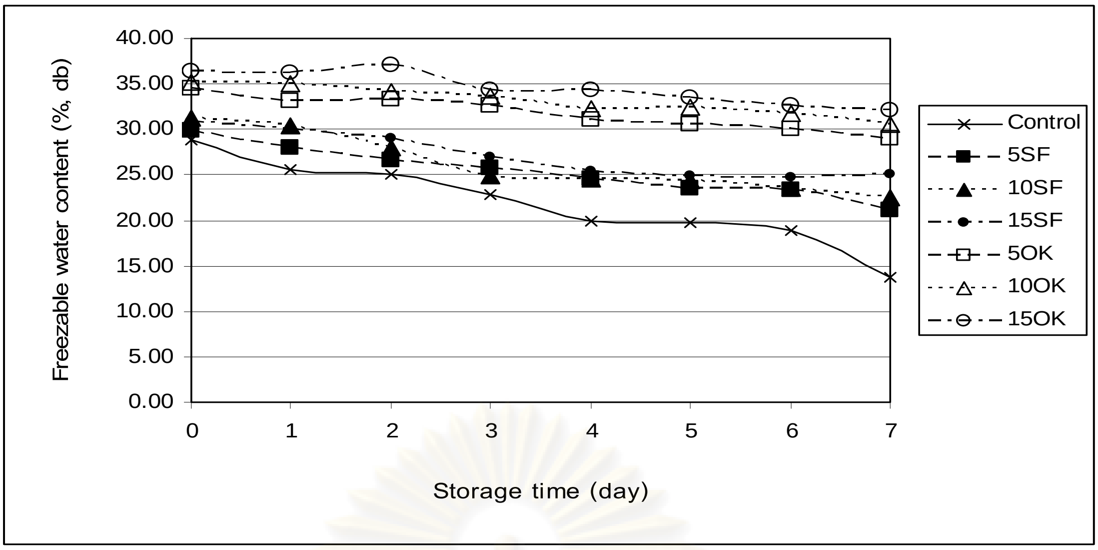


Figure 4.5 Freezable water content of wheat, soy flour-substituted and okara-substituted breads during 7-day storage.

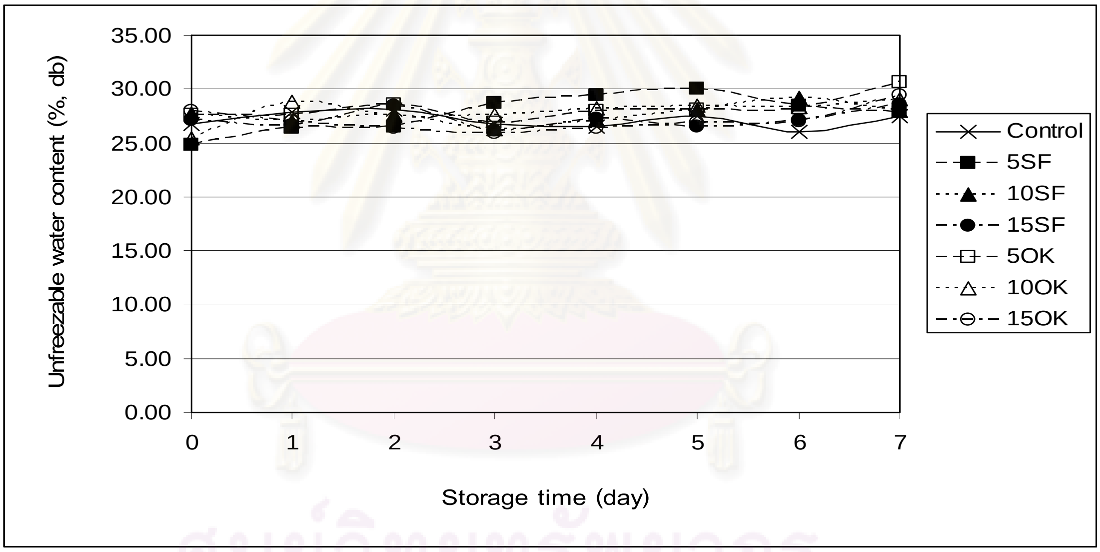


Figure 4.6 Unfreezable water content of wheat, soy flour-substituted and okara-substituted breads during 7-day storage.

Freshly baked control bread contained 28.86% freezable water on a dry basis (Figure 4.5). Upon substituting soy flour or okara, freezable water content became significantly increased (*p*≤0.05), with OK samples showing higher freezable water content. The 15OK bread contained highest freezable water (36.43%, db). Vittadini and Vodovotz (2003) reported similar finding and proposed that the higher freezable water content of soy-containing bread may be due to their higher moisture content as compared to the control.

Freezable water content decreased with increasing storage time for all samples (Figure 4.5). This was also in good agreement with those reported earlier by Baik and Chinachoti (2000, 2001). The control bread showed greater reduction in freezable water over time than the soy-containing breads did. A decrease in freezable water content of wheat bread during storage was common as reported by Rasmussen and Hansen (2001) and Vittadini and Vodovotz (2003). Lodi *et al.* (2007) investigated moisture distribution in bread using a magnetic resonance imaging (MRI) technique. It was found that soy ingredients helped promote homogeneous moisture distribution throughout the bread loaf. Minimal changes in freezable water content of soy-containing bread could be attributed to the low degree of moisture migration in bread during storage (Lodi and Vodovotz, 2008). This relationship between moisture migration and changes in freezable water content was also substantiated by the work of Baik and Chinachoti (2000). Crustless wheat bread was used in order to minimize moisture gradient within the loaf and thus prevent moisture migration. The authors reported that freezable water content of the bread remained unchanged during storage.

In the case of unfreezable water (Figure 4.6), the content in freshly baked breads was not significantly different ($p>0.05$). Moreover, it also remained relatively constant during 7-day storage. In an earlier study, Vittadini and Vodovotz (2003) also reported no significant changes in unfreezable water content during bread storage. The authors suggested that the freezable water, which is more available, was the fraction to undergo detectable changes during storage.

Up to this point, the role of soy ingredients, which were high in fiber, in retaining moisture became very obvious. This was demonstrated by the smaller changes in moisture and freezable water contents in SF and OK breads during storage as compared to the control. Hallberg and Chinachoti (2002) proposed that moisture distribution may play a role in bread staling. This statement was supported by the work of Leung, Magnuson and Bruinsma (1983) which found that bread staling was accompanied by an increase in water binding.

4.3.4 Amylopectin recrystallization

Starch is the major component of wheat constituting approximately 60% of the flour (Belitz and Grosch, 1986). Soybean contains approximately 34% of carbohydrates. However, this fraction is devoid of starch (Wijeratne, 1993). The presence of starch strongly influences quality as well as storage stability of those products made from wheat flour. As starch paste or gel cools, dehydration and insolubilization of starch take place. Starch molecules become reassociated via hydrogen bonding. The collective process of dissolved starch becoming less soluble is called retrogradation. In bread, amylose retrogradation occurs almost immediately after removal from the oven. On the other hand, amylopectin slowly retrogrades during storage, thus playing an important role on keeping quality of bread. Therefore, researchers have been focusing on amylopectin retrogradation or amylopectin recrystallization in an attempt to elucidate the mechanisms of bread staling.

DSC is a highly efficient technique that has been used to investigate amylopectin recrystallization. An endothermic peak observed around 40-70 ^{o}C indicates amylopectin recrystallization (Vittadini and Vodovotz, 2003). In this study, melting of amylopectin crystallite was observed around 65 ^{o}C, peaked at 75 ^{o}C, and ended at 80 ^{o}C (Figure 4.7). Bueso Ucles (2003) reported amylopectin crystallite melting temperature of around 50.3-57.4 ^{o}C for corn tortilla.

Energy required to melt amylopectin crystallite in the bread samples was illustrated in Figure 4.8. For all freshly baked bread samples, no endothermic peak was observed in the amylopectin crystallite melting range. This indicated negligible amount of amylopectin recrystallization in freshly baked bread. With increasing storage time, the melting enthalpy was found to increase, with the most pronounced in the control sample, followed by the 5%-substituted (5SF and 5OK), 10%-substituted (10SF and 10OK) and 15%-substituted (15SF and 15OK) samples. The 15OK exhibited the lowest amylopectin recrystallization throughout the storage time studied. In general, OK sample possessed slightly lower amylopectin recrystallization as compared to the SF sample of the same substitution level. Amylopectin recrystallization seemed to rapidly occur during the first two days of storage and leveled off after that.

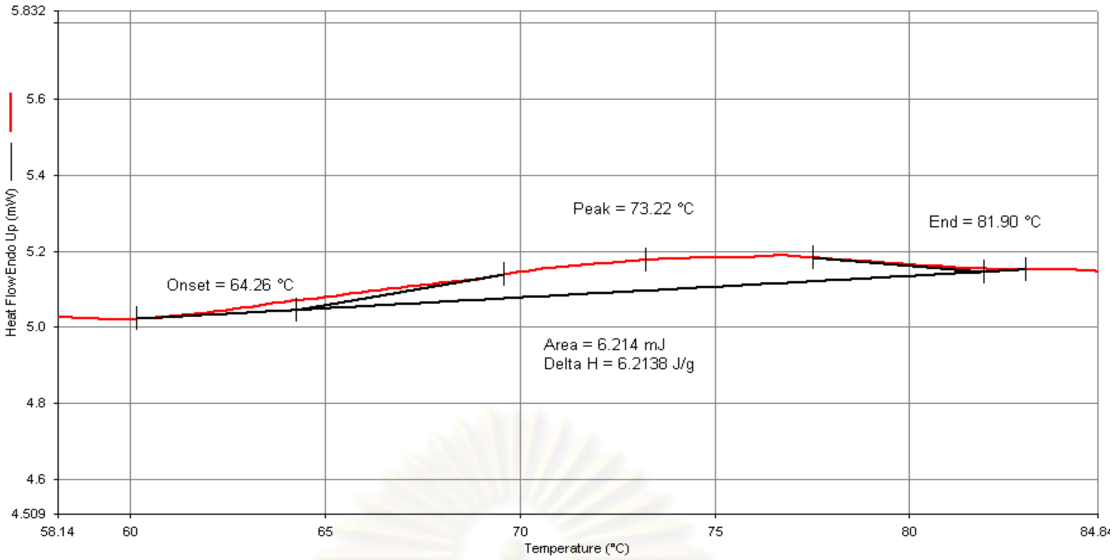


Figure 4.7 Typical DSC curve of amylopectin crystallite melting showing an endothermic transition at approximately 60-80 °C.

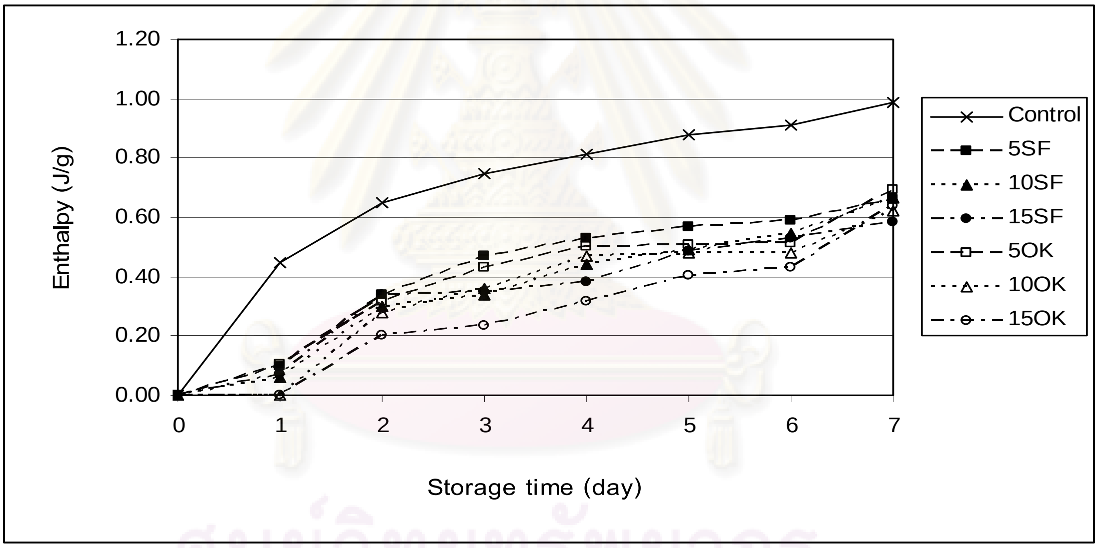


Figure 4.8 Melting enthalpy of recrystallized amylopectin in wheat, soy flour-substituted and okara-substituted breads during 7-day storage.

The role of soy ingredient in retarding amylopectin recrystallization was reported by Vittadini and Vodovotz (2003) who found that soy flour substitution at 20-40% helped decrease amylopectin recrystallization as compared to control wheat bread. Similar results were reported in previous studies (Longton and LeGrys, 1981; Roulet *et al.*, 1988). Recently, Zhou *et al.* (2008) reported that tea polysaccharide could lower retrogradation rate of wheat starch gel.

Baik and Chinachoti (2001) suggested that there was a correlation between lower amylopectin recrytallization and lower moisture gradient between bread crust and crumb. Soy ingredients helped promote homogeneous moisture distribution and thus lower moisture gradient within the loaf of bread (Section 4.3.3). This resulted in lower amylopectin recrystallization in soy-containing breads as compared to the control. Anil (2007) stated that the high water-holding capacity of soy components (soy protein and soy fiber) resulted in less water available for starch component, thus lowering starch recrystallization rate during storage.

Apart from water-holding and moisture-distributing effect of soy ingredient, Ryan *et al.* (2002) also proposed that soy components strongly interacted with starch, hence interfering starch-starch interaction and retarding amylopectin recrystallization.

Plant fibers such as wheat bran have been successfully used in various food products. One of the outstanding properties of these plant fibers is their excellent water-holding capacity. At present, a product made from cell wall fiber and protein of soybean cotyledon is available commercially (The production method is a proprietary procedure.). The manufacturer claims that this fiber product provides moisture absorption and control moisture migration in food products (Pacific Soybean & Grain, 2003).

To investigate the effect of different ingredients on moisture absorption, flour mixtures were determined for water-holding capacity and the result is shown in Figure 4.9. Different flour mixtures exhibited significantly different water-holding capacity ($p \leq 0.05$). Wheat flour/okara mixtures exhibited greater water-holding capacity than wheat flour/soy flour mixtures and wheat flour. Upon increasing level of soy substitution, water-holding capacity of the flour mixtures increased. This increased water-holding capacity of the soy-containing flour mixtures could be due to the higher fiber content of soy flour and okara as compared to wheat flour (Section 4.1).

4.3.5 Water soluble starch content

As starch retrogrades, the starch molecules (amylose and amylopectin) become reassociate via hydrogen bonding with a formation of ordered crystalline structure. The increase in crystallinity results in lowered solubility of starch. Therefore, water soluble starch content could be used as an indicator of starch retrogradation. Water soluble starch content of the bread samples is shown in Figure 4.10.

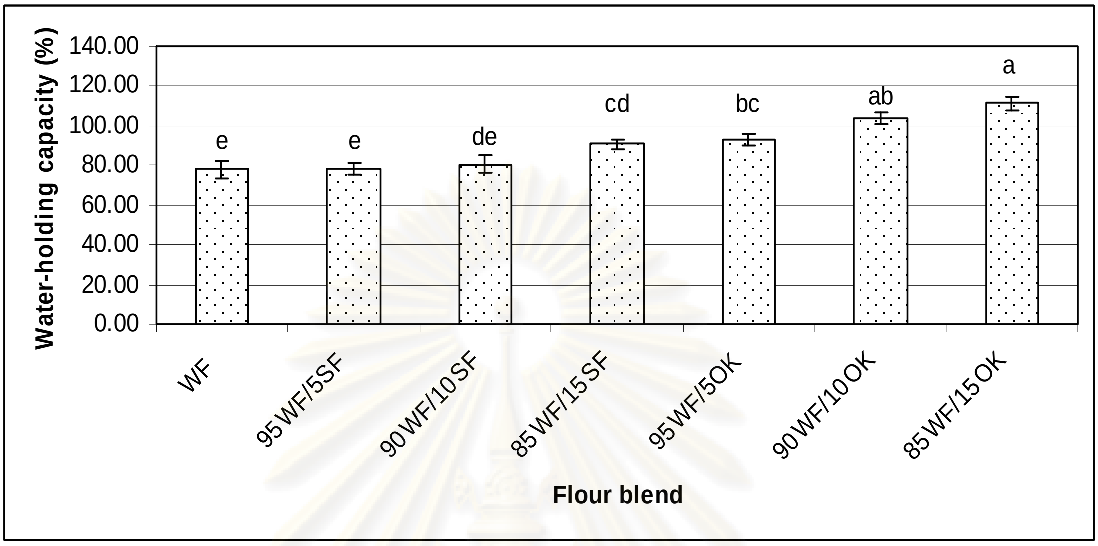


Figure 4.9 Water holding capacity of wheat flour (WF), wheat flour/soy flour mixtures (WF/SF) and wheat flour/okara mixtures (WF/OK). The number in each legend represents percentage of soy ingredient substitution in the flour mixture. Sample means which do not share a common letter differ significantly at *p*=0.05.

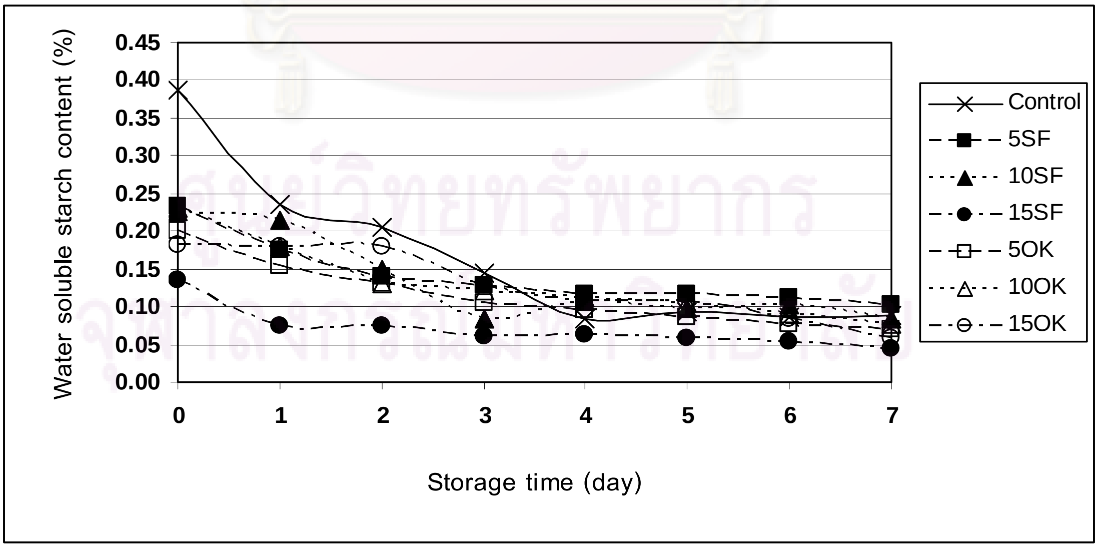


Figure 4.10 Water soluble starch content of wheat, soy flour-substituted and okara-substituted breads during 7-day storage.

Freshly baked control bread showed the highest water soluble starch content (0.39%). Upon substitution with soy flour or okara, which contained less starch, water soluble starch content was lowered significantly ($p \leq 0.05$). Water soluble starch content became lower with increasing storage time, with a sharper decrease in the control. The decrease in water soluble starch suggested the formation of water insoluble starch fraction (crystalline structure). This was also consistent with the increase in amylopectin recrystallization (Section 4.3.4).

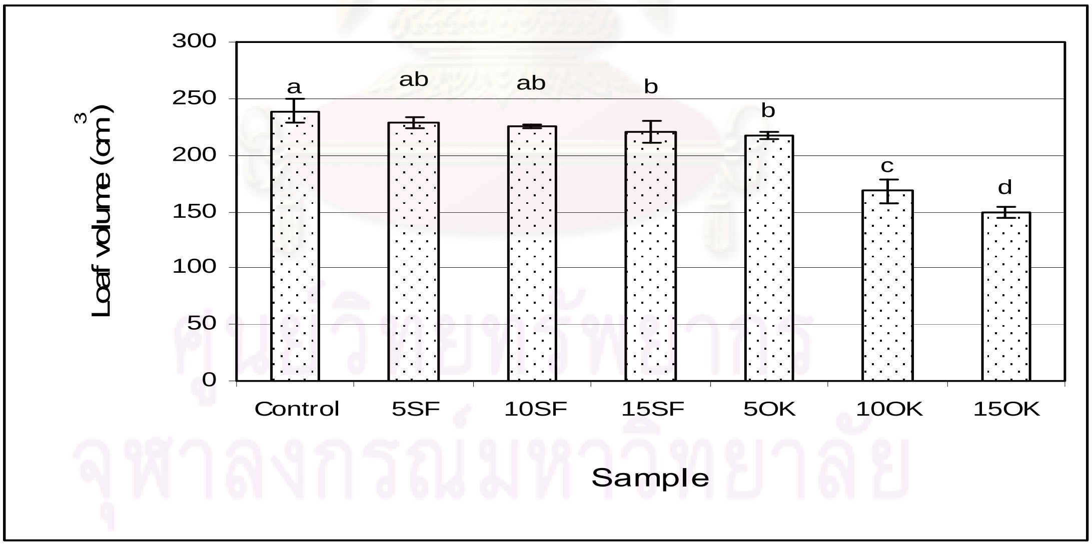
Shaikh *et al.* (2007) reported that water soluble starch content of Indian flat bread (*chapatti*) decreased from 5.23 to 0.30% during 1-month storage at room temperature. It was found that water soluble starch decreased sharply during the first 12-hour of storage. This was attributable to the retrogradation of amylose. After the first 12-hour, amylopectin slowly retrograded, resulted in a lower rate of soluble starch reduction.

For the effect of fiber substitution on the changes of water soluble starch content, Sidhu *et al.* (1997) measured water soluble starch content of commercial all-wheat Arabic bread (*khaboos*) and commercial wheat bran-added *khaboos*. It was reported that the fresh wheat bran-added bread exhibited lower water soluble starch content (2.17%) as compared to the all-wheat bread (2.35%). Upon 4-day storage, water soluble starch content of both breads decreased with the all-wheat bread showing a greater reduction in soluble starch than the bran-added bread (32.8% vs. 31.8% reduction).

4.3.6 Baking characteristics

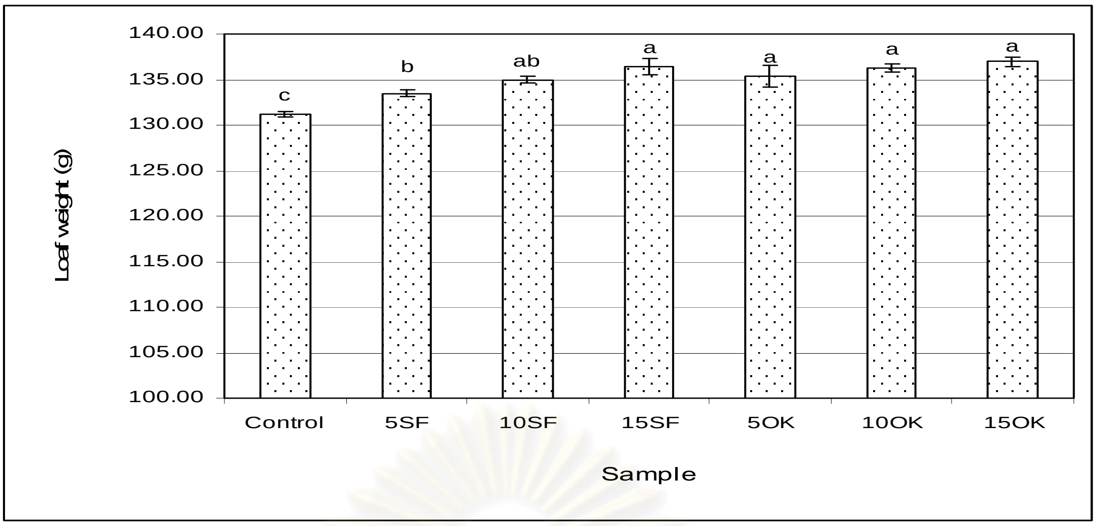
Baking characteristics such as loaf volume and loaf height pose a strong influence on consumer acceptance of bread product. Addition of soy ingredient to wheat bread has been known to negatively affect those attributes so it is essential to monitor changes in baking characteristics of soy-containing bread. Baking characteristics determined in this study include loaf volume, loaf weight, specific loaf volume and loaf height. These baking characteristics of freshly baked bread samples are presented in Figure 4.11.

A significant reduction in loaf volume ($p \leq 0.05$) was observed when soy ingredient was substituted (Figure 4.11a). At lower levels of soy flour substitution (5SF and 10SF), the bread samples had similar loaf volume to the control. However, with higher soy flour substitution (15SF), loaf volume became significantly decreased ($p \leq 0.05$) as compared to the control. Okara substitution was found to strongly affect loaf volume in a negative way. The 5OK had significantly lower loaf volume than the control. Loaf volume of okara-containing breads decreased with increasing level of substitution. The 15OK had the lowest loaf volume, a 38.08% reduction from the control. This depression in loaf volume upon substitution of non-wheat ingredient has been attributable to the dilution of gluten, a wheat protein necessary for bread structure and carbon dioxide retention (Dhingra and Jood, 2004). Moreover, the presence of high amount of fiber could also interfere with the formation gluten matrix, resulting in bread with inferior loaf volume (Hung, Maeda and Morita, 2007). Decrease in loaf volume upon addition of non-wheat ingredients have been reported by various researchers (Sharma, Bajwa and Nagi, 1999; Dhingra and Jood, 2004; Mohamed *et al.*, 2006).

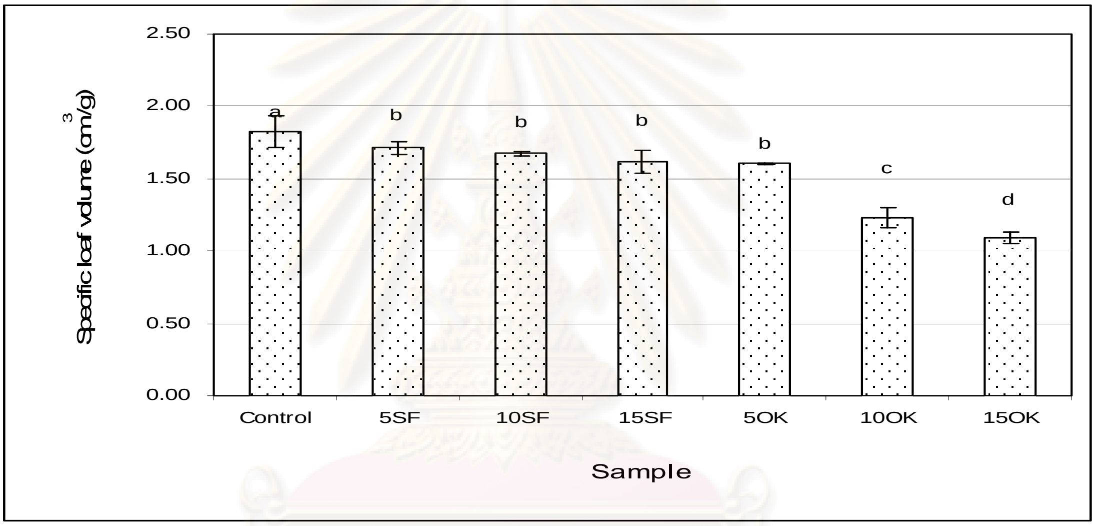


(a) Loaf volume

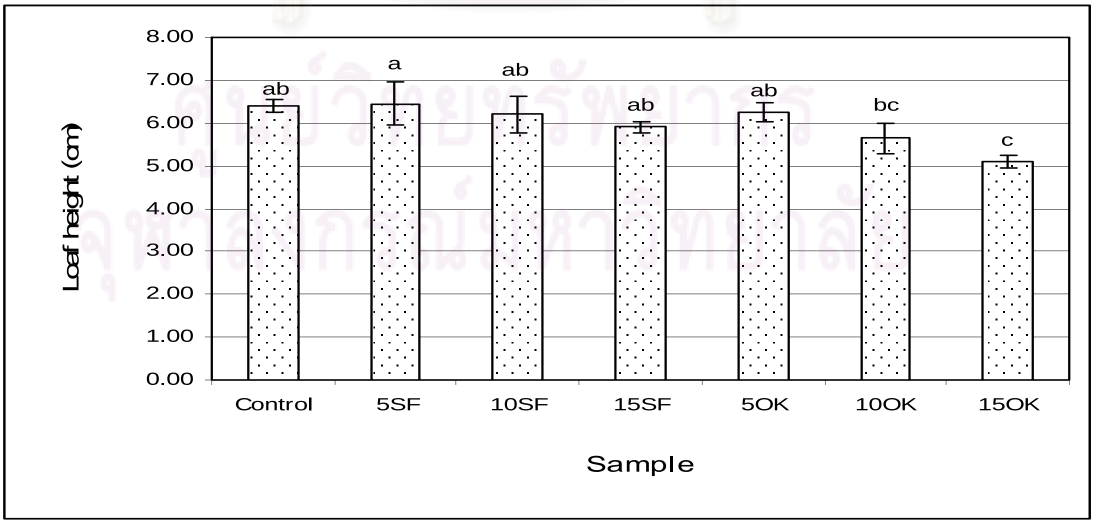
Figure 4.11 Baking characteristics of freshly baked wheat, soy flour-substituted and okara-substituted breads.



(b) Loaf weight



(c) Specific loaf volume



(d) Loaf height

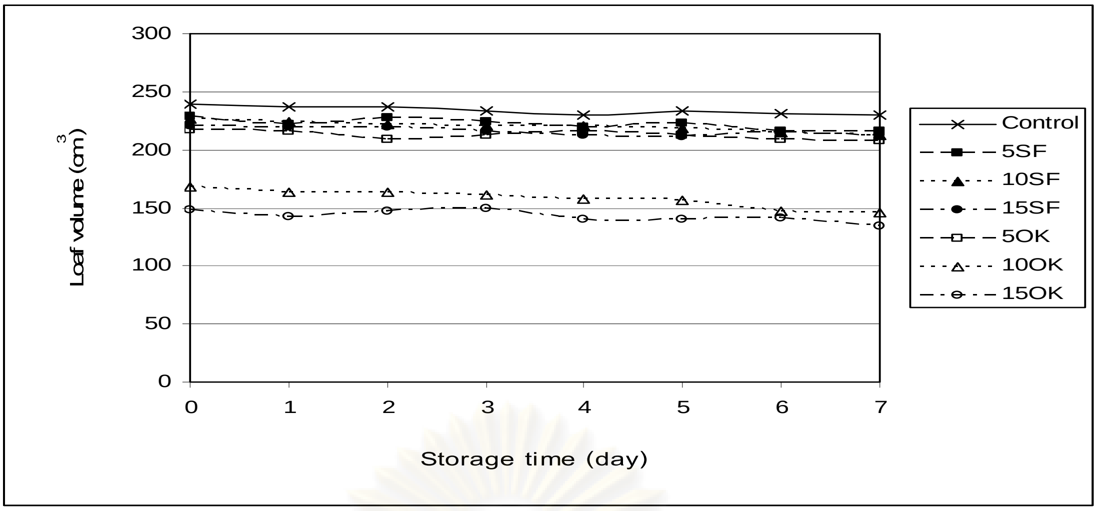
Figure 4.11 (Continued)

Loaf weight of soy-substituted breads was significantly higher than the control ($p \leq 0.05$) (Figure 4.11b). Upon increasing level of soy flour or okara substitution, loaf weight tended to increase. However, for okara-substituted samples, loaf weight did not differ significantly among different substitution levels ($p > 0.05$). Dhingra and Jood (2004) reported an increase in loaf weight with full-fat and defatted soy flour substitution. This increase in loaf weight could be due to that higher amount of moisture was retained by the substituted breads after baking (Rao and Hemamalini, 1991). Increase in loaf weight upon incorporation of non-wheat ingredients have also been reported by other researchers (Mohamed *et al*., 2006).

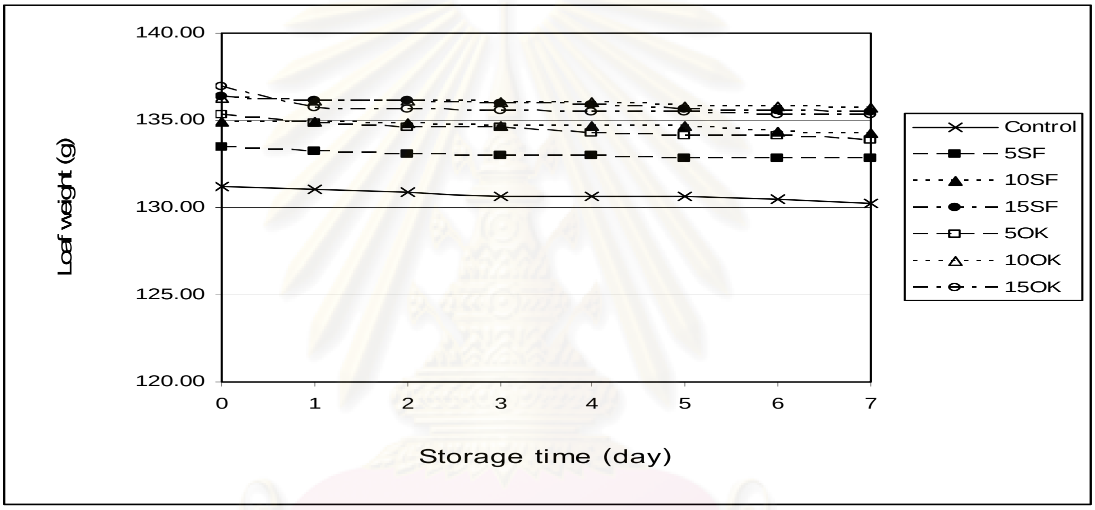
Specific loaf volume is obtained by dividing loaf volume by loaf weight and it is inversed with density. It was found that specific loaf volume differed significantly among bread samples ($p \leq 0.05$) (Figure 4.11c). SF breads had similar specific loaf volume to the control while OK breads had decreased specific loaf volume with increasing level of substitution. The decrease in specific loaf volume corresponded to the decrease in loaf volume and increase in loaf weight upon substitution of soy ingredients. Vittadini and Vodovotz (2003) demonstrated that loaf density directly correlated with level of soy flour substitution. Sangnark and Noomhorm (2004) reported similar results for breads substituted with dietary fiber from sugarcane bagasse. It was found that with 15% substitution of sugarcane fiber, the bread possessed decreased specific loaf volume (4.47 cm^3/g) as compared to the wheat control (6.81 cm^3/g).

Loaf height was found to decrease with substitution of soy ingredient (Figure 4.11d). However, loaf height of all SF breads (5SF, 10SF and 15SF) and the OK bread at low levels of substitution (5OK and 10OK) was not significantly different from that of the control.

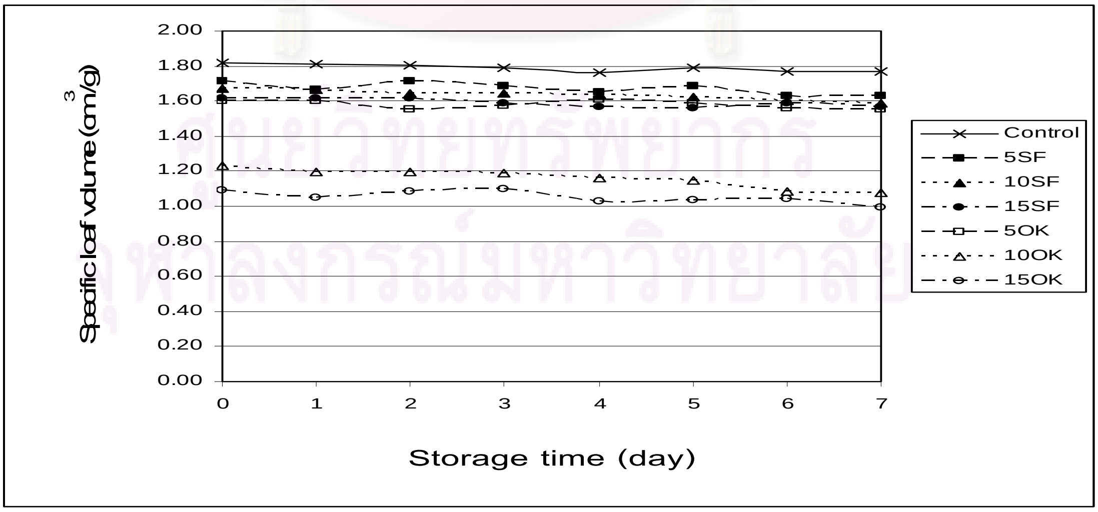
Figure 4.12 shows the loaf volume, loaf weight, specific loaf volume and loaf height during 7-day storage. It was found that all the baking characteristics decreased slightly during storage. However, these changes were not statistically significant ($p > 0.05$).



(a) Loaf volume

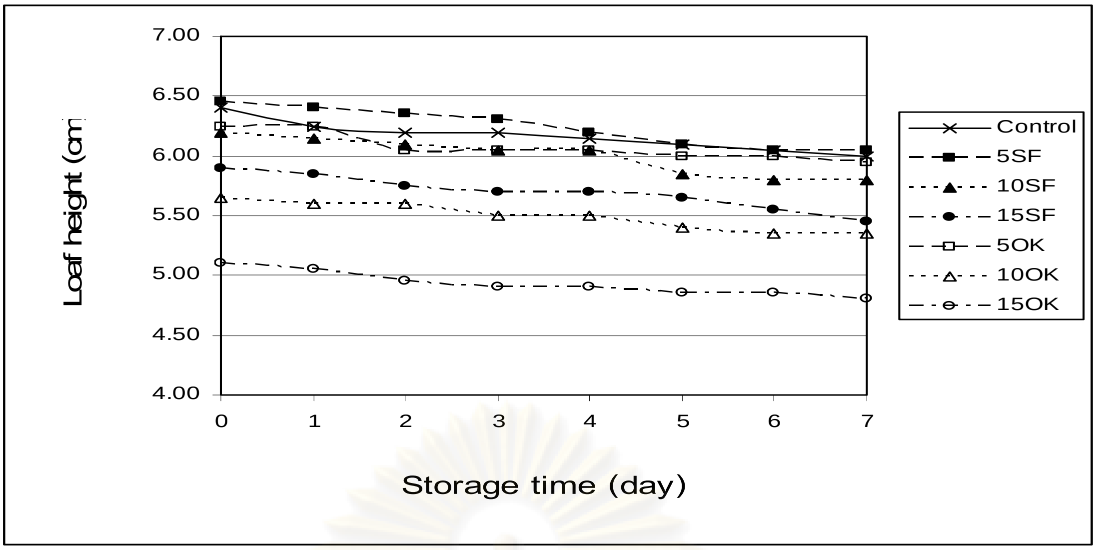


(b) Loaf weight



(b) Specific loaf volume

Figure 4.12 Baking characteristics of wheat, soy flour-substituted and okara-substituted breads during 7-day storage.



(d) Loaf height

Figure 4.12 (Continued)

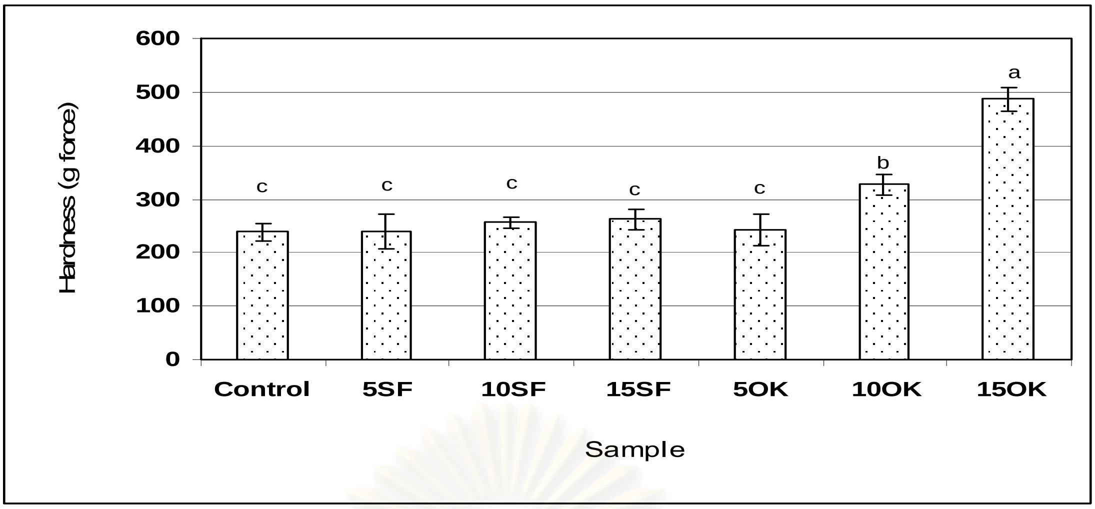
4.3.7 Textural characteristics

Texture is one of the important characteristics of bread. Different ingredients added to bread recipe play an important role in modifying its texture. It is generally recognized that bread becomes firmer as it is aging. For this reason, consumers have been using a "squeeze test" to evaluate bread freshness.

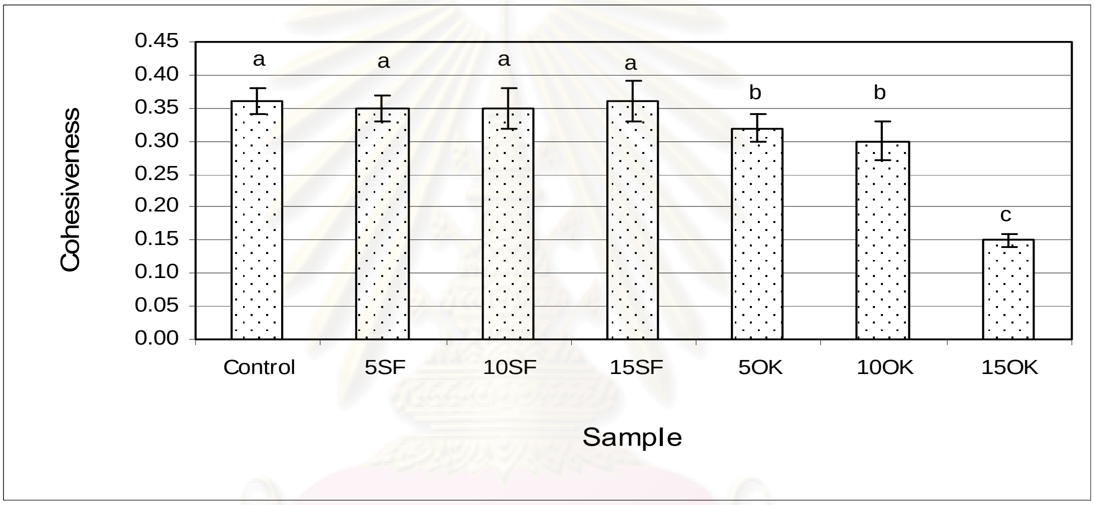
In this study, a Texture Profile Analysis (TPA) was used to evaluate textural parameters of bread. TPA, also called a "two bite test", is a popular technique for characterizing food structure. TPA provides textural parameters which highly correlate with parameters obtained by sensory evaluation (Stable Micro Systems, 1996). Seven parameters, namely, hardness, cohesiveness, springiness, fracturability, chewiness, gumminess and resilience, could be obtained from a TPA curve (Bourne, 1982). Typical TPA curve is shown in Appendix B4. In the current study, hardness, cohesiveness and springiness were reported for bread samples (Figure 4.13). Hardness is the peak force of the first compression of the product. Cohesiveness reflects the ability of the product to withstand the second deformation relative to the first deformation. Springiness indicates the ability of the product to spring back after the first deformation (Bourne, 1982).

Addition of ingredient high in fiber has been known to affect texture, especially hardness of bread. Upon addition of soy flour (5SF, 10SF and 15SF) and addition of okara at low level (5OK), hardness of the freshly baked bread samples was similar to the control (p>0.05) (Figure 4.13a). However, at higher okara substitutions (10OK and 15OK), hardness dramatically increased ($p \leq 0.05$). Waliszewski *et al.* (2002) substituted okara in corn tortilla and reported that hardness of the okara-added tortillas, as evaluated organoleptically, tended to increase with increasing level of okara substitution. However, it was reported that as high as 25% of okara could be substituted without any detectable changes in hardness of tortilla. This could be due to the fact that tortilla is a flat bread which is normally harder in texture than leavened bread. Therefore, the increase in hardness did not pose a significant effect on tortilla as it did on white bread.

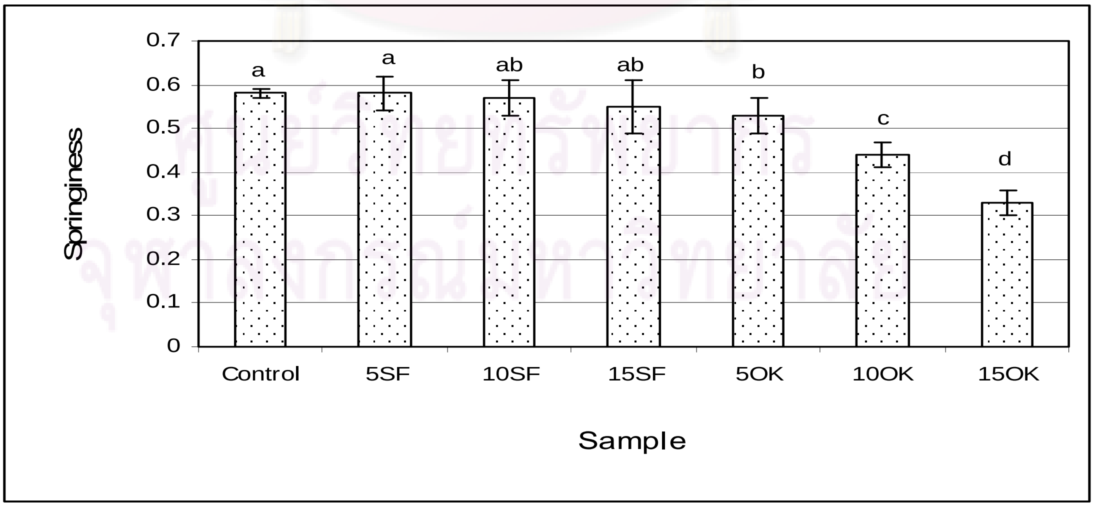
Similar changes in cohesiveness and springiness upon addition of soy ingredients were observed (Figure 4.13b and c). It was found that upon increasing level of soy flour substitution, the value of each textural parameter was similar to the control (p>0.05). On the other hand, those parameters of OK samples became decreasing with increasing level of substitution. The decrease in value of those textural parameters of OK breads could be due to decreasing gluten concentration. Upon substitution of soy flour or okara, the concentration of gluten responsible for good structure and proper porosity of bread was decreased. In the case of SF samples, even though there was a gluten dilution effect, soy flour itself was high in protein content. Soy protein has been known for its structure providing effect in various foods such as tofu (Watanabe and Kishi, 1984), yuba (Watanabe and Kishi, 1984) and meat products (Songkanlayanawat, 2007; Herrero *et al.*, 2008). This, therefore, helped alleviate the negative effect of soy flour substitution on bread texture.



(a) Hardness



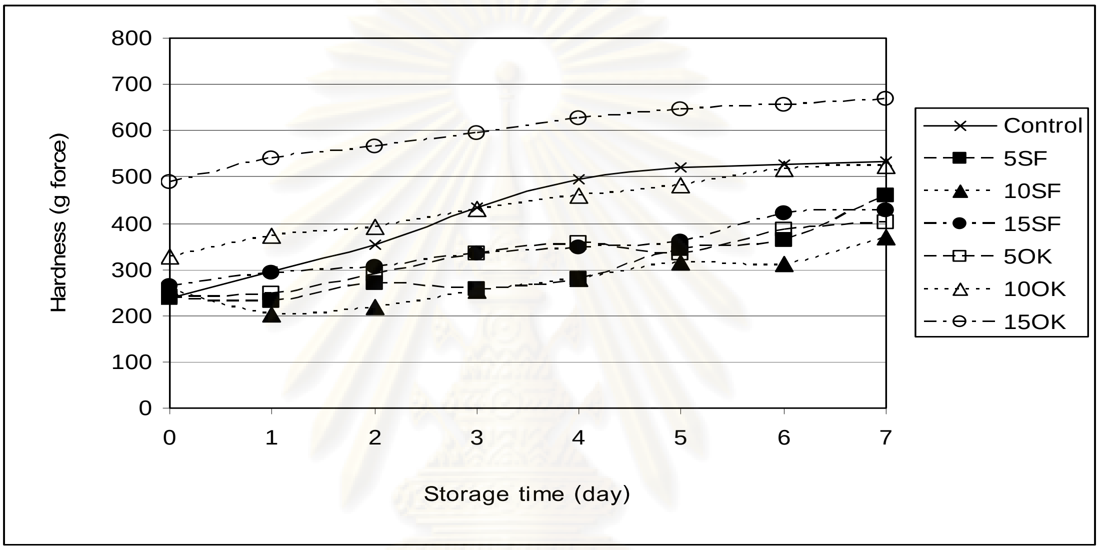
(b) Cohesiveness



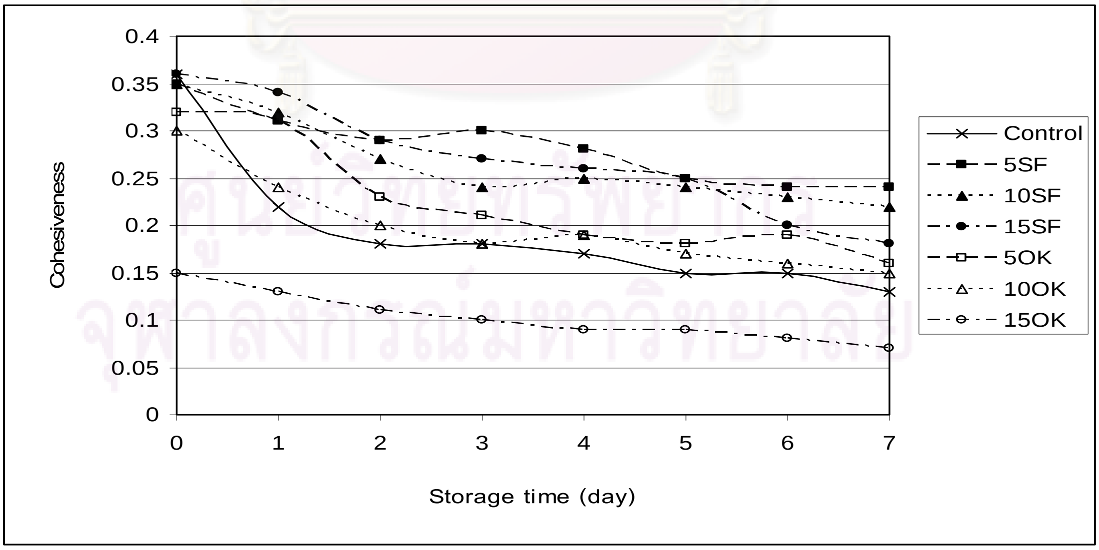
(c) Springiness

Figure 4.13 Textural characteristics of freshly baked wheat, soy flour-substituted and okara-substituted breads.

Changes of textural parameters of the bread samples during 7-day storage are shown in Figure 4.14. Hardness of all bread samples increased during storage (Figure 4.14a). The increasing rate was lower in SF and OK samples than the control. This was likely to be due to greater changes regarding moisture and starch during storage of the control bread (Section 4.3.1-4.3.5). All breads exhibited decreasing cohesiveness and springiness with increasing storage time, which was also more pronounced in the control bread (Figure 4.14b and c).

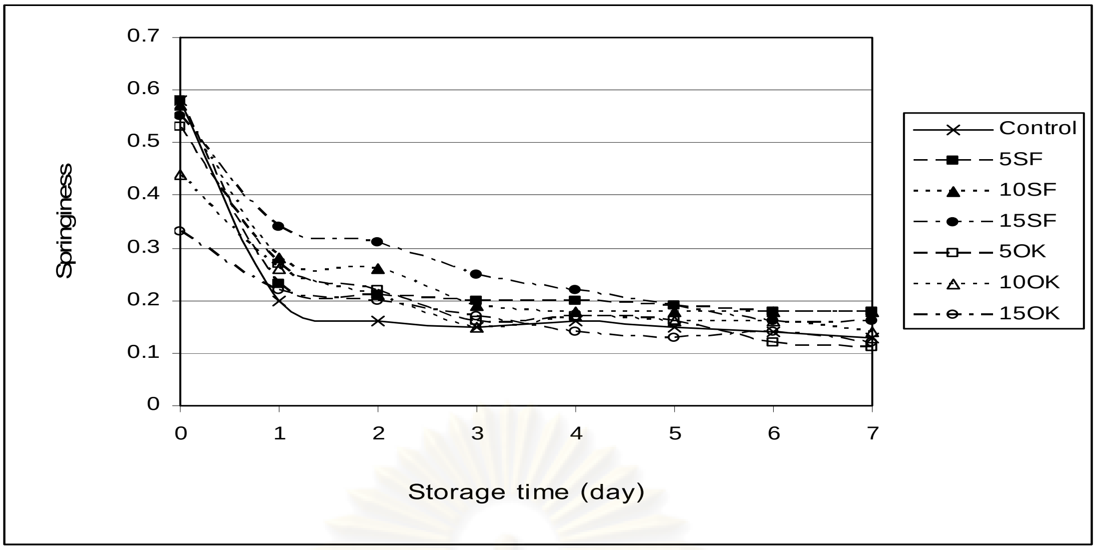


(a) Hardness



(b) Cohesiveness

Figure 4.14 Textural characteristics of wheat, soy flour-substituted and okara-substituted breads during 7-day storage.

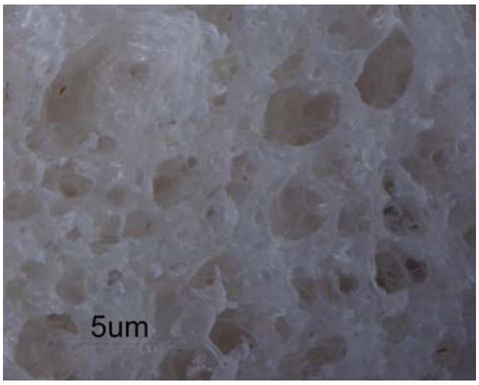


(b) Springiness

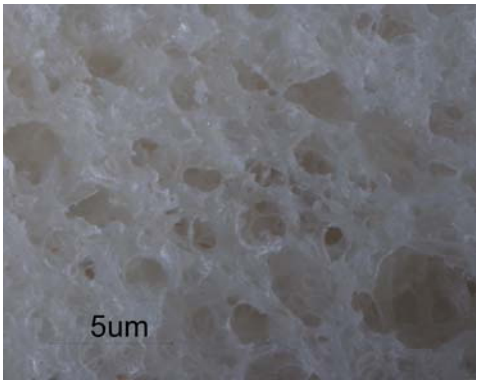
Figure 4.14 (Continued)

4.3.8 Crumb grain structure

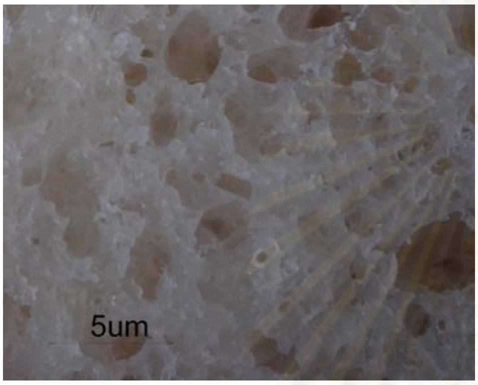
Grain structure of the freshly baked bread crumbs and those stored for a period of 7 days was investigated using a stereomicroscope (Figure 4.15). The freshly baked control bread crumb (Figure 4.15a) appeared whiter and more transparent as compared to the soy-substituted crumbs. Crumb alveoli (air cells) of the control bread were round, uniform in size and evenly distributed throughout the crumb matrix. Upon soy substitution (Figure 4.15c, e, g, i, k, m), the crumb was increased in yellowness, especially for the SF samples. Crumb alveoli became smaller and varying in shape and size. The 15OK exhibited the smallest alveoli among the samples. The less porous structure of soy-containing bread was due to dilution of gluten. Upon adding soy ingredient, the concentration of gluten necessary for dough elasticity and gas retention was decreased. All samples became more opaque as they were staling (Figure 4.15b, d, f, h, j, l, n). However, changes in grain structure upon storage were not readily noticeable.



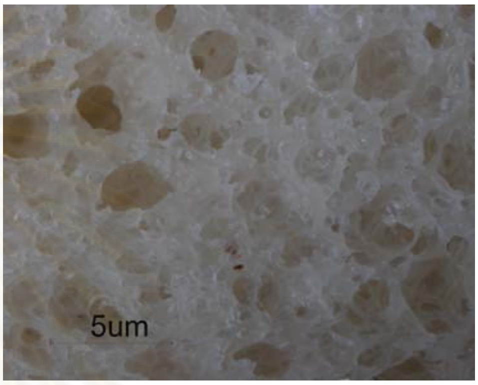
(a) Control, Day 0



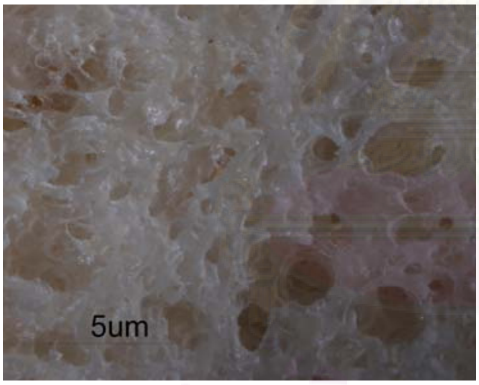
(b) Control, Day 7



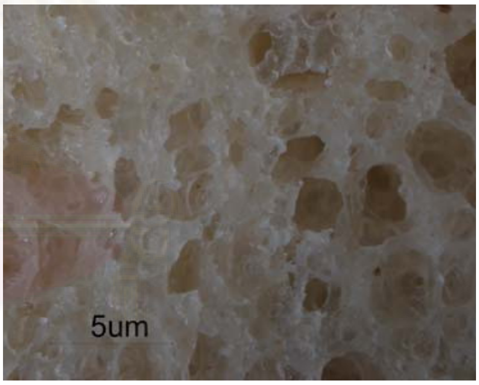
(c) 5SF, Day 0



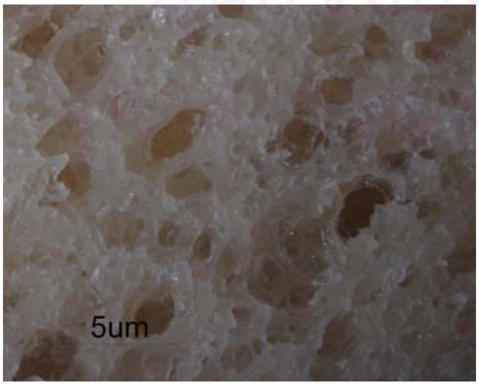
(d) 5SF, Day 7



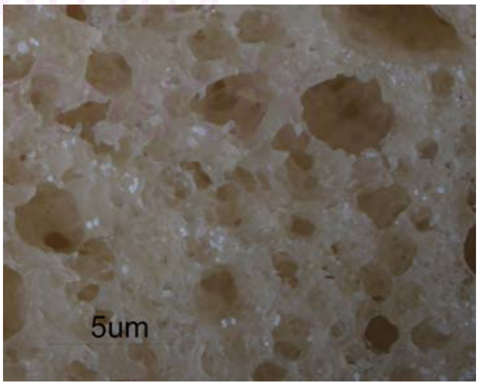
(e) 10SF, Day 0



(f) 10SF, Day 7

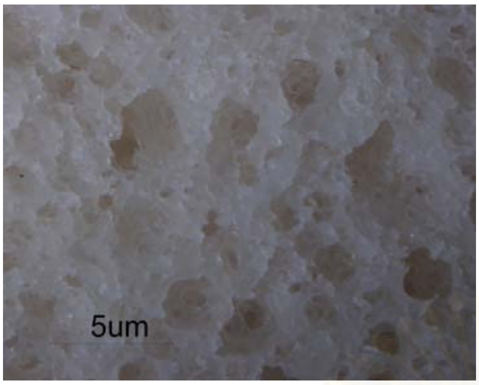


(g) 15SF, Day 0

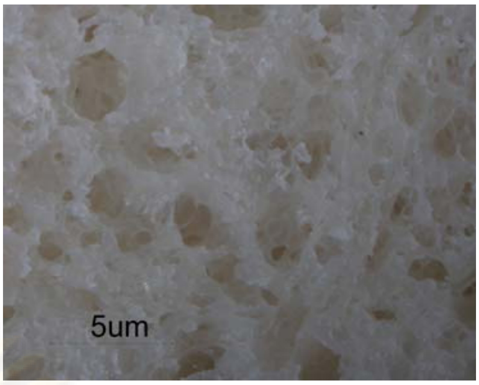


(h) 15SF, Day 7

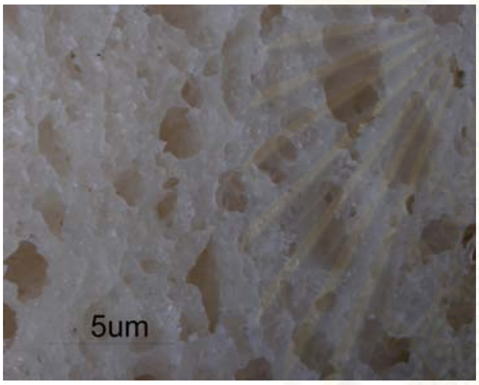
Figure 4.15 Crumb grain of wheat, soy flour-substituted and okara-substituted breads at Day 0 and Day 7 of storage.



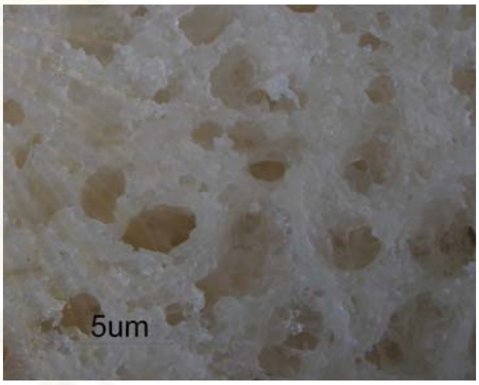
(i) 5OK, Day 0



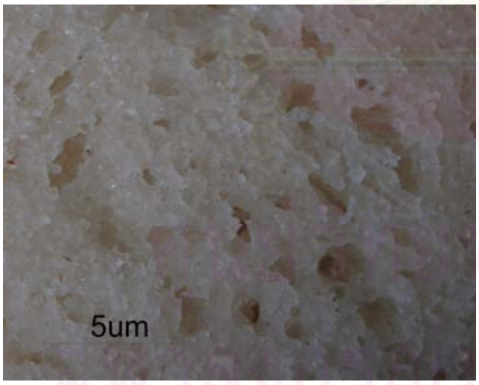
(j) 5OK, Day 7



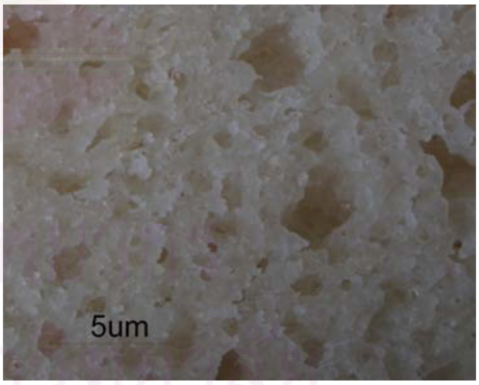
(k) 10OK, Day 0



(l) 10OK, Day 7



(m) 15OK, Day 0



(n) 15OK, Day 7

Figure 4.15 (Continued)

4.3.9 Color

Bread color is one of the crucial factors affecting consumer acceptance. In the case of basic white bread, crumb and crust with lighter color are generally preferred. In this study, CIE L^* a^* b^* were measured. However, humans perceive color as a combination of these values as they are represented in the color space. Therefore, hue angle, chroma and whiteness index were calculated for bread crumb while hue angle and chroma were calculated for bread crust. Color parameters of freshly baked crumbs and crusts were reported in Tables 4.2 and 4.3.

Table 4.2 Color parameters of freshly baked wheat, soy flour-substituted and okara-substituted bread crumbs

Crumb sample	Hue angle	Chroma	Whiteness index	L^*
Control	$91.50^{a}\pm2.56$	$12.18^{f}\pm0.80$	$68.02^{a}\pm0.50$	$70.43^{c}\pm0.65$
5SF	$91.05^{a}\pm0.99$	$15.05^{d}\pm1.10$	$64.09^{c}\pm0.96$	$67.40^{d}\pm0.87$
10SF	$89.75^{bc}\pm0.76$	$17.60^{c}\pm0.69$	$62.12^{d}\pm0.65$	$66.46^{e}\pm0.56$
15SF	$87.89^{d}\pm0.99$	$21.29^{a}\pm0.78$	$59.43^{e}\pm1.03$	$65.47^{f}\pm1.03$
5OK	$90.67^{ab}\pm0.55$	$13.79^{e}\pm0.33$	$67.48^{ab}\pm0.26$	$70.55^{c}\pm0.35$
10OK	$89.87^{a}\pm1.00$	$17.12^{c}\pm0.47$	$67.11^{b}\pm1.04$	$71.91^{b}\pm1.37$
15OK	$89.50^{c}\pm0.79$	$19.04^{b}\pm0.38$	$67.26^{b}\pm0.84$	$73.36^{a}\pm1.07$

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Table 4.3 Color parameters of freshly baked wheat, soy flour-substituted and okara-substituted bread crusts

Crust sample	Hue angle	Chroma	L^*
Control	$60.51^{b}\pm0.86$	$32.79^{b}\pm0.47$	$46.50^{b}\pm0.58$
5SF	$56.15^{d}\pm1.65$	$28.26^{cd}\pm1.04$	$41.14^{d}\pm1.10$
10SF	$55.73^{d}\pm0.78$	$27.51^{d}\pm1.18$	$38.59^{e}\pm1.04$
15SF	$54.15^{e}\pm1.84$	$26.58^{e}\pm0.98$	$36.24^{f}\pm0.80$
5OK	$56.83^{d}\pm1.24$	$28.47^{c}\pm0.67$	$42.87^{c}\pm0.64$
10OK	$58.65^{c}\pm0.82$	$32.77^{b}\pm0.66$	$46.88^{b}\pm1.13$
15OK	$62.77^{a}\pm1.09$	$35.96^{a}\pm0.93$	$52.61^{a}\pm2.44$

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Hue is the actual color of an object such as red, yellow and blue (Figure 4.16). Chroma is the intensity or purity of a color. High chroma color looks rich and full while low chroma color looks dull and grayish (Adamson, 2009).

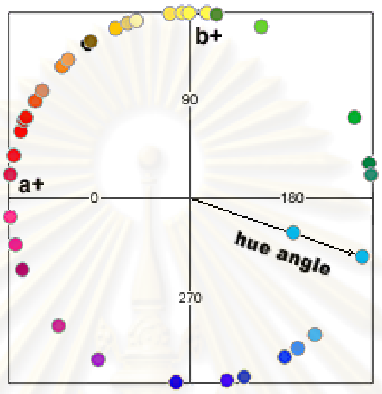
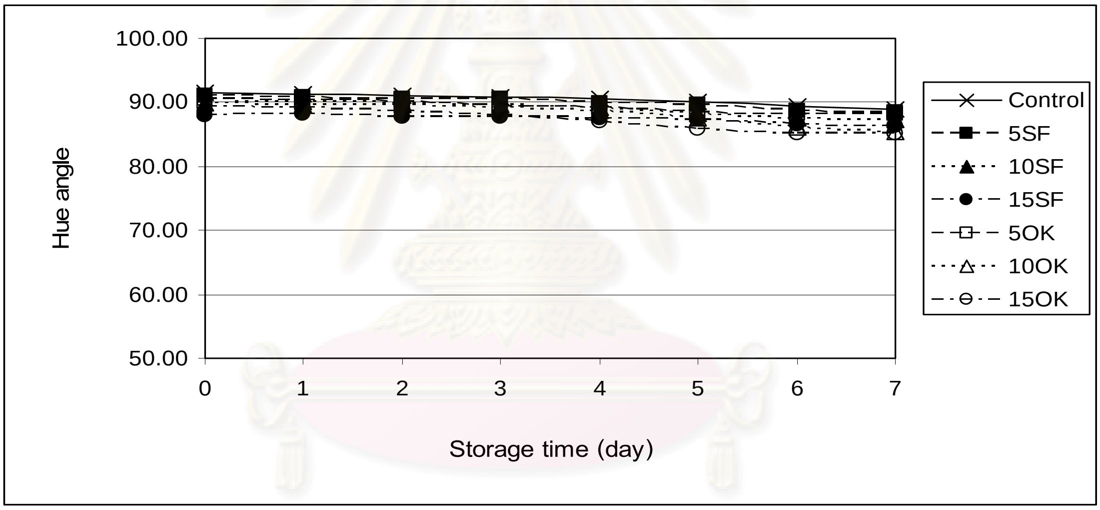


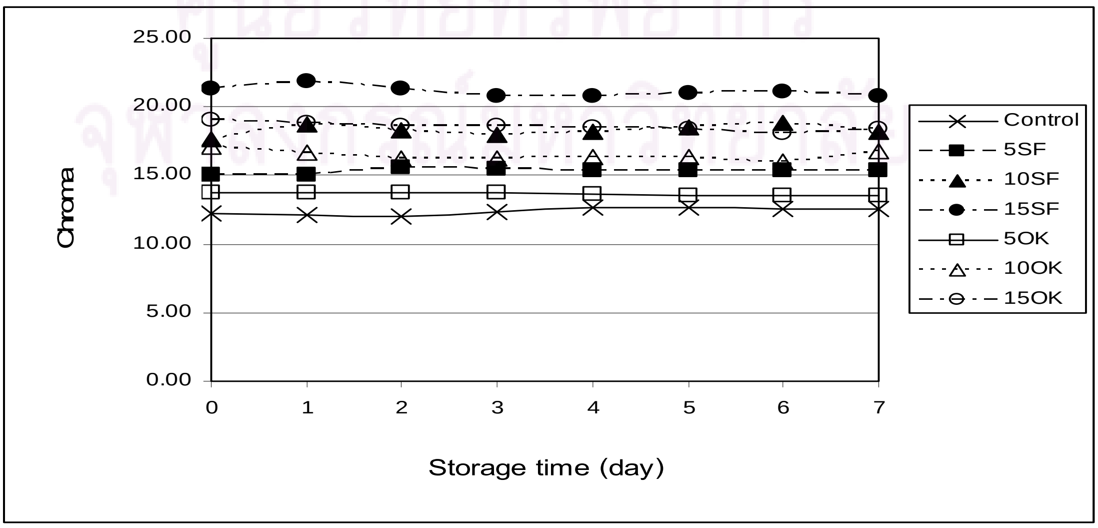
Figure 4.16 Visual color wheel with hue angle (Source: Handprint Media, 2001)

Hue angle of all crumb samples were approximately 90° (Table 4.2), representing yellow color. The hue angle reported in this study was in good agreement with that reported for okara-substituted corn tortilla (Waliszewski *et al.*, 2002). Chroma of bread crumb was obviously affected by soy substitution. The intensity of yellowness became increased with increasing substitution level. Watanabe and Kishi (1984) stated that isoflavones are responsible for the pale yellow color of soybean. Isoflavones are water soluble and therefore could be leached out from okara upon soymilk extraction. This explained the more intense yellowness of SF crumb as compared to the OK crumb of the same substitution level. Whiteness index decreased with increasing level of soy substitution. Similar results were also reported by Waliszewski *et al.* (2002). Substitution of soy flour resulted in a decrease in lightness (L^*) while substitution of okara caused an increase in lightness. L^* value was significantly different among the samples (p<0.05). However, it varied within quite a narrow range (65.47-73.36).

Bread crusts exhibited hue angle in the 60° range (Table 4.3), representing orange-yellow color. Two major mechanisms imparting color of bread crust include caramelization of sugars and interaction between sugars and protein materials (Maillard browning) (Pomeranz and Shallenberger, 1971). The control crust exhibited a chroma of 32.79. Chroma of the crust slightly decreased upon substitution of soy flour (5SF, 10SF and 15SF) and substitution of okara at low levels (5OK and 10OK). However, at higher substitution of okara (15OK), increasing chroma was observed. The changes in chroma with no definite trend could be due to the fact that, with soy substitution, concentration of sugars available for browning reactions (caramelization and Maillard reactions) decreased, even though the content of lysine available for Maillard reactions increased (Hallén, İbanoğlu and Ainsworth, 2004). Changes in lightness (L^*) of bread crusts upon soy-substitution were of similar trend. All crumb and crust samples exhibited no significant changes in color attributes during storage (Figures 4.17 and 4.18).

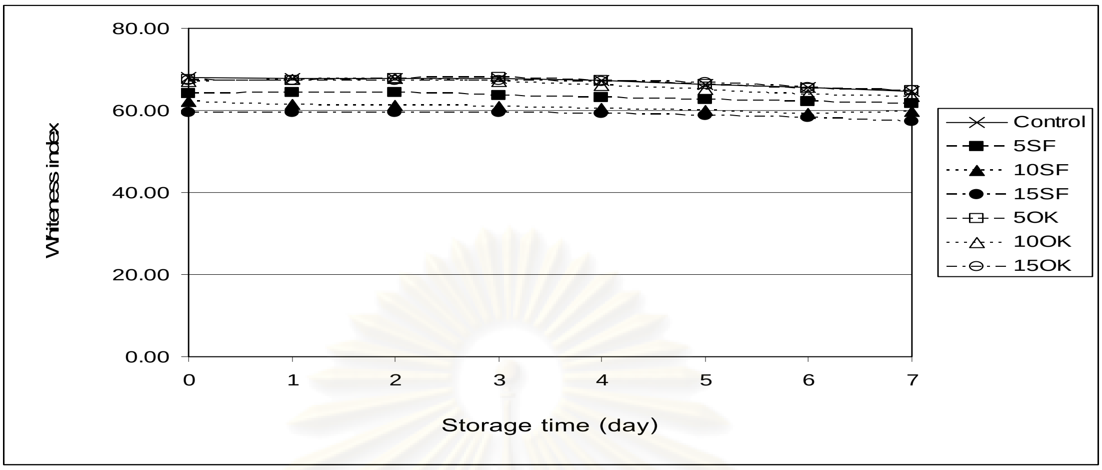


(a) Hue angle

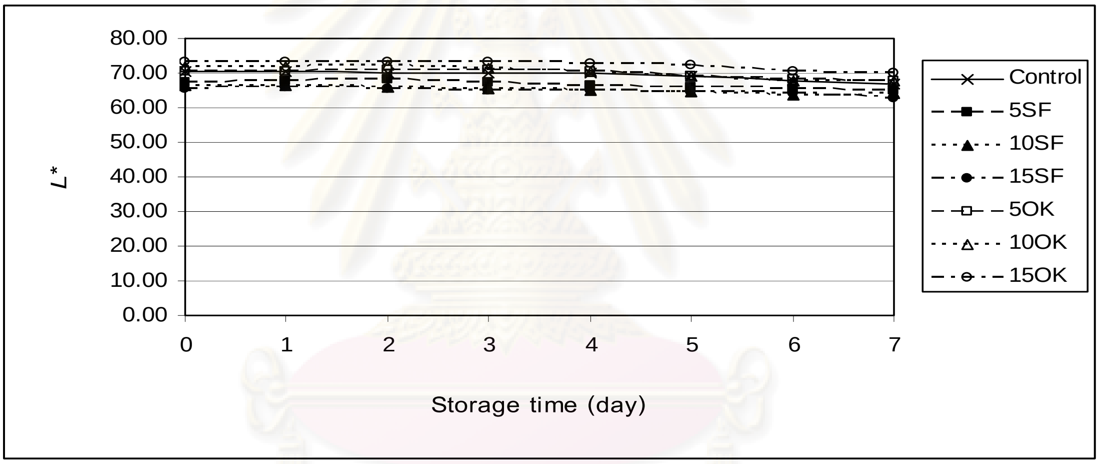


(b) Chroma

Figure 4.17 Color parameters of wheat, soy flour-substituted and okara-substituted crumbs during 7-day storage.

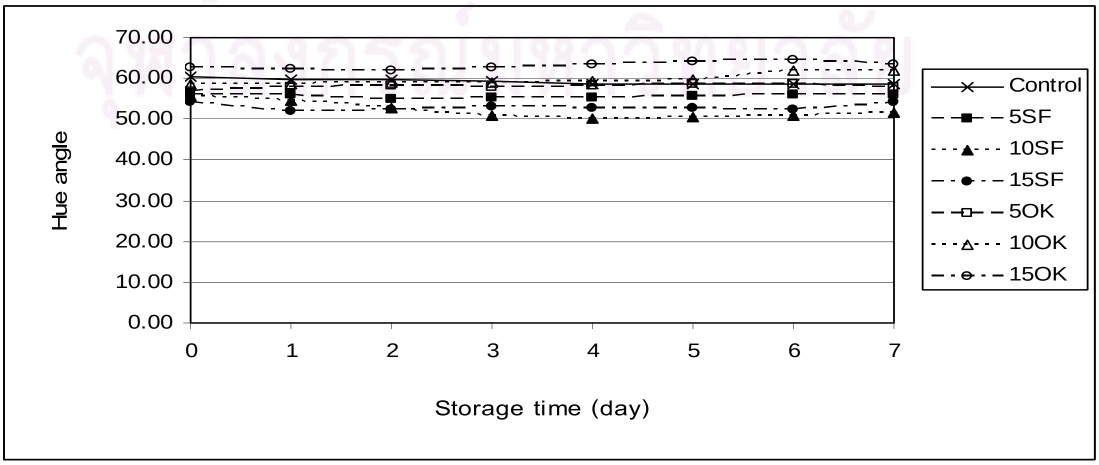


(c) Whiteness index



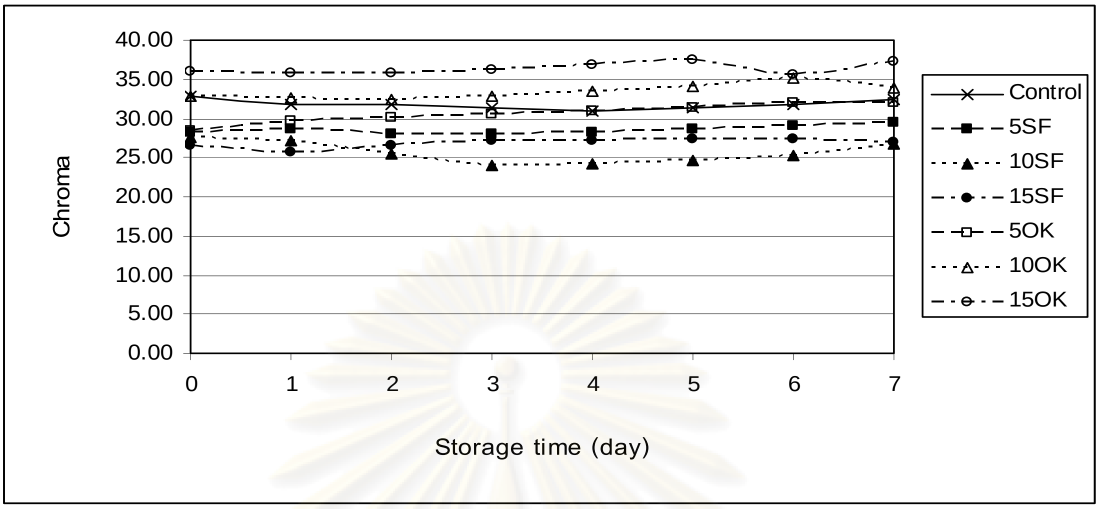
(d) L^*

Figure 4.17 (Continued)

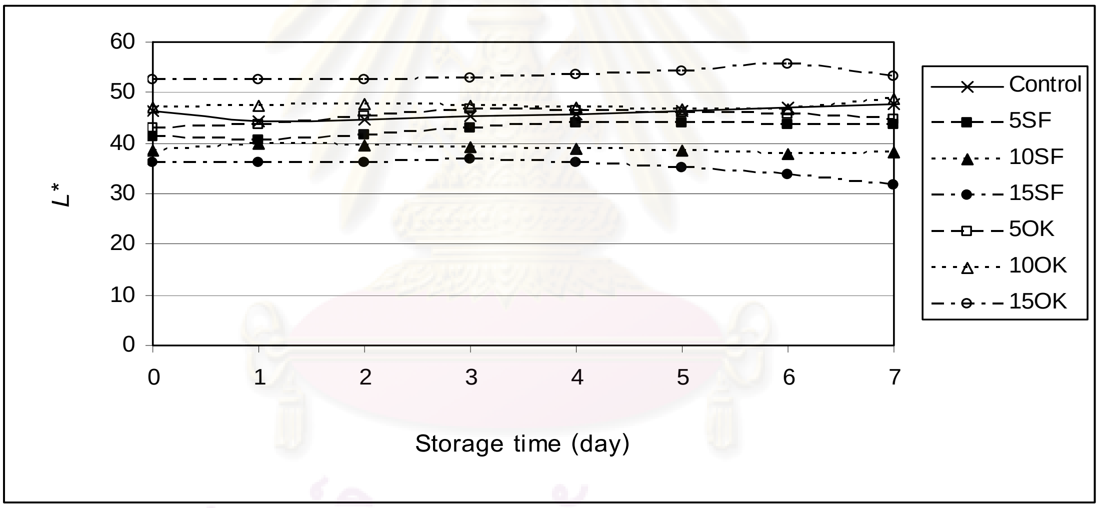


(a) Hue angle

Figure 4.18 Color parameters of wheat, soy flour-substituted and okara-substituted crusts during 7-day storage.



(c) Chroma



(d) *L**

Figure 4.18 (Continued)

4.3.10 Sensory attributes

4.3.10.1 Descriptive analysis (DA)

The bread samples were evaluated for the intensity of the following attributes: crust color (orange-brown), crumb color (yellow), porosity (pore homogeneity and pore size), texture (hardness, cohesiveness and springiness) and flavor (beany flavor) (Appendix B2). The results are shown in Table 4.4-4.11.

From sensory evaluation of crust color, it was found that the intensity of orange-brown color was not different among the samples (p>0.05) (Table 4.4), even though the chroma values obtained using a color meter differed significantly (p≤0.05) (Section 4.3.9). On the other hand, yellowness intensity of crumb samples as evaluated organoleptically (Table 4.5) strongly agreed with the result obtained objectively (Section 4.3.9). Upon substitution of soy ingredients, the yellowness intensity increased significantly (p≤0.05). This was especially pronounced in the SF samples. Upon storing for 5 days, the panel rated the intensity of both crust and crumb color similarly to those of the freshly baked sample (Day 0).

Table 4.4 Crust color (orange-brown), as evaluated using DA, of wheat, soy flour-substituted and okara-substituted breads during 5-day storage

samples	Day 0 ^{ns}	Day 1 ^{ns}	Day 2 ^{ns}	Day 3 ^{ns}	Day 4 ^{ns}	Day 5 ^{ns}
Control	9.43±0.82	9.15±0.56	9.25±1.38	9.50±0.94	9.51±1.52	9.80±1.68
5SF	9.88±0.84	9.66±1.55	10.45±1.09	9.65±1.28	9.83±1.78	10.00±1.53
10SF	10.44±0.94	10.52±1.53	10.60±0.89	10.66±1.76	11.49±1.59	10.30±1.80
15SF	10.63±1.50	9.94±1.68	10.75±1.37	10.55±1.58	9.61±1.78	10.35±1.60
5OK	9.72±0.89	9.61±1.10	10.45±1.22	9.76±0.71	9.92±1.24	10.25±1.44
10OK	9.76±1.58	10.42±1.28	10.80±1.46	9.45±1.28	9.92±1.51	9.80±1.29
15OK	9.33±1.08	10.21±1.47	10.55±1.80	10.11±0.82	9.95±1.03	9.55±1.14

Values are means ± SD of three different determinations.

"ns" indicates no significant difference among sample means at p=0.05.

Pore homogeneity and pore size were also evaluated for bread samples (Tables 4.6 and 4.7). The soy-substituted samples received a significantly lower score for pore homogeneity than the control (p≤0.05). It was indicated that pores of the SF samples were more evenly distributed as compared to those of the OK sample of the same substitution level. There was no difference regarding to pore size among the SF samples and the control (p>0.05). However, the OK samples had smaller pore size than the control. The lower pore homogeneity and smaller pore size of the OK samples were due to gluten dilution effect (Dhingra and Jood, 2004) and the lower concentration of proteins necessary for structure and gas retention.

Table 4.5 Crumb color (yellow), as evaluated using DA, of wheat, soy flour-substituted and okara-substituted breads during 5-day storage

samples	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Control	$2.33^{d}\pm0.74$	$2.54^{e}\pm0.99$	$1.90^{e}\pm0.96$	$2.87^{e}\pm0.66$	$3.03^{f}\pm1.11$	$2.40^{e}\pm0.46$
5SF	$11.07^{b}\pm1.76$	$10.18^{b}\pm1.39$	$11.25^{b}\pm1.53$	$9.71^{b}\pm1.30$	$10.28^{c}\pm1.63$	$10.45^{b}\pm1.80$
10SF	$12.58^{a}\pm1.35$	$12.44^{a}\pm1.35$	$12.70^{a}\pm1.45$	$12.56^{a}\pm1.16$	$11.88^{b}\pm1.27$	$12.35^{a}\pm1.63$
15SF	$13.65^{a}\pm1.35$	$13.35^{a}\pm1.66$	$13.40^{a}\pm1.43$	$12.47^{a}\pm1.55$	$13.41^{a}\pm1.34$	$13.20^{a}\pm1.62$
5OK	$4.34^{c}\pm1.40$	$3.88^{d}\pm1.29$	$3.85^{d}\pm1.08$	$4.59^{d}\pm1.37$	$4.38^{e}\pm0.90$	$4.20^{d}\pm1.04$
10OK	$7.77^{c}\pm1.64$	$8.51^{c}\pm1.72$	$8.95^{c}\pm1.67$	$7.65^{c}\pm1.51$	$7.87^{d}\pm1.28$	$7.75^{c}\pm1.03$
15OK	$10.88^{b}\pm1.17$	$10.74^{b}\pm1.68$	$11.05^{b}\pm1.29$	$11.59^{a}\pm1.31$	$10.98^{bc}\pm1.66$	$11.25^{b}\pm1.80$

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Table 4.6 Pore homogeneity, as evaluated using DA, of wheat, soy flour-substituted and okara-substituted breads during 5-day storage

samples	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Control	$13.24^{a}\pm0.91$	$13.19^{a}\pm1.25$	$13.65^{a}\pm1.66$	$14.24^{a}\pm0.91$	$13.55^{a}\pm1.94$	$13.78^{a}\pm1.28$
5SF	$10.51^{b}\pm1.47$	$9.85^{c}\pm1.58$	$10.15^{c}\pm1.33$	$9.88^{c}\pm0.90$	$10.39^{c}\pm1.41$	$10.90^{b}\pm1.27$
10SF	$4.11^{d}\pm0.84$	$4.24^{d}\pm1.14$	$4.75^{e}\pm1.02$	$5.63^{d}\pm0.94$	$4.87^{de}\pm1.68$	$5.75^{c}\pm1.88$
15SF	$5.66^{c}\pm1.55$	$4.34^{d}\pm1.13$	$4.75^{e}\pm1.78$	$6.19^{d}\pm1.59$	$5.65^{d}\pm1.52$	$4.60^{d}\pm1.21$
5OK	$10.45^{b}\pm1.22$	$10.82^{b}\pm1.96$	$11.50^{b}\pm1.43$	$11.48^{b}\pm1.43$	$11.95^{b}\pm1.47$	$11.35^{b}\pm1.40$
10OK	$3.33^{d}\pm0.99$	$3.64^{d}\pm1.53$	$3.10^{f}\pm0.91$	$3.74^{e}\pm1.32$	$4.34^{e}\pm1.53$	$4.20^{d}\pm0.87$
15OK	$1.60^{e}\pm0.70$	$1.48^{e}\pm0.68$	$1.30^{g}\pm1.23$	$1.18^{f}\pm0.55$	$1.69^{f}\pm0.54$	$1.48^{f}\pm1.03$

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Hardness, cohesiveness and springiness, as evaluated organoleptically (Table 4.8-4.10), were found to be of similar trend as those determined using an Instron universal materials testing machine (Section 4.3.7). At low level of substitution (5%), hardness of the soy-substituted samples (5SF and 5OK) was not significantly different from that of the control (p>0.05) (Table 4.8). Hardness of the bread increased with increasing level of soy substitution, with OK samples being higher in hardness than the SF sample of the same substitution level. Hardness of all samples was found to increase with increasing storage time, with the control showing a greater degree of hardening. At Day 5, the control exhibited a hardness score of 10.60, a 103% increase from that of Day 0, while the 5-day old 15SF and 15OK demonstrated an increase in hardness score of 12 and 28%, respectively.

Table 4.7 Pore size, as evaluated using DA, of wheat, soy flour-substituted and okara-substituted breads during 5-day storage

samples	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Control	12.08^{a}±1.10	13.44^{a}±1.40	13.56^{a}±1.35	12.84^{ab}±1.83	11.23^{b}±1.52	13.85^{a}±1.46
5SF	12.11^{ab}±1.57	11.84^{b}±1.34	12.45^{b}±1.54	12.66^{ab}±1.20	11.95^{ab}±1.50	12.00^{b}±1.20
10SF	12.54^{ab}±0.76	13.36^{a}±1.70	12.60^{ab}±1.13	13.40^{a}±1.11	12.84^{a}±1.36	12.55^{b}±1.70
15SF	11.82^{ab}±1.29	12.53^{ab}±0.96	10.85^{c}±1.58	12.13^{b}±1.63	11.57^{b}±1.55	12.33^{b}±1.30
5OK	11.45^{b}±1.65	11.60^{b}±1.79	11.65^{bc}±1.33	12.44^{ab}±1.08	11.31^{b}±1.38	10.85^{c}±1.41
10OK	9.38^{c}±1.31	9.23^{c}±1.21	9.65^{d}±1.14	9.54^{c}±1.04	10.07^{c}±1.28	10.15^{c}±1.53
15OK	7.73^{d}±0.23	7.64^{d}±0.14	7.76^{e}±0.26	6.88^{d}±0.46	6.64^{d}±0.32	7.75^{d}±0.41

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Table 4.8 Hardness, as evaluated using DA, of wheat, soy flour-substituted and okara-substituted breads during 5-day storage

samples	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Control	5.22^{c}±1.46	7.35^{d}±1.92	8.00^{c}±1.70	9.64^{c}±1.74	10.57^{b}±1.94	10.60^{b}±1.57
5SF	5.23^{c}±1.15	5.48^{e}±1.16	5.70^{d}±1.48	6.66^{d}±0.91	7.33^{de}±1.42	7.55^{d}±1.54
10SF	4.96^{c}±1.04	5.63^{e}±0.84	6.15^{d}±0.75	7.43^{d}±0.87	7.83^{d}±0.91	8.05^{d}±1.68
15SF	8.30^{b}±1.82	8.74^{c}±1.34	8.88^{c}±1.61	9.13^{c}±1.78	9.44^{c}±1.48	9.30^{c}±1.41
5OK	5.54^{c}±1.43	5.76^{e}±1.19	6.28^{d}±1.23	6.75^{d}±1.72	6.53^{e}±1.11	8.25^{cd}±1.81
10OK	8.67^{b}±1.57	9.84^{b}±1.65	10.75^{b}±1.58	10.81^{b}±1.45	11.20^{b}±1.49	11.45^{b}±1.54
15OK	10.30^{a}±1.47	11.63^{a}±1.08	12.92^{a}±1.26	13.68^{a}±1.56	13.30^{a}±1.56	13.20^{a}±1.21

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Table 4.9 Cohesiveness, as evaluated using DA, of wheat, soy flour-substituted and okara-substituted breads during 5-day storage

samples	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Control	13.11^{a}±1.51	13.82^{a}±1.04	12.04^{a}±1.51	11.57^{a}±1.36	11.61^{a}±1.20	11.50^{a}±1.18
5SF	10.88^{ab}±1.85	10.69^{b}±1.60	10.66^{b}±1.73	10.25^{bc}±1.47	9.33^{b}±1.26	10.23^{b}±1.50
10SF	10.54^{ab}±1.72	10.35^{bc}±1.81	10.31^{bc}±1.86	10.45^{ab}±1.31	10.32^{b}±1.52	10.50^{ab}±1.39
15SF	9.87^{abc}±1.60	9.51^{cd}±1.48	9.45^{cd}±1.59	9.19^{cd}±1.39	9.68^{b}±1.83	9.50^{bc}±1.66
5OK	9.55^{bc}±1.37	9.24^{d}±1.11	8.50^{de}±1.45	8.66^{de}±1.51	9.38^{b}±1.30	8.52^{c}±1.59
10OK	8.76^{c}±1.56	7.34^{e}±1.57	8.25^{e}±1.49	7.81^{e}±1.57	7.67^{c}±1.51	8.74^{c}±1.32
15OK	8.59^{c}±1.40	6.65^{e}±1.28	8.12^{e}±1.04	7.88^{e}±1.55	6.72^{c}±1.24	8.65^{c}±1.40

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

For cohesiveness (Table 4.9), there was no significant difference among the SF samples and the control (p>0.05). With increasing okara substitution, cohesiveness was found to decrease. This was also due to the gluten dilution effect. Cohesiveness of all samples was perceived to stay relatively constant over a period of 5 days.

Table 4.10 Springiness, as evaluated using DA, of wheat, soy flour-substituted and okara-substituted breads during 5-day storage

samples	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Control	12.67^{a}±1.83	8.54^{b}±1.30	8.88^{a}±1.03	7.31^{a}±1.61	5.22^{ab}±1.47	4.36^{b}±1.39
5SF	10.49^{b}±1.01	6.30^{cd}±1.04	5.55^{c}±1.55	5.85^{b}±1.49	4.74^{abc}±1.24	4.33^{b}±1.69
10SF	10.36^{b}±1.62	9.76^{a}±1.71	7.49^{b}±1.45	6.11^{b}±1.40	5.01^{abc}±1.20	4.15^{b}±1.54
15SF	9.86^{bc}±0.94	5.34^{d}±1.55	4.10^{d}±0.98	4.51^{c}±1.33	4.06^{cd}±0.83	3.70^{b}±1.74
5OK	9.55^{bc}±1.82	8.47^{b}±1.79	7.70^{b}±1.70	6.44^{ab}±1.56	5.62^{a}±1.50	4.43^{b}±1.30
10OK	8.59^{c}±1.73	6.78^{c}±1.65	5.05^{c}±1.54	4.18^{c}±0.94	3.66^{d}±1.48	3.65^{a}±1.26
15OK	6.32^{d}±0.76	5.20^{d}±1.61	5.20^{c}±1.32	4.86^{c}±1.64	4.53^{bcd}±1.44	3.50^{b}±1.70

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at *p*=0.05.

Table 4.11 Beany flavor, as evaluated using DA, of wheat, soy flour-substituted and okara-substituted breads during 5-day storage

samples	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Control	$0.40^{e}\pm0.17$	$0.40^{e}\pm0.14$	$0.30^{f}\pm0.13$	$0.50^{d}\pm0.14$	$0.40^{g}\pm0.12$	$0.50^{e}\pm0.11$
5SF	$3.46^{c}\pm1.28$	$3.15^{d}\pm0.82$	$3.60^{d}\pm1.73$	$3.28^{c}\pm1.18$	$4.19^{e}\pm1.37$	$3.05^{d}\pm1.17$
10SF	$12.54^{a}\pm1.55$	$11.42^{b}\pm1.26$	$11.75^{b}\pm1.26$	$12.18^{a}\pm1.82$	$11.66^{c}\pm1.43$	$11.95^{b}\pm1.63$
15SF	$12.94^{a}\pm1.68$	$11.65^{b}\pm1.67$	$13.40^{a}\pm1.51$	$12.43^{a}\pm1.56$	$13.59^{a}\pm0.87$	$12.50^{ab}\pm1.11$
5OK	$1.72^{d}\pm0.41$	$2.49^{d}\pm0.67$	$2.50^{e}\pm0.83$	$1.11^{d}\pm0.62$	$2.33^{f}\pm1.13$	$3.55^{d}\pm1.27$
10OK	$7.18^{b}\pm1.25$	$6.77^{c}\pm1.46$	$6.50^{c}\pm1.03$	$8.01^{b}\pm1.59$	$7.48^{d}\pm1.00$	$8.10^{c}\pm1.44$
15OK	$12.65^{a}\pm1.42$	$12.71^{a}\pm1.92$	$12.41^{b}\pm1.18$	$11.69^{a}\pm1.50$	$12.71^{b}\pm1.56$	$12.85^{a}\pm1.29$

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at *p*=0.05.

The panel indicated decreased springiness upon substitution of soy ingredients (Table 4.10), with OK samples showing a greater decrease in springiness. Springiness of all samples decreased with increasing storage time, with the control showing a greatest reduction in springiness (66%).

Beany flavor was noticed upon substitution of soy ingredients (Table 4.11). In general, the panel indicated that beany flavor was more intense in the SF samples than the OK sample of the same substitution level. The lower beany flavor of OK samples could be due to the fact that the okara was prepared using a hot-water blanching method which could inactivate lipoxygenase, an enzyme responsible for beany flavor in processed soy products (Wilkens *et al.*, 1967). Beany flavor of all samples remained unchanged during 5-day storage.

4.3.10.2 Acceptance test

The freshly baked bread samples were evaluated using a 9-point hedonic scale, ranging from “dislike extremely (1)”, to “neither like nor dislike (5)”, to “like extremely

(9)." A sample received a score of 5 or higher was therefore considered acceptable. The bread samples were evaluated for the following attributes: crust color, crumb color, texture, flavor, taste, and overall acceptance (Appendix B3). The results are shown in Table 4.12.

Table 4.12 Hedonic scores* of freshly baked wheat, soy flour-substituted and okara-substituted breads

Sample	Crust color	Crumb color	Texture	Flavor	Taste ^{ns}	Overall acceptance
Control	6.74^{a}±1.87	7.80^{a}±0.70	6.82^{a}±1.19	6.12^{a}±1.24	6.28±1.09	6.92^{a}±1.05
5SF	6.10^{bc}±1.80	6.76^{c}±1.04	6.44^{ab}±1.15	6.04^{a}±1.55	5.82±1.67	5.98^{b}±1.61
10SF	5.06^{d}±1.65	5.44^{e}±1.31	6.00^{bc}±1.01	5.38^{b}±1.61	5.50±1.47	5.48^{c}±1.23
15SF	5.34^{e}±1.29	4.90^{f}±1.37	5.62^{c}±1.37	5.42^{b}±1.51	5.43±1.32	5.20^{c}±1.37
5OK	6.54^{ab}±1.47	7.26^{b}±0.92	6.22^{b}±1.53	6.16^{a}±1.06	5.84±1.13	6.36^{b}±1.17
10OK	6.48^{ab}±1.23	5.98^{d}±1.50	4.30^{d}±1.18	4.62^{c}±1.51	5.64±1.48	4.68^{d}±1.42
15OK	5.82^{c}±1.79	4.90^{f}±1.83	3.10^{e}±1.36	3.18^{d}±1.29	5.36±1.55	3.12^{e}±1.38

* A 9-point scale, with 1 being "dislike extremely" and 9 being "like extremely"

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

The score for crust color was significantly different among the samples ($p \leq 0.05$), with that of the control being the highest (6.74). However the scores remained in relatively narrow range (5.06-6.74). In contrast, the score for crumb color decreased sharply with increasing level of soy substitution. This implied that the panel preferred a lighter crumb color.

Upon substitution of okara, the score for texture decreased significantly. The 15OK sample received the lowest score of 3.10. The score for the SF samples also decreased with increasing level of substitution, but to a lesser extent than that of the OK breads.

At low level of substitution (5%), the flavor score for both soy flour- and okara-substituted breads was similar to that of the control ($p>0.05$). With increasing level of substitution, the score became decreasing, indicating that the panel preferred bread with less beany flavor.

Taste was the only attribute that was not significantly different among the samples (*p*>0.05). This could be due to the fact that both soy flour and okara used for substitution were rather bland in taste.

For the overall acceptance, the control received the highest score (6.92) while the 15OK received the lowest score (3.12). The overall acceptance score tended to decrease with increasing level of soy substitution, with the OK samples exhibiting a greater decrease in overall acceptance than the SF sample of the same substitution level. However, all samples, except the 10OK and 15OK, received a score of higher than 5 and were therefore considered acceptable.

Several researchers reported that soy substitution posed a negative effect on the organoleptic quality of bread. However, all of those researchers suggested that low level of soy ingredient can be substituted without resulting in an unacceptable final product. Dhingra and Jood (2001, 2004) reported that up to 10% of soy flour can be added and still yielded a bread with high overall acceptablility score (approximately 7.5 as compared to 7.7 for the control). The 10% soy flour-substituted bread received a hedonic score not significantly different from the control in crust color, appearance, flavor, crust texture and taste. Olaoye *et al.* (2006) reported that up to 5% of full-fat soy flour could be added to bread and still received a score similar to the control in crumb texture and general acceptability. Meanwhile, the authors also reported that 15% soy flour-containing sample received a score similar to the control in aroma and taste. Waliszewski *et al.* (2002) reported that up to 10% fortification of okara did not significantly affect sensory attributes of corn tortilla, including aroma, flavor, after taste, appearance, manual texture and oral texture.

4.4 Consumer acceptance and staling of soy-substituted breads

In the U. S. A., bread sold in a supermarket is usually removed from the shelf after about 2-3 days (Mikkelson, 2007) (Data for bread sold in Thailand could not be obtained.). From the current study, it was found that a 2-day old control exhibited a

47.8% increase in hardness as compared to the freshly baked bread (Figure 4.19). Therefore, if one could assume that an increase in hardness of over 50% compared to the freshly baked control would result in an "unsalable" bread, the 5SF, 10SF, 15SF and 5OK could be displayed on the shelf up to approximately 5 days without unacceptable changes in hardness. At higher substitution levels of okara, the breads were quite hard even at Day 0. Hardness of the 1-day old 10OK was 55.5% higher than that of the freshly baked control while that of the freshly baked 15OK was already 104.2% higher than that of the freshly baked control.

Consumer acceptance of the product must also be taken into consideration. From this study, it was found that even though the 5SF, 10SF, 15SF and 5OK received a significantly lower overall acceptance score ($p \leq 0.05$) than the control, those samples still received a score of 5 or higher, which was considered acceptable. Therefore, it could be concluded that substitution of up to 15% of soy flour and 5% of okara would result in organoleptically acceptable bread which stayed fresh for a longer time.

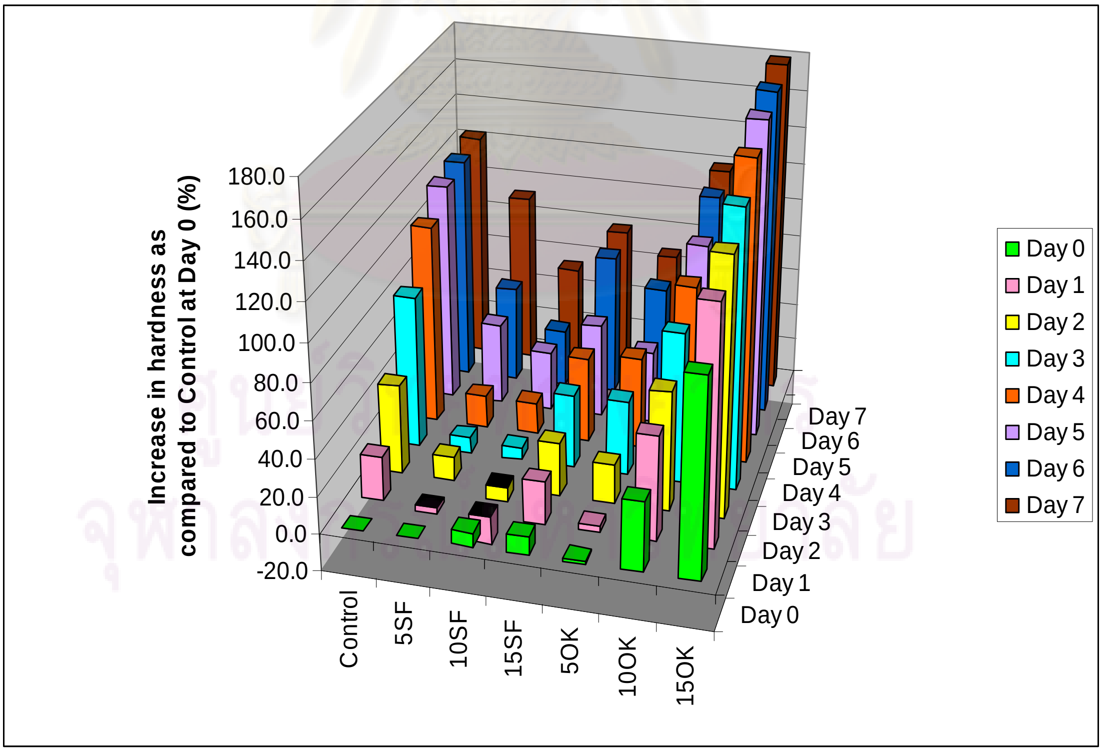


Figure 4.19 Increase in hardness of bread samples as compared to that of the freshly baked control

CHAPTER 5

CONCLUSION AND SUGGESTION FOR FURTHER STUDY

Conclusion

The substitution of soy flour and okara appeared to have mixed impact on the quality of freshly baked and stored white bread. A negative effect was observed on the quality of freshly baked bread which was especially pronounced in the okara-substituted bread. Freshly baked soy-substituted bread generally exhibited a decrease in loaf volume, specific loaf volume and loaf height, with an increase in loaf weight. Okara-containing samples exhibited an increase in hardness and a decrease in cohesiveness and springiness as compared to the control. Crumb alveoli of soy-containing crumb were smaller and varying in shape and size. This negative effect was mainly due to the dilution of gluten upon soy ingredient substitution. Crumb color of the soy-containing bread became more yellow, especially for the soy flour-containing bread, and the soy breads also possessed a beany flavor.

In contrast, soy substitution was proved to have a positive effect on storage quality of bread. In this study, it was demonstrated that addition of soy ingredients helped retard bread staling, as shown through the decreases in moisture loss, freezable water reduction and amylopectin recrystallization. The role in controlling moisture was mainly attributable to the high fiber content of soy ingredients, especially okara. With its high water-holding capacity, fiber helped promote homogeneus moisture distribution throughout the loaf and thus minimized moisture migration in bread during storage. Apart from the excellent water-holding capacity, soy ingredients (i. e., soy fiber and soy protein) were also reported to interact strongly with starch, hence interfering starch-starch interaction and reducing amylopectin recrystallization.

Substitution of up to 15% of soy flour and 5% of okara resulted in organoleptically acceptable bread which could be kept for 5 days without unacceptable changes in hardness.

Suggestion for further study

Different soy compositions (i. e., fiber, protein, fat) appeared to have a role in determining the quality of freshly baked bread and the staling of stored bread. It is therefore interesting to investigate the role of each composition on such quality of bread.

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APPENDICES

APPENDIX A

ANALYTICAL PROCEDURES

A.1 Moisture content (AOAC, 2000)

<u>Instrument</u>

1. Hot air oven, Model 600 (Memmert, Schwabach, Germany)

<u>Method</u>

1. Weigh an aluminum pan which has previously been dried and cooled to room temperature.

2. Accurately weigh approximately 3 g of sample into the pan.

3. Dry the sample pan in a hot air oven at 105 °C to a constant weight.

4. Remove the sample pan from the oven. Place the pan in a desiccator and cool to room temperature. Weigh the sample pan.

5. Calculate moisture content using Equation A.1:

$$\%\text{Moisture (wb)} = \frac{W_1 - W_2}{W_1} \times 100 \qquad (A.1)$$

Where W_1 is g of sample before drying.

W_2 is g of sample after drying.

A.2 Crude protein content (AOAC, 2000)

<u>Instruments</u>

1. Kjeldahl digestion unit, Model K-424 (Buchi, Flawil, Switzerland)
2. Kjeldahl distillation unit, Model B-324 (Buchi, Flawil, Switzerland)

<u>Chemical reagents</u>

1. Boric acid, 4% (w/w)
2. Hydrochloric acid, 0.1 N
3. Indicator (0.1% methylene blue + 0.2% methyl red)

4. Selenium reagent mixture
5. Sulfuric acid, concentrated (96.5%, w/w)
6. Sulfuric acid, 0.1 N
7. Sodium hydroxide, 50% (w/w)

Method

1. Accurately weigh 0.7-2.2 g of sample onto a Whatman No. 54 filter paper and place in a sample tube.

2. Add 5 g of selenium reagent mixture.

3. Add 30 ml of concentrated H_2SO_4.

4. Place the rack holding sample tubes in the Kjeldahl digestion unit. Connect fume exhaust unit to the tubes and turn on water pump. Set the thermostat to 400 °C and digest the sample for 45 minutes until the solution turns clear.

5. Remove the sample tubes from the digestion unit. Leave the water pump on and the fume exhaust unit connected. Allow the sample tube to cool.

6. Remove the exhaust unit from the sample tube and place the tube in the Kjeldahl distillation unit. Add 80 ml of distilled water and 120 ml of 50% sodium hydroxide solution.

7. Place a conical flask containing 50 ml of 4% boric acid and 4 drops of indicator.

8. Run the distillation unit according to the instrument manual.

9. Remove the flask from the distillation unit. Titrate ammonia in the solution with 0.1 N HCl until purplish color is obtained.

10. Calculate crude protein content using Equation A.2:

$$\%\text{Crude protein (wb)} = \frac{\text{ml of titer} \times \text{N of HCl} \times 14 \times \text{conversion factor}}{\text{g of sample} \times 10} \qquad \text{(A.2)}$$

Note: The following conversion factors were used: 5.70 for wheat flour, 5.71 for soy flour and okara, and 6.25 for bread.

A.3 Crude fat content (AOAC, 2000)

Instruments and apparatus

1. Hot air oven, Model 600 (Memmert, Schwabach, Germany)
2. Rotary evaporator, Eyela® Model N-N (Tokyo Rikakikai, Tokyo, Japan)
3. Soxhlet extraction apparatus (Gerhardt, Bonn, Germany)

Chemical reagent

1. Petroleum ether

Method

1. Weigh a round bottom flask which has previously been dried and cooled to room temperature.

2. Accurately weigh 2-3 g of dried sample onto Whatman No. 1 filter paper and place into a thimble. Place the thimble into the extraction chamber of the Soxhlet apparatus.

3. Add 250 ml of petroleum ether into the round bottom flask.

4. Heat the solvent and allow the extraction process to proceed for 4 hours.

5. Evaporate the solvent from the solvent-fat mixture using a rotary evaporator.

6. Dry the remaining liquid in a hot air oven at 60 °C for 30 minutes and then cool to room temperature in a desiccator. Weigh the crude fat obtained.

7. Calculate crude fat content using Equation A.3:

$$\%\text{Crude fat (db)} = \frac{\text{g of crude fat}}{\text{g of dried sample}} \times 100 \qquad \text{(A.3)}$$

A.4 Crude fiber content (AOAC, 2000)

Instruments

1. Hot air oven, Model 600 (Memmert, Schwabach, Germany)
2. Muffle furnace, Model CWF1200 (Carbolite, Hope Valley, U. K.)

Chemical reagents

1. Ethyl alcohol, 95% (w/w)
2. Sodium hydroxide, 1.25% (w/w)

3. Sulfuric acid, 1.25% (w/w)

Method

1. Accurately weigh approximately 5 g of dried defatted sample into a beaker.
2. Add 200 ml of 1.25% sulfuric acid and boil for 30 minutes.
3. Filter through Whatman No. 1 filter paper. Rinse with copious amount of hot water to neutralize the residue.
4. Transfer the residue to a beaker. Add 200 ml of 1.25% sodium hydroxide and boil for 30 minutes.
5. Filter through Whatman No. 1 filter paper. Rinse with copious amount of hot water to neutralize the residue.
6. Rinse the residue with 95% ethyl alcohol.
7. Dry the residue in a hot air oven at 105 °C to a constant weight.
8. Cool to room temperature in a desiccator and weigh.
9. Weigh a crucible which has previously been ignited at 550 °C and cooled to room temperature.
10. Transfer the dried residue into the crucible. Heat the residue using a burner until it is charred. Incinerate the sample in a muffle furnace at 550 °C for 4 hours or until grayish-white ash is obtained.
11. Cool to room temperature in a desiccator and weigh.
12. Obtain the amount of crude fiber by subtracting the weight after incineration from the weight before incineration.
13. Calculate crude fiber content using Equation A.4:

$$\%\text{Crude fiber (wb)} = \frac{\text{g of crude fiber}(100 - \text{M} - \text{L})}{\text{g of dried defatted sample}} \tag{A.4}$$

Where M is moisture content of the sample (% wb).

L is crude fat content of the sample (% wb).

A.5 Ash content (AOAC, 2000)

Instrument

1. Muffle furnace, Model CWF1200 (Carbolite, Hope Valley, U. K.)

Method

1. Weigh a crucible which has previously been ignited at 550 °C and cooled to room temperature.

2. Accurately weigh 3-5 g of dried sample into the crucible.

3. Heat the sample using a burner until it is charred.

4. Incinerate the sample in a muffle furnace at 550 °C for 4 hours or until grayish-white ash is obtained.

5. Cool to room temperature in a desiccator and weigh.

6. Calculate ash content using Equation A.5:

$$\%\text{Ash (db)} = \frac{\text{g of ash}}{\text{g of dried sample}} \times 100 \qquad (A.5)$$

APPENDIX B

STANDARD CURVE AND BALLOTS

B.1 Water soluble starch content: standard curve

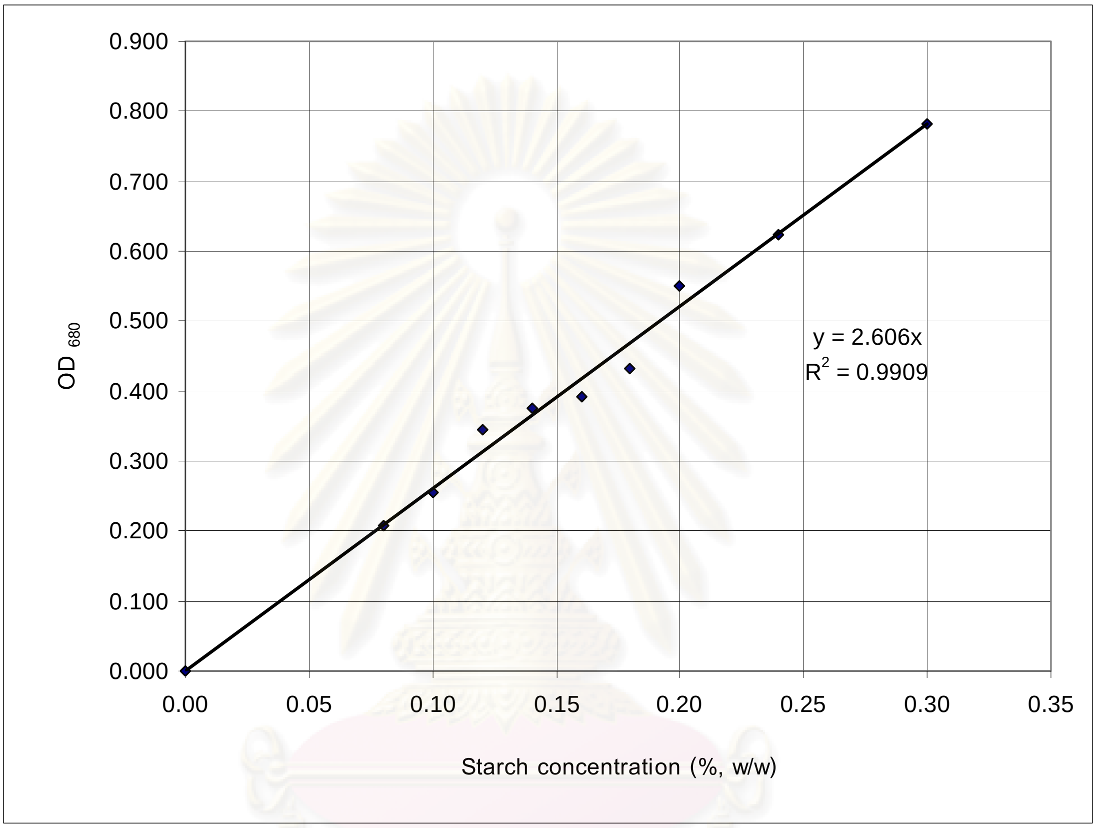

Figure B.1 Standard curve for water soluble starch determination

B.2 Descriptive analysis: ballot

QUESTIONNAIRE FOR BREAD

Sample ….…….   Name ……………………………Date ………………

Please rinse your mouth with rinsing water before testing a sample.

Please indicate the intensity of each attribute by putting a mark on the line scale at the point best represented the intensity you have perceived.

1. Crust color (สีเปลือกนอก)

Orange-brown (สีน้ำตาลส้ม)

slight   intense

2. Crumb color (สีเนื้อใน)

Yellow (สีเหลือง)

slight   intense

3. Porosity (ความเป็นรูพรุน)

Pore homogeneity (ความสม่ำเสมอของรูพรุน)

slight   very

Pore size (ขนาดของรูพรุน)

small   large

4. Texture (เนื้อสัมผัส)

Hardness (ความแข็ง)

slight   very

Cohesiveness (การเกาะตัวกันของเนื้อขนมปัง)

slight   very

Springiness (ความยืดหยุ่น)

slight   very

5. Flavor (กลิ่นรส)

Beany flavor (กลิ่นรสถั่ว)

slight   intense

B.3 Hedonic test: ballot

QUESTIONNAIRE FOR BREAD

Sample…………………..........   Name ……………………………Date ………………

Please evaluate the bread samples from left t right by indicate how much you like or dislike each sample by checking the appropriate phrase.

โปรดทดสอบตัวอย่างขนมปังจากซ้ายไปขวา ระบุระดับความชอบหรือไม่ชอบโดยใส่เครื่องหมาย √ หน้าข้อความที่ตรงกับระดับความชอบหรือไม่ชอบของท่าน

Crust color
(สีเปลือกนอก)
xxx
_like extremely
_like very much
_like moderately
_like slightly
_neither like
nor dislike
_dislike slightly
_dislike moderately
_dislike very much
_dislike extremely

Crumb color
(สีเนื้อใน)
xxx
_like extremely
_like very much
_like moderately
_like slightly
_neither like
nor dislike
_dislike slightly
_dislike moderately
_dislike very much
_dislike extremely

Texture
(เนื้อสัมผัส)
xxx
_like extremely
_like very much
_like moderately
_like slightly
_neither like
nor dislike
_dislike slightly
_dislike moderately
_dislike very much
_dislike extremely

Flavor
(กลิ่นรส)
xxx
_like extremely
_like very much
_like moderately
_like slightly
_neither like
nor dislike
_dislike slightly
_dislike moderately
_dislike very much
_dislike extremely

Taste
(รสชาติ)
xxx
_like extremely
_like very much
_like moderately
_like slightly
_neither like
nor dislike
_dislike slightly
_dislike moderately
_dislike very much
_dislike extremely

Overall acceptance
(การยอมรับโดยรวม)
xxx
_like extremely
_like very much
_like moderately
_like slightly
_neither like
nor dislike
_dislike slightly
_dislike moderately
_dislike very much
_dislike extremely

B.4 Texture Profile Analysis (TPA) curve

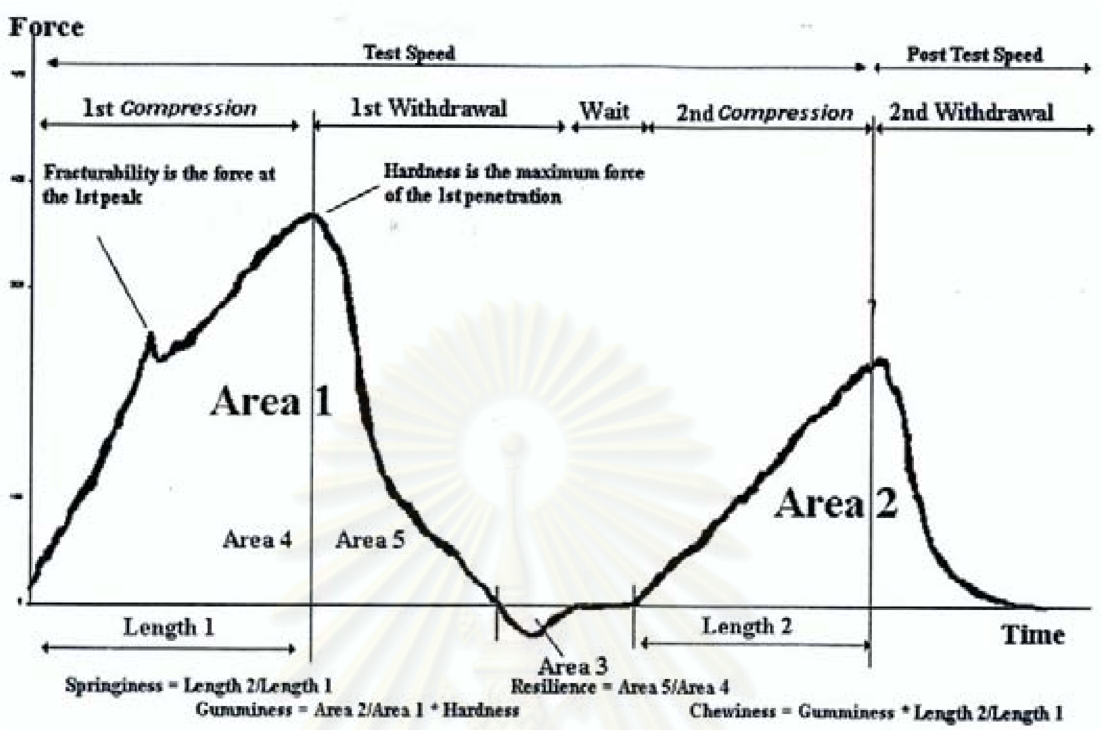

Figure B.2 Typical TPA curve and textural parameters that could be obtained from the curve (Source: Texture Technologies, 2009)

APPENDIX C

DATA TABLES

Table C.1 Proximate compositions (%wb) of wheat, soy flour-substituted and okara-substituted breads

Samples	Moisture	Protein	Fat	Ash	Crude fiber	Available Carbohydrates
Control	39.08^{c}±0.53	9.63^{b}±0.80	4.73^{e}±0.13	1.40^{b}±0.15	0.67^{c}±0.02	48.56^{a}±1.36
5SF	39.14^{c}±1.04	10.00^{b}±0.73	11.76^{c}±0.66	1.57^{ab}±0.13	0.79^{c}±0.08	40.95^{b}±0.29
10SF	39.83^{ab}±0.54	11.28^{ab}±1.01	13.10^{b}±0.49	1.78^{ab}±0.19	0.85^{c}±0.10	37.87^{b}±1.74
15SF	39.63^{bc}±0.23	12.04^{a}±0.76	15.76^{a}±0.91	1.91^{a}±1.91	1.03^{bc}±1.03	34.01^{c}±2.18
5OK	40.15^{ab}±0.18	9.94^{b}±0.61	4.93^{e}±0.33	1.47^{ab}±0.13	1.55^{ab}±0.16	46.74^{a}±1.83
10OK	40.34^{a}±0.34	10.53^{ab}±1.00	9.37^{d}±0.11	1.58^{ab}±0.16	1.64^{a}±0.10	41.22^{b}±1.72
15OK	40.38^{a}±0.39	11.00^{ab}±1.00	9.91^{d}±0.44	1.63^{ab}±0.09	1.82^{a}±0.16	39.47^{b}±2.77

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Table C.2 Moisture content (%db) of wheat, soy flour-substituted and okara-substituted breads during 7-day storage

Day	Control	5SF	10SF	15SF	5OK	10OK	15OK
0	64.15^{a}±1.41	64.25^{a}±2.95	65.37^{a}±1.67	65.05^{a}±1.89	65.91^{a}±2.83	66.21^{a}±3.05	67.73^{a}±1.09
1	60.21^{b}±1.01	61.02^{ab}±4.46	62.47^{ab}±4.87	61.70^{b}±3.57	62.20^{ab}±4.34	63.14^{ab}±3.34	63.48^{b}±2.70
2	59.01^{bc}±0.78	59.99^{bc}±3.66	60.93^{bc}±3.09	60.13^{bc}±1.76	61.40^{bc}±3.92	62.45^{ab}±3.82	62.55^{bc}±3.08
3	58.38^{bcd}±1.85	60.00^{bc}±2.93	58.80^{cd}±2.48	60.20^{bc}±3.64	60.89^{bc}±4.12	61.26^{b}±4.35	61.42^{bcd}±2.83
4	56.96^{cd}±4.73	58.40^{bcd}±2.50	58.07^{cd}±2.40	57.94^{cd}±1.55	58.49^{bc}±2.80	60.35^{b}±3.24	60.07^{bcd}±2.93
5	56.49^{cd}±3.80	57.18^{cd}±1.11	58.06^{cd}±2.56	57.43^{cd}±1.43	57.60^{bc}±1.99	60.03^{b}±4.76	60.22^{bcd}±3.71
6	56.45^{cd}±1.66	56.80^{cd}±1.37	57.42^{cd}±1.86	56.84^{d}±1.38	58.02^{c}±3.22	59.38^{b}±3.37	59.13^{cd}±3.38
7	55.13^{d}±2.32	56.08^{d}±1.68	56.89^{d}±1.97	56.15^{d}±2.11	57.38^{c}±2.15	58.53^{b}±2.26	58.21^{d}±2.86

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Table C.3 Water activity of wheat, soy flour-substituted and okara-substituted breads during 7-day storage

Day	Control^{ns}	5SF^{ns}	10SF^{ns}	15SF^{ns}	5OK^{ns}	10OK^{ns}	15OK^{ns}
0	0.92±0.00	0.92±0.03	0.93±0.01	0.93±0.01	0.93±0.00	0.94±0.01	0.94±0.01
1	0.91±0.01	0.92±0.00	0.92±0.00	0.92±0.01	0.93±0.01	0.93±0.01	0.94±0.01
2	0.91±0.01	0.92±0.00	0.92±0.00	0.92±0.00	0.93±0.00	0.93±0.00	0.94±0.01
3	0.91±0.00	0.92±0.01	0.92±0.00	0.92±0.00	0.93±0.00	0.93±0.00	0.94±0.00
4	0.91±0.00	0.92±0.00	0.92±0.00	0.92±0.01	0.93±0.01	0.93±0.00	0.93±0.01
5	0.91±0.01	0.92±0.00	0.92±0.00	0.92±0.00	0.92±0.00	0.93±0.01	0.93±0.01
6	0.91±0.00	0.91±0.00	0.92±0.01	0.92±0.00	0.92±0.00	0.93±0.01	0.93±0.01
7	0.90±0.00	0.91±0.00	0.92±0.01	0.92±0.01	0.92±0.01	0.93±0.01	0.93±0.00

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at *p*=0.05.

"ns" indicates no significant difference among sample means at *p*=0.05.

Table C.4 Freezable water content (%db) of wheat, soy flour-substituted and okara-substituted breads during 7-day storage

Day	Control	5SF	10SF	15SF^{ns}	5OK	10OK^{ns}	15OK^{ns}
0	28.86^{a}±1.30	29.92^{a}±1.84	31.28^{a}±2.31	30.86±1.81	34.44^{a}±0.71	35.15±0.51	36.43±0.33
1	25.59^{ab}±1.75	27.98^{ab}±2.52	30.40^{a}±2.56	30.08±3.34	33.10^{ab}±0.47	34.95±0.62	36.22±0.28
2	25.03^{ab}±4.83	26.66^{ab}±2.39	28.01^{ab}±3.30	29.01±3.36	33.22^{ab}±1.87	34.22±1.55	37.01±1.58
3	22.85^{bc}±1.91	25.79^{abc}±2.03	24.83^{b}±0.96	27.04±3.48	32.62^{abc}±0.49	33.66±0.89	34.27±0.99
4	19.91^{c}±2.57	24.59^{bc}±1.41	24.61^{b}±0.58	25.33±2.18	31.10^{abc}±1.38	32.36±1.20	34.33±0.02
5	19.71^{c}±2.28	23.60^{bc}±1.00	24.29^{b}±0.88	24.85±2.19	30.55^{abc}±2.09	32.43±1.49	33.42±0.99
6	18.89^{c}±3.59	23.36^{bc}±2.45	23.57^{b}±1.28	24.75±1.31	30.00^{bc}±2.80	31.75±0.01	32.59±1.06
7	13.72^{d}±1.68	21.06^{c}±2.81	22.49^{b}±0.67	25.07±0.60	29.09^{c}±0.51	30.52±0.62	32.05±0.48

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at *p*=0.05.

"ns" indicates no significant difference among sample means at *p*=0.05.

Table C.5 Unfreezable water content (%db) of wheat, soy flour-substituted and okara-substituted breads during 7-day storage

Day	Control^{ns}	5SF^{ns}	10SF^{ns}	15SF^{ns}	5OK^{ns}	10OK^{ns}	15OK^{ns}
0	26.77±2.26	24.81±1.12	27.69±1.74	27.09±1.46	27.65±0.26	25.49±0.45	27.97±0.88
1	27.91±0.79	26.35±2.16	26.97±1.37	26.68±1.03	27.59±0.03	28.78±3.27	27.06±0.50
2	28.14±1.82	26.57±3.95	27.61±2.01	28.44±2.56	28.59±0.01	27.45±0.81	26.44±0.46
3	26.75±2.45	28.76±2.36	26.24±0.11	26.15±1.05	26.85±1.15	27.66±0.06	25.95±1.17
4	26.50±1.17	29.37±1.36	27.16±0.60	27.25±1.25	27.96±1.46	28.18±0.62	26.43±0.98
5	27.54±2.63	30.07±3.05	28.14±0.15	26.51±1.86	28.12±0.97	28.51±1.13	26.09±0.76
6	26.05±3.87	28.47±0.76	29.18±1.95	27.17±0.49	28.27±0.49	28.34±0.31	27.01±0.55
7	27.49±1.94	27.86±1.34	28.03±1.05	28.54±0.49	30.68±1.12	29.06±0.29	29.37±2.49

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at *p*=0.05.

“ns” indicates no significant difference among sample means at *p*=0.05.

Table C.6 Melting enthalpy of recrystallized amylopectin (J/g) in wheat, soy flour-substituted and okara-substituted breads during 7-day storage

Day	Control	5SF	10SF	15SF	5OK	10OK	15OK
0	0.00^{e}±0.00	0.00^{d}±0.00	0.00^{e}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{d}±0.00	0.00^{e}±0.00
1	0.45^{d}±0.03	0.10^{d}±0.14	0.06^{e}±0.10	0.07^{d}±0.10	0.10^{d}±0.03	0.00^{d}±0.00	0.00^{e}±0.00
2	0.66^{c}±0.11	0.34^{c}±0.08	0.30^{d}±0.07	0.34^{c}±0.08	0.31^{c}±0.03	0.28^{c}±0.03	0.20^{d}±0.04
3	0.77^{bc}±0.12	0.47^{bc}±0.04	0.34^{cd}±0.16	0.35^{c}±0.07	0.43^{bc}±0.10	0.36^{bc}±0.06	0.23^{d}±0.10
4	0.81^{bc}±0.06	0.53^{ab}±0.06	0.44^{bcd}±0.12	0.38^{bc}±0.06	0.50^{b}±0.09	0.47^{b}±0.02	0.32^{cd}±0.02
5	0.88^{ab}±0.07	0.57^{ab}±0.04	0.49^{bc}±0.01	0.49^{ab}±0.09	0.51^{b}±0.01	0.48^{b}±0.10	0.40^{bc}±0.06
6	0.91^{ab}±0.09	0.59^{ab}±0.63	0.55^{ab}±0.05	0.53^{a}±0.03	0.51^{b}±0.11	0.48^{b}±0.06	0.43^{b}±0.06
7	0.99^{a}±0.14	0.66^{a}±0.17	0.67^{a}±0.00	0.58^{a}±0.03	0.69^{a}±0.06	0.62^{a}±0.04	0.64^{a}±0.02

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at *p*=0.05.

Table C.7 Water soluble starch content (%db) of wheat, soy flour-substituted and okara-substituted breads during 7-day storage

Day	Control	5SF	10SF	15SF	5OK	10OK	15OK
0	0.39^{a}±0.08	0.23^{a}±0.05	0.23^{a}±0.01	0.14^{a}±0.00	0.20^{a}±0.02	0.23^{a}±0.00	0.18^{a}±0.01
1	0.24^{b}±0.02	0.17^{b}±0.03	0.21^{b}±0.01	0.07^{b}±0.00	0.15^{b}±0.00	0.18^{b}±0.00	0.18^{a}±0.00
2	0.20^{c}±0.01	0.14^{c}±0.01	0.15^{c}±0.00	0.07^{b}±0.00	0.13^{c}±0.00	0.13^{c}±0.00	0.18^{a}±0.00
3	0.14^{d}±0.01	0.13^{cd}±0.00	0.08^{d}±0.01	0.06^{d}±0.00	0.10^{d}±0.01	0.12^{d}±0.00	0.13^{b}±0.01
4	0.08^{e}±0.01	0.12^{de}±0.00	0.11^{d}±0.01	0.06^{d}±0.00	0.10^{e}±0.00	0.11^{e}±0.00	0.11^{c}±0.01
5	0.09^{e}±0.01	0.12^{de}±0.00	0.10^{e}±0.00	0.06^{d}±0.00	0.09^{f}±0.00	0.10^{f}±0.00	0.11^{c}±0.01
6	0.09^{e}±0.01	0.11^{de}±0.00	0.10^{f}±0.00	0.05^{d}±0.00	0.08^{fg}±0.00	0.09^{g}±0.00	0.08^{d}±0.00
7	0.09^{e}±0.01	0.10^{e}±0.01	0.08^{f}±0.01	0.04^{e}±0.00	0.07^{g}±0.01	0.08^{h}±0.00	0.12^{e}±0.00

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Table C.8 Loaf volume (cm^{3}) of wheat, soy flour-substituted and okara-substituted breads during 7-day storage

Day	Control	5SF^{ns}	10SF	15SF^{ns}	5OK^{ns}	10OK	15OK^{ns}
0	239.11^{a}±1.26	228.53±4.99	226.44^{a}±6.45	220.57±9.29	217.08±2.72	168.33^{a}±9.43	148.54±5.01
1	237.52^{ab}±5.77	221.56±5.01	224.50^{a}±5.15	219.51±12.59	215.57±6.46	163.00^{ab}±11.38	142.53±2.36
2	236.48^{abc}±2.02	227.36±3.18	221.55^{ab}±0.96	220.08±6.58	209.60±4.65	163.00^{ab}±8.91	147.52±1.16
3	233.41^{abc}±3.15	224.32±4.62	221.83^{ab}±6.76	215.50±5.66	212.24±3.17	161.67^{ab}±6.60	149.66±0.69
4	230.34^{abc}±1.74	219.44±1.56	220.50^{ab}±3.69	213.00±5.59	215.41±2.25	157.65^{ab}±11.10	139.52±4.74
5	234.73^{bc}±2.42	223.58±11.43	218.36^{ab}±2.56	211.61±5.11	213.06±3.45	156.87^{ab}±2.22	140.35±7.50
6	231.29^{bc}±1.48	216.07±1.47	215.73^{ab}±0.49	215.00±6.32	209.50±2.33	147.15^{b}±6.43	141.83±12.39
7	230.68^{c}±0.45	216.50±4.85	213.00^{b}±4.27	212.36±7.18	207.93±2.93	146.71^{b}±2.98	134.50±4.95

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

"ns" indicates no significant difference among sample means at p=0.05.

Table C.9 Loaf weight (g) of wheat, soy flour-substituted and okara-substituted breads during 7-day storage

Day	Control^{ns}	5SF^{ns}	10SF^{ns}	15SF^{ns}	5OK^{ns}	10OK^{ns}	15OK^{ns}
0	131.20±0.28	133.50±3.80	134.95±0.35	136.40±0.85	135.35±1.20	136.30±0.42	136.95±0.49
1	131.05±0.35	133.28±5.32	134.91±3.56	136.10±0.57	134.85±0.78	135.18±6.58	135.71±2.76
2	122.81±4.65	130.07±2.39	135.82±4.82	136.24±3.86	133.62±2.14	136.44±3.68	135.66±3.78
3	127.66±0.35	132.93±3.17	134.60±13.65	135.93±4.95	131.74±3.39	135.87±5.83	134.83±5.53
4	130.62±0.95	131.99±3.05	133.64±1.74	133.41±6.80	134.35±3.34	136.13±4.40	135.52±2.56
5	128.67±6.07	129.74±6.26	134.86±1.34	135.76±1.77	133.15±5.26	134.76±2.29	134.41±4.34
6	130.50±0.42	132.81±4.81	134.15±2.81	135.57±10.62	133.97±3.80	135.54±7.64	135.48±4.51
7	130.24±7.50	132.77±3.56	134.33±4.60	135.42±4.09	133.86±5.13	135.73±7.42	135..32±7.30

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at *p*=0.05.

“ns” indicates no significant difference among sample means at *p*=0.05.

Table C.10 Specific loaf volume (cm^3/g) of wheat, soy flour-substituted and okara-substituted breads during 7-day storage

Day	Control	5SF^{ns}	10SF^{ns}	15SF^{ns}	5OK^{ns}	10OK^{ns}	15OK^{ns}
0	1.82^{a}±0.11	1.71±0.01	1.68±0.04	1.62±0.08	1.60±0.01	1.23±0.07	1.08±0.03
1	1.81^{ab}±0.05	1.66±0.03	1.67±0.08	1.61±0.10	1.60±0.06	1.21±0.14	1.05±0.00
2	1.93^{a}±0.09	1.75±0.01	1.63±0.07	1.62±0.00	1.57±0.06	1.20±0.10	1.09±0.04
3	1.83^{ab}±0.02	1.69±0.08	1.65±0.12	1.59±0.02	1.61±0.02	1.19±0.10	1.11±0.05
4	1.76^{b}±0.00	1.66±0.05	1.65±0.01	1.60±0.12	1.60±0.02	1.16±0.04	1.03±0.02
5	1.83^{ab}±0.10	1.73±0.17	1.62±0.04	1.56±0.06	1.60±0.09	1.16±0.04	1.04±0.02
6	1.77^{ab}±0.01	1.63±0.05	1.61±0.03	1.59±0.08	1.56±0.03	1.09±0.11	1.05±0.13
7	1.77^{ab}±0.10	1.63±0.08	1.59±0.09	1.57±0.01	1.55±0.08	1.08±0.04	1.00±0.09

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at *p*=0.05.

“ns” indicates no significant difference among sample means at *p*=0.05.

Table C.11 Loaf height (cm) of wheat, soy flour-substituted and okara-substituted breads during 7-day storage

Day	Control^{ns}	5SF^{ns}	10SF^{ns}	15SF^{ns}	5OK^{ns}	10OK^{ns}	15OK^{ns}
0	6.42±0.13	6.45±0.49	6.23±0.38	5.94±0.08	6.22±0.25	5.67±0.33	5.13±0.10
1	6.25±0.27	6.41±0.57	6.15±0.35	5.88±0.18	6.27±0.40	5.61±0.35	5.55±0.30
2	6.27±0.18	6.33±0.00	6.14±0.55	5.73±0.57	6.65±1.06	5.66±0.65	4.95±0.35
3	6.22±0.23	6.31±0.33	6.05±0.42	5.72±0.68	6.15±0.28	5.45±0.51	4.98±0.52
4	6.16±0.08	6.23±0.76	6.11±0.41	5.70±0.57	6.06±0.83	5.50±0.71	4.93±0.25
5	6.13±0.23	6.15±0.40	5.88±0.66	5.67±0.57	6.00±0.16	5.43±0.61	4.88±0.17
6	6.05±0.34	6.00±0.14	5.81±0.58	5.55±0.07	6.16±0.51	5.33±0.31	4.82±0.76
7	6.00±0.42	6.05±0.64	5.84±0.47	5.46±0.54	5.97±0.66	5.33±0.52	4.80±0.28

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

"ns" indicates no significant difference among sample means at p=0.05.

Table C.12 Hardness (g force) of wheat, soy flour-substituted and okara-substituted breads during 7-day storage

Day	Control	5SF	10SF	15SF	5OK	10OK	15OK
0	238.76^{f}±16.57	238.95^{e}±31.62	256.78^{e}±10.57	262.58^{e}±18.92	242.26^{e}±28.88	326.82^{e}±18.52	487.54^{e}±22.09
1	294.86^{e}±28.63	231.75^{de}±17.38	202.45^{e}±23.13	293.11^{d}±27.71	247.57^{e}±19.34	371.19^{d}±16.75	541.17^{d}±17.82
2	352.82^{d}±16.01	268.40^{cd}±15.05	219.87^{d}±18.00	306.23^{d}±26.53	288.15^{d}±14.82	390.67^{d}±26.23	566.59^{cd}±21.21
3	434.92^{c}±29.57	258.43^{c}±19.85	254.13^{d}±14.42	333.40^{c}±27.27	334.75^{c}±16.04	431.08^{c}±37.41	593.55^{c}±30.99
4	495.78^{b}±35.80	280.76^{c}±21.65	279.81^{c}±18.70	348.08^{bc}±29.35	357.02^{bc}±19.68	458.99^{b}±23.00	625.75^{b}±41.54
5	521.48^{ab}±39.07	344.00^{b}±18.63	314.96^{b}±30.51	360.34^{b}±19.92	332.92^{b}±21.45	481.31^{b}±30.64	646.27^{ab}±26.94
6	526.61^{a}±25.32	362.61^{b}±24.12	312.76^{b}±29.47	420.98^{a}±18.47	386.39^{a}±33.82	516.58^{a}±25.27	656.96^{ab}±23.34
7	533.77^{a}±31.02	458.95^{a}±36.05	367.93^{a}±28.19	427.13^{a}±15.68	400.80^{a}±25.26	523.84^{a}±24.58	667.52^{a}±65.29

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Table C.13 Cohesiveness of wheat, soy flour-substituted and okara-substituted breads during 7-day storage

Day	Control	5SF	10SF	15SF	5OK	10OK	15OK
0	0.36^{a}±0.03	0.35^{a}±0.03	0.35^{a}±0.03	0.36^{a}±0.03	0.32^{a}±0.02	0.30^{a}±0.03	0.15^{a}±0.01
1	0.22^{b}±0.02	0.31^{b}±0.01	0.32^{b}±0.03	0.34^{b}±0.03	0.31^{a}±0.03	0.24^{b}±0.02	0.13^{a}±0.02
2	0.18^{c}±0.01	0.29^{bc}±0.02	0.27^{c}±0.02	0.29^{c}±0.02	0.23^{b}±0.02	0.20^{c}±0.02	0.11^{b}±0.01
3	0.18^{c}±0.01	0.30^{cd}±0.02	0.24^{cd}±0.02	0.27^{cd}±0.02	0.21^{c}±0.02	0.18^{cd}±0.01	0.10^{c}±0.01
4	0.17^{c}±0.01	0.28^{d}±0.02	0.25^{de}±0.02	0.26^{d}±0.02	0.19^{d}±0.02	0.19^{de}±0.01	0.09^{cd}±0.01
5	0.15^{d}±0.01	0.25^{e}±0.01	0.24^{de}±0.02	0.25^{d}±0.02	0.18^{d}±0.02	0.17^{e}±0.01	0.09^{cd}±0.02
6	0.15^{d}±0.01	0.24^{e}±0.02	0.23^{de}±0.01	0.20^{e}±0.01	0.19^{d}±0.01	0.16^{e}±0.01	0.08^{de}±0.01
7	0.13^{e}±0.01	0.24^{e}±0.02	0.22^{e}±0.02	0.18^{f}±0.01	0.16^{e}±0.01	0.15^{f}±0.01	0.07^{e}±0.01

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Table C.14 Springiness of wheat, soy flour-substituted and okara-substituted breads during 7-day storage

Day	Control	5SF	10SF	15SF	5OK	10OK	15OK
0	0.58^{a}±0.05	0.58^{a}±0.05	0.57^{a}±0.05	0.55^{a}±0.05	0.53^{a}±0.03	0.44^{a}±0.03	0.33^{a}±0.03
1	0.20^{b}±0.02	0.23^{b}±0.02	0.28^{b}±0.02	0.34^{b}±0.03	0.27^{b}±0.02	0.26^{b}±0.02	0.22^{b}±0.02
2	0.16^{c}±0.01	0.21^{c}±0.02	0.26^{b}±0.02	0.31^{c}±0.03	0.22^{c}±0.02	0.21^{c}±0.02	0.20^{c}±0.02
3	0.15^{c}±0.01	0.20^{cd}±0.02	0.19^{c}±0.01	0.25^{d}±0.02	0.16^{d}±0.01	0.15^{d}±0.01	0.17^{d}±0.01
4	0.16^{c}±0.01	0.20^{cd}±0.02	0.18^{c}±0.01	0.22^{e}±0.02	0.17^{d}±0.01	0.17^{de}±0.01	0.14^{e}±0.01
5	0.15^{cd}±0.01	0.19^{cd}±0.01	0.18^{c}±0.01	0.19^{f}±0.01	0.16^{d}±0.01	0.16^{de}±0.01	0.13^{ef}±0.01
6	0.14^{cd}±0.01	0.18^{d}±0.01	0.18^{c}±0.01	0.16^{fg}±0.01	0.12^{e}±0.01	0.16^{ef}±0.01	0.14^{ef}±0.01
7	0.13^{d}±0.01	0.18^{d}±0.01	0.18^{c}±0.01	0.16^{f}±0.01	0.11^{e}±0.01	0.14^{f}±0.01	0.12^{f}±0.01

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Table C.15 Hue angle (degree) of wheat, soy flour-substituted and okara-substituted bread crumbs during 7-day storage

Day	Control	5SF	10SF	15SF	5OK	10OK	15OK
0	91.50^{a}±2.56	91.05^{a}±0.99	89.75^{a}±0.76	87.89^{a}±0.99	90.67^{a}±0.55	89.87^{a}±0.85	89.50^{a}±0.79
1	91.24^{ab}±2.10	90.83^{ab}±1.25	90.10^{a}±0.87	88.16^{a}±1.42	90.26^{ab}±1.22	89.54^{ab}±0.88	89.15^{a}±0.64
2	91.07^{ab}±2.87	90.63^{ab}±1.40	89.90^{ab}±0.65	87.77^{a}±1.29	90.19^{ab}±1.40	89.58^{ab}±1.37	88.79^{ab}±0.78
3	90.88^{ab}±2.50	90.65^{ab}±1.54	89.70^{ab}±1.08	87.63^{ab}±1.02	89.29^{bc}±1.28	89.19^{ab}±0.89	88.07^{b}±0.62
4	90.65^{abc}±1.78	89.88^{abc}±1.86	89.13^{b}±0.80	87.51^{ab}±0.95	89.05^{cd}±1.42	88.76^{b}±0.52	86.96^{c}±1.30
5	90.21^{abc}±1.02	89.51^{bcd}±1.71	88.31^{c}±0.59	87.32^{abc}±0.94	88.44^{cd}±1.56	87.43^{c}±0.57	85.82^{d}±2.36
6	89.35^{bc}±0.65	88.74^{cd}±1.52	87.51^{d}±0.76	86.62^{bc}±1.18	88.23^{cd}±1.35	86.17^{d}±0.90	85.15^{d}±1.31
7	88.87^{c}±2.04	88.55^{d}±1.31	87.18^{d}±0.79	86.42^{c}±1.58	88.12^{d}±0.71	85.47^{d}±1.40	85.03^{d}±1.22

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Table C.16 Chroma of wheat, soy flour-substituted and okara-substituted bread crumbs during 7-day storage

Day	Control	5SF	10SF	15SF	5OK^{ns}	10OK	15OK
0	12.18^{ab}±0.80	15.05^{a}±1.10	17.60^{d}±0.69	21.29^{b}±0.78	13.79±0.33	17.12^{a}±0.47	19.04^{a}±0.38
1	12.12^{ab}±0.82	15.02^{a}±0.58	18.68^{ab}±0.97	21.90^{a}±0.79	13.78±0.33	16.66^{bc}±0.28	18.82^{ab}±0.44
2	11.99^{b}±0.47	15.64^{a}±0.67	18.33^{abc}±0.58	21.32^{b}±0.54	13.75±0.19	16.21^{d}±0.24	18.61^{ab}±0.69
3	12.31^{ab}±0.40	15.48^{ab}±0.53	17.98^{cd}±0.42	20.74^{b}±0.68	13.71±0.43	16.24^{d}±0.17	18.56^{ab}±0.26
4	12.63^{a}±0.53	15.31^{ab}±0.61	18.18^{abcd}±0.30	20.82^{b}±0.58	13.65±0.35	16.29^{d}±0.37	18.50^{ab}±0.75
5	12.64^{a}±0.22	15.32^{ab}±0.39	18.49^{abc}±0.25	20.96^{b}±0.61	13.56±0.38	16.35^{cd}±0.65	18.45^{ab}±1.53
6	12.58^{a}±0.46	15.33^{ab}±0.24	18.80^{a}±0.40	21.09^{b}±0.82	13.48±0.56	15.97^{d}±0.42	18.10^{b}±0.65
7	12.53^{a}±0.78	15.35^{ab}±0.52	18.15^{bcd}±1.25	20.74^{b}±0.61	13.55±0.59	16.80^{ab}±0.55	18.41^{ab}±1.12

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

"ns" indicates no significant difference among sample means at p=0.05.

Table C.17 Whiteness index of wheat, soy flour-substituted and okara-substituted bread crumbs during 7-day storage

Day	Control	5SF	10SF	15SF	5OK	10OK	15OK
0	68.02^{a}±0.50	64.09^{b}±0.96	62.12^{a}±0.65	59.43^{ab}±1.03	67.48^{ab}±0.26	67.11^{a}±1.04	67.26^{a}±0.84
1	67.90^{ab}±1.79	64.34^{a}±0.61	61.48^{ab}±0.86	59.50^{a}±0.75	67.40^{ab}±0.72	67.45^{a}±0.50	67.31^{a}±0.37
2	67.75^{ab}±1.99	64.39^{a}±0.65	61.27^{ab}±0.94	59.51^{a}±0.60	67.70^{ab}±1.04	67.77^{a}±0.55	67.35^{a}±0.64
3	67.69^{ab}±2.38	63.74^{ab}±0.86	61.04^{b}±1.71	59.51^{a}±0.92	67.99^{a}±1.70	67.10^{a}±0.56	67.23^{a}±0.46
4	67.32^{ab}±1.78	63.09^{abc}±1.71	60.59^{bc}±1.27	59.19^{ab}±0.81	67.35^{ab}±1.76	66.09^{b}±0.68	67.03^{a}±0.91
5	66.42^{bc}±1.48	62.74^{bcd}±1.74	59.91^{cd}±0.79	58.71^{bc}±0.72	66.37^{bc}±2.12	65.06^{c}±0.89	66.83^{a}±1.58
6	65.53^{cd}±1.92	62.21^{cd}±1.91	59.21^{cd}±0.87	58.22^{c}±0.76	65.36^{c}±2.66	64.02^{d}±1.34	65.50^{b}±1.63
7	64.63^{d}±1.03	61.68^{d}±2.20	59.67^{d}±1.49	57.41^{d}±1.15	64.99^{c}±1.34	63.51^{d}±0.87	64.99^{b}±1.07

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Table C.18 CIE L^* of wheat, soy flour-substituted and okara-substituted bread crumbs during 7-day storage

Day	Control	5SF	10SF	15SF	5OK	10OK	15OK
0	70.43^{a}±0.68	67.40^{ab}±0.87	66.46^{a}±0.56	65.47^{ab}±1.02	70.55^{ab}±0.34	71.91^{a}±1.37	73.36^{a}±1.06
1	70.27^{a}±0.93	67.66^{ab}±0.78	66.31^{a}±0.73	65.93^{a}±0.71	70.46^{ab}±0.83	72.03^{a}±0.63	73.27^{a}±0.43
2	70.06^{a}±0.67	68.00^{a}±0.85	65.88^{ab}±1.12	65.58^{ab}±0.63	70.77^{ab}±1.14	72.14^{a}±0.65	73.17^{a}±0.56
3	70.13^{a}±0.88	67.21^{abc}±0.95	65.44^{abc}±2.13	65.22^{ab}±0.92	71.08^{a}±1.91	71.39^{a}±0.60	72.99^{ab}±0.51
4	69.86^{a}±1.15	66.42^{bcd}±1.73	65.03^{bc}±1.58	64.90^{bc}±0.77	70.34^{ab}±1.90	70.26^{b}±0.66	72.72^{ab}±0.72
5	68.89^{b}±1.02	66.04^{cde}±1.83	64.42^{cd}±0.92	64.42^{cd}±0.67	69.22^{bc}±2.24	69.12^{c}±0.85	72.43^{b}±1.05
6	67.91^{c}±0.70	65.46^{de}±1.76	63.80^{d}±0.93	63.93^{d}±0.74	68.09^{c}±2.85	67.76^{d}±1.83	70.63^{c}±0.71
7	66.92^{d}±0.99	64.88^{e}±2.01	63.99^{d}±0.95	62.80^{e}±1.33	67.72^{c}±1.61	67.61^{d}±1.05	70.22^{c}±1.03

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Table C.19 Hue angle (degree) of wheat, soy flour-substituted and okara-substituted bread crust during 7-day storage

Day	Control	5SF^{ns}	10SF	15SF	5OK	10OK	15OK
0	60.51^{a}±0.86	56.15±1.65	55.73^{a}±0.78	54.15^{a}±1.84	56.83^{b}±1.24	58.65^{b}±0.82	62.77^{bcd}±1.09
1	59.70^{ab}±0.48	56.10±1.24	54.69^{b}±1.73	51.90^{b}±1.12	57.72^{ab}±1.08	58.75^{b}±0.63	62.24^{cd}±0.53
2	59.68^{ab}±1.83	54.98±1.42	52.52^{c}±0.88	52.44^{b}±0.53	58.12^{a}±0.69	58.85^{b}±1.09	62.01^{d}±0.97
3	59.16^{bc}±1.03	55.22±1.70	50.99^{cd}±1.14	52.91^{ab}±1.03	57.97^{a}±1.17	59.07^{b}±1.02	62.47^{cd}±0.92
4	58.62^{c}±0.61	55.44±2.24	50.19^{de}±0.75	52.77^{ab}±0.49	58.21^{a}±0.74	59.38^{b}±1.26	63.27^{bcd}±1.34
5	58.61^{c}±0.64	55.66±1.64	50.47^{e}±0.63	52.54^{b}±0.72	58.51^{a}±0.72	59.69^{b}±1.73	64.02^{bc}±2.03
6	58.59^{c}±0.82	55.92±0.91	50.74^{e}±1.03	52.28^{b}±1.62	58.76^{a}±1.34	61.76^{a}±1.17	64.47^{b}±3.53
7	58.56^{c}±1.06	56.12±0.87	51.70^{e}±1.16	54.10^{c}±2.15	57.74^{ab}±1.40	61.72^{a}±0.91	63.28^{a}±1.65

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

"ns" indicates no significant difference among sample means at p=0.05.

Table C.20 Chroma of wheat, soy flour-substituted and okara-substituted bread crust during 7-day storage

Day	Control	5SF	10SF	15SF	5OK	10OK	15OK
0	32.79^{a}±0.47	28.26^{ab}±1.04	27.51^{a}±1.18	26.58^{ab}±0.98	28.47^{e}±0.67	32.77^{d}±0.66	35.96^{c}±0.93
1	31.81^{bc}±0.23	28.70^{ab}±0.81	27.22^{a}±1.39	25.77^{b}±1.48	29.74^{d}±0.58	32.63^{d}±0.58	35.71^{c}±0.36
2	31.85^{bc}±0.82	27.95^{b}±1.20	25.57^{b}±0.46	26.47^{ab}±0.77	30.02^{cd}±0.38	32.49^{cd}±1.13	35.86^{c}±0.45
3	31.39^{cd}±0.39	28.08^{ab}±1.70	23.97^{d}±0.72	27.18^{a}±0.86	30.47^{cd}±1.09	32.92^{cd}±0.92	36.15^{bc}±0.41
4	30.94^{d}±0.40	28.21^{ab}±2.24	24.26^{d}±0.53	27.22^{a}±0.45	30.86^{bc}±0.48	33.56^{bc}±0.84	36.83^{ab}±0.70
5	31.32^{cd}±0.44	28.54^{ab}±1.35	24.71^{cd}±0.33	27.29^{a}±0.51	31.46^{ab}±0.76	34.20^{b}±1.09	37.53^{a}±1.21
6	31.88^{bc}±0.99	29.03^{ab}±0.48	25.16^{bc}±0.40	27.36^{a}±1.16	32.07^{a}±1.75	35.16^{a}±0.96	35.67^{c}±1.17
7	32.45^{ab}±1.63	29.$^{54a±}$1.56	26.78^{a}±1.19	26.91^{c}±1.00	32.05^{a}±0.69	33.98^{b}±0.42	37.36^{a}±0.70

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Table C.21 CIE L^* of wheat, soy flour-substituted and okara-substituted bread crust during 7-day storage

Day	Control	5SF	10SF	15SF	5OK	10OK	15OK
0	$46.50^{bc}\pm0.58$	$41.14^{c}\pm1.10$	$38.59^{cde}\pm1.04$	$36.24^{ab}\pm0.80$	$42.87^{e}\pm0.64$	$46.88^{b}\pm1.13$	$52.61^{b}\pm2.44$
1	$44.35^{f}\pm0.31$	$40.45^{c}\pm0.98$	$39.88^{a}\pm1.20$	$36.27^{ab}\pm3.47$	$43.71^{e}\pm0.57$	$47.26^{b}\pm0.89$	$52.53^{b}\pm1.72$
2	$44.77^{ed}\pm1.31$	$41.69^{bc}\pm1.76$	$39.60^{ab}\pm0.44$	$36.01^{ab}\pm0.67$	$45.24^{cd}\pm0.62$	$47.64^{b}\pm1.10$	$52.46^{b}\pm1.45$
3	$45.28^{def}\pm0.69$	$42.81^{ab}\pm1.97$	$39.31^{abc}\pm0.71$	$36.97^{a}\pm1.03$	$46.76^{a}\pm1.71$	$47.42^{b}\pm0.79$	$52.75^{b}\pm1.14$
4	$45.78^{cde}\pm0.33$	$43.93^{a}\pm2.43$	$38.94^{bcd}\pm0.60$	$36.20^{ab}\pm0.61$	$46.46^{ab}\pm1.02$	$47.10^{b}\pm0.55$	$53.43^{b}\pm0.83$
5	$46.29^{bcd}\pm0.70$	$43.86^{a}\pm1.68$	$38.38^{de}\pm0.47$	$35.03^{bc}\pm0.36$	$46.02^{abc}\pm0.44$	$46.77^{b}\pm0.82$	$54.12^{ab}\pm1.10$
6	$47.05^{ab}\pm1.30$	$43.74^{a}\pm0.83$	$37.82^{e}\pm0.45$	$33.87^{c}\pm0.93$	$45.57^{bcd}\pm1.31$	$46.69^{b}\pm1.15$	$55.61^{a}\pm3.71$
7	$47.80^{a}\pm1.92$	$43.63^{a}\pm1.22$	$38.35^{de}\pm0.91$	$31.72^{d}\pm1.03$	$44.76^{d}\pm1.66$	$48.90^{a}\pm2.48$	$53.04^{b}\pm1.58$

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

Table C.22 Crust color (orange-brown), as evaluated using DA, of wheat, soy flour-substituted and okara-substituted breads during 5-day storage

Day	Control^{ns}	5SF^{ns}	10SF	15SF^{ns}	5OK^{ns}	10OK	15OK
0	9.43±0.82	9.88±0.84	$10.44^{ab}\pm0.94$	10.63±1.50	9.72±0.89	$9.76^{c}\pm1.58$	$9.33^{b}\pm1.08$
1	9.15±0.56	9.66±1.55	$10.52^{b}\pm1.53$	9.94±1.68	9.61±1.10	$10.42^{ab}\pm1.28$	$10.21^{ab}\pm1.47$
2	9.25±1.38	10.45±1.09	$10.60^{ab}\pm0.89$	10.75±1.37	10.45±1.22	$10.80^{a}\pm1.46$	$10.55^{a}\pm1.80$
3	9.50±0.94	9.65±1.28	$10.66^{ab}\pm1.76$	10.55±1.58	9.76±0.71	$9.45^{c}\pm1.28$	$10.11^{ab}\pm0.82$
4	9.51±1.52	9.83±1.78	$11.49^{a}\pm1.59$	9.61±1.78	9.92±1.24	$9.92^{ab}\pm1.51$	$9.95^{ab}\pm1.03$
5	9.80±1.68	10.00±1.53	$10.30^{b}\pm1.80$	10.35±1.60	10.25±1.44	$9.80^{c}\pm1.29$	$9.55^{b}\pm1.14$

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

"ns" indicates no significant difference among sample means at p=0.05.

Table C.23 Crumb color (yellow), as evaluated using DA, of wheat, soy flour-substituted and okara-substituted breads during 5-day storage

Day	Control	5SF	10SF	15SF	5OK^{ns}	10OK	15OK^{ns}
0	$2.33^{ab}\pm0.74$	$11.07^{a}\pm1.76$	$12.58^{ab}\pm1.35$	$13.65^{a}\pm1.35$	4.34±1.40	$7.77^{b}\pm1.64$	10.88±1.17
1	$2.54^{ab}\pm0.99$	$10.18^{ab}\pm1.39$	$12.44^{ab}\pm1.35$	$13.35^{ab}\pm1.66$	3.88±1.29	$8.51^{ab}\pm1.72$	10.74±1.68
2	$1.90^{b}\pm0.96$	$11.25^{a}\pm1.53$	$12.70^{ab}\pm1.45$	$13.40^{ab}\pm1.43$	3.85±1.08	$8.95^{a}\pm1.67$	11.05±1.29
3	$2.87^{a}\pm0.66$	$9.71^{b}\pm1.30$	$12.56^{ab}\pm1.16$	$12.47^{b}\pm1.55$	4.59±1.37	$7.65^{b}\pm1.51$	11.59±1.31
4	$3.03^{a}\pm1.11$	$10.28^{ab}\pm1.63$	$11.88^{b}\pm1.27$	$13.41^{ab}\pm1.34$	4.38±0.90	$7.87^{b}\pm1.28$	10.98±1.66
5	$2.40^{ab}\pm0.46$	$10.45^{ab}\pm1.80$	$12.35^{a}\pm1.63$	$13.20^{ab}\pm1.62$	4.20±1.04	$7.75^{b}\pm1.03$	11.25±1.80

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

"ns" indicates no significant difference among sample means at p=0.05.

Table C.24 Pore homogeneity, as evaluated using DA, of wheat, soy flour-substituted and okara-substituted breads during 5-day storage

Day	Control	5SF^{ns}	10SF	15SF	5OK^{ns}	10OK	15OK
0	13.24^{b}±0.91	10.51±1.47	4.11^{c}±0.84	5.66^{ab}±1.55	10.45±1.22	3.33^{bc}±0.99	1.60^{ab}±0.70
1	13.19^{b}±1.25	9.85±1.58	4.24^{c}±1.14	4.34^{c}±1.13	10.82±1.96	3.64^{abc}±1.53	1.48^{ab}±0.68
2	13.65^{ab}±1.66	10.15±1.33	4.75^{bc}±1.02	4.75^{bc}±1.78	11.50±1.43	3.10^{c}±0.91	1.30^{ab}±1.23
3	14.24^{a}±0.91	9.88±0.90	5.63^{ab}±0.94	6.19^{a}±1.59	11.48±1.43	3.74^{abc}±1.32	1.18^{b}±0.55
4	13.55^{ab}±1.94	10.39±1.41	4.87^{abc}±1.68	5.65^{ab}±1.52	11.95±1.47	4.34^{a}±1.53	1.69^{a}±0.54
5	13.78^{ab}±1.28	10.90±1.27	5.75^{a}±1.88	4.60^{bc}±1.21	11.35±1.40	4.20^{ab}±0.87	1.48^{ab}±1.03

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

“ns” indicates no significant difference among sample means at p=0.05.

Table C.25 Pore size, as evaluated using DA, of wheat, soy flour-substituted and okara-substituted breads during 5-day storage

Day	Control	5SF^{ns}	10SF^{ns}	15SF	5OK	10OK^{ns}	15OK
0	12.08^{a}±1.10	12.11±1.57	12.54±0.76	11.82^{ab}±1.29	11.45^{ab}±1.65	9.38±1.31	7.73^{a}±0.23
1	13.44^{a}±1.40	11.84±1.34	13.36±1.70	12.53^{a}±0.96	11.60^{ab}±1.79	9.23±1.21	7.64^{a}±0.14
2	13.56^{a}±1.35	12.45±1.54	12.60±1.13	10.85^{b}±1.58	11.65^{ab}±1.33	9.65±1.14	7.76^{a}±0.26
3	12.84^{a}±1.83	12.66±1.20	13.40±1.11	12.13^{a}±1.63	12.44^{a}±1.08	9.54±1.04	6.88^{ab}±0.46
4	11.23^{b}±1.52	11.95±1.50	12.84±1.36	11.57^{ab}±1.55	11.31^{ab}±1.38	10.07±1.28	6.64^{b}±0.32
5	13.85^{a}±1.46	12.00±1.20	12.55±1.70	12.33^{a}±1.30	10.85^{b}±1.41	10.15±1.53	7.75^{a}±0.41

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

“ns” indicates no significant difference among sample means at p=0.05.

Table C.26 Hardness, as evaluated using DA, of wheat, soy flour-substituted and okara-substituted breads during 5-day storage

Day	Control	5SF	10SF	15SF^{ns}	5OK	10OK	15OK
0	5.22^{c}±1.46	5.23^{c}±1.15	4.96^{c}±1.04	8.30±1.82	5.54^{c}±1.43	8.67^{c}±1.57	10.30^{c}±1.47
1	7.35^{b}±1.92	5.48^{c}±1.16	5.63^{bc}±0.84	8.74±1.34	5.76^{bc}±1.19	9.84^{b}±1.65	11.63^{b}±1.08
2	8.00^{b}±1.70	5.70^{bc}±1.48	6.15^{b}±0.75	8.88±1.61	6.28^{bc}±1.23	10.75^{ab}±1.58	12.92^{a}±1.26
3	9.64^{a}±1.74	6.66^{ab}±0.91	7.43^{a}±0.87	9.13±1.78	6.75^{b}±1.72	10.81^{ab}±1.45	13.68^{a}±1.56
4	10.57^{a}±1.94	7.33^{a}±1.42	7.83^{a}±0.91	9.44±1.48	6.53^{bc}±1.11	11.20^{a}±1.49	13.30^{a}±1.56
5	10.60^{a}±1.57	7.55^{a}±1.54	8.05^{a}±1.68	9.30±1.41	8.25^{a}±1.81	11.45^{a}±1.54	13.20^{a}±1.21

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at p=0.05.

“ns” indicates no significant difference among sample means at p=0.05.

Table C.27 Cohesiveness, as evaluated using DA, of wheat, soy flour-substituted and okara-substituted breads during 5-day storage

Day	Control^{ns}	5SF	10SF^{ns}	15SF^{ns}	5OK^{ns}	10OK	15OK
0	13.11±1.51	10.88^{a}±1.85	10.54±1.72	9.87±1.60	9.55±1.37	8.76^{a}±1.56	8.59^{a}±1.40
1	13.82±1.04	10.69^{ab}±1.60	10.35±1.81	9.51±1.48	9.24±1.11	7.34^{b}±1.57	6.65^{b}±1.28
2	12.04±1.51	10.66^{ab}±1.73	10.31±1.86	9.45±1.59	8.50±1.45	8.25^{ab}±1.49	8.12^{a}±1.04
3	11.57±1.36	10.25^{ab}±1.47	10.45±1.31	9.19±1.39	8.66±1.51	7.81^{ab}±1.57	7.88^{a}±1.55
4	11.61±1.20	9.33^{b}±1.26	10.32±1.52	9.68±1.83	9.38±1.30	7.67^{ab}±1.51	6.72^{b}±1.24
5	11.50±1.18	10.23^{ab}±1.50	10.50±1.39	9.50±1.66	8.52±1.59	8.74^{a}±1.32	8.65^{a}±1.40

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at *p*=0.05.

"ns" indicates no significant difference among sample means at *p*=0.05.

Table C.28 Springiness, as evaluated using DA, of wheat, soy flour-substituted and okara-substituted breads during 5-day storage

Day	Control	5SF	10SF	15SF	5OK	10OK	15OK
0	12.67^{a}±1.83	10.49^{a}±1.01	10.36^{a}±1.62	9.86^{a}±0.94	9.55^{a}±1.82	8.59^{a}±1.73	6.32^{a}±0.76
1	8.54^{b}±1.30	6.30^{b}±1.04	9.76^{a}±1.71	5.34^{b}±1.55	8.47^{b}±1.79	6.78^{b}±1.65	5.20^{b}±1.61
2	8.88^{b}±1.03	5.55^{bc}±1.55	7.49^{b}±1.45	4.10^{c}±0.98	7.70^{b}±1.70	5.05^{c}±1.54	5.20^{b}±1.32
3	7.31^{c}±1.61	5.85^{b}±1.49	6.11^{c}±1.40	4.51^{c}±1.33	6.44^{c}±1.56	4.18^{cd}±0.94	4.86^{b}±1.64
4	5.22^{d}±1.47	4.74^{cd}±1.24	5.01^{d}±1.20	4.06^{c}±0.83	5.62^{c}±1.50	3.66^{d}±1.48	4.53^{b}±1.44
5	4.36^{d}±1.39	4.33^{d}±1.69	4.15^{d}±1.54	3.70^{c}±1.74	4.43^{d}±1.30	3.65^{b}±1.26	3.50^{c}±1.70

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at *p*=0.05.

Table C.29 Beany flavor, as evaluated using DA, of wheat, soy flour-substituted and okara-substituted breads during 5-day storage

Day	Control	5SF	10SF^{ns}	15SF	5OK	10OK	15OK
0	0.40^{ab}±0.17	3.46^{c}±1.28	12.54±1.55	12.94^{a}±1.68	1.72^{ab}±0.41	7.18^{bcd}±1.25	12.65^{b}±1.42
1	0.40^{ab}±0.14	3.15^{b}±0.82	11.42±1.26	11.65^{a}±1.67	2.49^{c}±0.67	6.77^{cd}±1.46	12.71^{a}±1.92
2	0.30^{b}±0.13	3.60^{b}±1.73	11.75±1.26	13.40^{b}±1.51	2.50^{ab}±0.83	6.50^{d}±1.03	12.41^{a}±1.18
3	0.50^{a}±0.14	3.28^{d}±1.18	12.18±1.82	12.43^{b}±1.56	1.11^{c}±0.62	8.01^{ab}±1.59	11.69^{ab}±1.50
4	0.40^{ab}±0.12	4.19^{b}±1.37	11.66±1.43	13.59^{a}±0.87	2.33^{a}±1.13	7.48^{abc}±1.00	12.71^{a}±1.56
5	0.50^{a}±0.11	3.05^{a}±1.17	11.95±1.63	12.50^{a}±1.11	3.55^{c}±1.27	8.10^{a}±1.44	12.85^{ab}±1.29

Values are means ± SD of three different determinations.

Sample means within a column which do not share a common superscript letter differ significantly at *p*=0.05.

"ns" indicates no significant difference among sample means at *p*=0.05.

VITA

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