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THESIS

DEVELOPMENT OF EXTRUDED SNACKS FROM GERMINATED KHAO DAWK MALI 105 AND HOM NIN BROWN RICE FLOUR FORTIFIED WITH XANTHONES

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The objectives of this study were to determine effects of storage duration of paddy rice and pH levels of steeping water during germination on physicochemical properties of germinated brown rice flour (GBRF) prepared from two rice varieties (Khao Dawk Mali 105 (KM) and Hom Nin (HN)) and to develop extruded snacks from those flours by a twin-screw extruder that were acceptable to consumers. A 6x2 factorial arrangement in a completely randomized design (CRD) with six storage periods (2, 4, 6, 8, 10, and 12 months) and two steeping conditions (in a buffer solution at pH 3 and in reverse osmosis water at pH 6.8) for 48 h were used. Both germinated brown rice flour (GBRF) obtained from paddy rice stored for 6 to 10 months and germinated at pH 3 yielded the high free gamma-aminobutyric acid (GABA) content (83 to 99 mg/100 g flour for GBRF from KM (GKMF) and 30 to 42 mg/100 g flour for GBRF from HN (GHNF)). Extruded snack formulation 90.5% of GBRF (obtained from steeping at pH 3 for 48 h), 9.05% of soy protein isolate and 0.45% of $CaCO_3$ was used to study the effect of extrusion conditions on physicochemical and sensory properties of extruded snacks. A 3x3 factorial arrangement in CRD was used: three levels of feed moisture (14, 18 and 22%) and three levels of screw speed (300, 350 and 400 rpm). The results showed that increasing the feed moisture caused an increase in bulk density and hardness, but a decrease in expansion ratio. Increasing feed moisture increased retention of free GABA. Total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity and ferric reducing antioxidant power (FRAP) of extrudates from both rice varieties were decreased when compared with those of the non-extruded. Optimum conditions to produce extruded snack obtain high free GABA and acceptable for consumer predicted by regression model for GKMF and GHNF were 16 to 19% of feed moisture and 300 to 310 rpm of screw speed and 14 to 19 % of feed moisture and 300 to 400 rpm of screw speed, respectively. Both GBRFs mixed with crude xanthone powder was used to study the effect of barrel temperature (120, 140 and 160°C) on physicochemical properties of extrudates. The result showed that increasing barrel temperature caused an increased in expansion ratio, but a decrease in a_w, bulk density and hardness. Increasing barrel temperature increased retention of TPC, DPPH radical-scavenging activity and FRAP. However, free GABA and α-mangostin content of extrudates were decreased. Regarding consumer acceptability (n=200 for Thai and 100 for US. consumers), the mean overall liking scores of developed snack from GKMF was slightly higher (7.4 for both Thai and US. consumers) than the GHNF (7.0 for both Thai and US. consumers). This study demonstrated the feasibility of producing highly nutritious extruded snacks from GBRF that were acceptable to both Thai and US. consumers.

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LIST OF ABBREVIATIONS

KM	=	Khao Dawk Mali 105
HN	=	Hom Nin
KMR	=	KM rice
HNR	=	HN rice
BR	=	brown rice
GBR	=	germinated brown rice
GBRF	=	germinated brown rice flour
GKMF	=	germinated brown rice flour from Khao Dawk Mali 105
GHNF	=	germinated brown rice flour from Hom Nin
GKME	=	extrudates from germinated brown rice flour from Khao Dawk Mali 105
GHNE	=	extrudates from germinated brown rice flour from Hom Nin
˚C	=	degree Celsius
˚B	=	Brix
h	=	hour
sec	=	second
min	=	minute
g	=	gram
mL	=	milliliter
µL	=	microliter
M	=	molar
mg/L	=	milligram per liter
µm	=	micrometer
µg	=	microgram
mm	=	millimeter
mmol	=	millimolar
nm	=	nanometer
ppm	=	parts per million

LIST OF ABBREVIATIONS (Continued)

v/v	=	volume per volume
w/v	=	weight per volume
w/w	=	weight per weight
etc.	=	et cetera (and so on)
e.g.	=	exempli gratia (for example)
i.e.	=	id est (that is)

DEVELOPMENT OF EXTRUDED SNACKS FROM GERMINATED KHAO DAWK MALI 105 AND HOM NIN BROWN RICE FLOUR FORTIFIED WITH XANTHONES

INTRODUCTION

Snack as a food not eaten at a recognized mealtime and one which made a minor contribution to the day's dietary intake. They are made primarily from corn, wheat, oats, or rice, in about that order of the quantities produced (Robert and Elwood, 2000). Food extrusion has become a very important process and decisive role in the innovation and development of snack products. Several unit operations including mixing, shearing, conveying, cooking and forming are combined in the extruder. Many researchers have reported the positive and negative effects of the extrusion process on the nutritional quality of food and feed mixtures using different extruder conditions (temperature, feed moisture, and screw speed) and raw material characteristics (composition and particle size). Extrusion cooking is the destruction of antinutritional factors, especially trypsin inhibitors, tannins and phytates, all of which inhibit protein digestibility (Singh *et al*., 2007). In addition, extrusion cooking was found effective in improving protein and starch digestibility and increasing fiber solubility (Shimelis and Rakshit 2007; Repo-Carrasco-Valencia *et al*., 2009). However, the negative effect of extrusion process was reported to reduce the antioxidant activities in the extrudate products of grain, cereal and beans (Ozer *et al*., 2006; Anton *et al*., 2009; Repo-Carrasco-Valencia *et al*., 2009). In contrast, some studies showed the retention of some antioxidant compounds during extrusion for rice (Ohtsubo *et al*., 2005), wheat, barley, and rye (Zielinski *et al*., 2001). Greater consumer demand for nutritious extruded food product with enhanced bioactive compounds has shifted research focus towards incorporation of bioactive rich ingredients with traditionally extruded starch materials (Brennan *et al*., 2011).

Rice, as a raw material for extrusion, offers a relatively good puffing quality with attractive white color, ease of digestion, bland flavor, and is suitable for coating with a variety of flavorings (Yagci and Gogus, 2009). Germinated brown rice (GBR)

is considered as innovative rice by preserving all the nutrients in the rice grain for consumption to create the highest value from rice. It has high content of gamma-aminobutyric acid (GABA), phenolic acid content, ferulic acid (Tian *et al*., 2004), tocopherols and γ-oryzanol (Shoichi *et al*., 2004) and a decrease in some antinutrients, such as phytic acid (Ghavidel and Prakash, 2007). GABA functions as an inhibitory neurotransmitter in the central nervous system, prevents Alzheimer's disease (Kinnersley and Turano, 2000), and regulates blood pressure in both animals and humans (Zhang *et al*., 2006). Several methods are used to increase the GABA content in brown rice, such as soaking the rice in deionized water or acidic solution, controlling under anaerobic condition, substrate and enzyme addition and microbiological fermentation.

Xanthones, a particular class of plant phytochemicals from mangosteen (*Garcinia mangostana*), are highly biologically active, posses antioxidant antibacterial, antifungal, antitumor, antiplatelet aggregation, antithrombotic, (Chaivisuthangkura *et al.*, 2009), and have cardiovascular protective effects (Miwako *et al*., 2009). The α-mangostin, one of the major and much-studied xanthones found in mangosteen, was proven to be a stronger antioxidant (Jung *et al*., 2006). Nowadays, products developed from xanthones are popular and variety in the market such as concentrate drink, food supplements and herbal cosmetic because of their powerful antioxidant potential. However, the comprehensive data on physicochemical properties of snack from germinated brown rice flour (GBRF) has been rarely studied. It is interesting to know how the extrusion conditions influence the bioactive compounds including GABA content, α-mangostin content and the antioxidant activity of the extrudates from GBRF. Furthermore, the health benefit from GBRF and xanthone can be added value to Thai agricultural products. Therefore, the objectives of this research were to study the effect of extrusion conditions on the free GABA content, α-mangostin content and antioxidant activity of extruded snack made from GBRF and developed snack containing GABA that were acceptable to consumers.

OBJECTIVES

1. To study the effect of pH conditions of steeping water and storage time of paddy rice on physicochemical properties of germinated brown rice flour.

2. To evaluate the effect of extrusion process on physicochemical properties of extruded snack from germinated brown rice flour fortified with crude xanthone powder.

3. To develop the formulation of extruded snack from germinated brown rice flour.

4. To determine the qualities and study the consumer acceptance of developed snack from germinated brown rice flour.

LITERATURE REVIEW

1. Extrusion cooking and food nutrition

Extrusion cooking, as a multi-step, multi-functional and thermal/mechanical process, has permitted a large number of food applications. Physical, chemical and sensory properties of extruded snack can vary considerably depending on operating conditions and properties of raw material (Liu *et al*., 2000). Effects of extrusion cooking on nutritional quality are interesting to study. Beneficial effects include destruction of antinutritional factors, gelatinisation of starch, increased soluble dietary fiber and reduction of lipid oxidation. On the other hand, Maillard reactions between protein and sugars reduce the nutritional value of the protein, depending on the raw material types, their composition and process conditions. Heat-labile vitamins may be lost to varying extents. Changes in proteins and amino acid profile, carbohydrates, dietary fibre, vitamins, mineral content and some non-nutrient healthful components of food may be either beneficial or deleterious (Singh *et al*., 2006).

1.1 Effect of extrusion variables on product quality

Extrusion cooking is accomplished by using a combination of moisture, pressure, temperature and mechanical shear (Barrows and Hardy, 2001). Several extrusion process variables can influence the composition of finished products. These include raw material characteristics, mixing and conditioning of raw material, barrel temperature, pressure, screw speed, moisture content, flow rate, energy input, residence type and screw configuration influences physicochemical properties of extrudates. Critical extrusion process variables such as temperature profile, feed moisture content and screw speed may induce desirable modifications, thus improving palatability and technological properties of extruded products (Gui and Ryu 2008).

1.1.1 Temerature profile

The barrel temperature profile affects the product temperature, which in turn affects degree of cook and the melt viscosity. An increase in the barrel

temperature will decrease the melt viscosity (Mercier and Feillet, 1975). The reduced viscosity effect would favour the bubble growth during extrusion. Moreover, the degree of superheating of water in the extruder would increase at higher temperature, also leading to greater expansion (Ding *et al*., 2005; Ding *et al*., 2006; Badrie and Mellowes, 2006). Consequently, increased barrel temperature caused the reduction of bulk density and hardness of rice extrudates (Ding *et al*., 2005), wheat-based extrudates (Ding *et al*., 2006) and cassava extrudates (Badrie and Mellowes, 2006). The water absorption index (WAI) measure the amount of water absorbed, mainly by starch (Anderson, 1969). According to Ding *et al*. (2006), increasing barrel temperature caused decreased in values of WAI. Ding *et al*. (2006) also stated that the WAI decreases with increasing temperature if dextrinization or starch melting predominate over the gelatinization phenomenon. Furthermore, a decrease in WAI with increasing temperature was probably due to decomposition or degradation of starch (Pelembe *et al*., 2002).

The water solubility index (WSI) measures the amount of soluble components relased from the starch upon extrusion cooking (Kirby *et al*., 1988) and has been used as an indicator of degradation of molecular components (Ding *et al*., 2005). Hagenimana *et al*. (2006) showed that values of WSI of rice flour extrudates increased with increasing both the severity of the screw profile and temperature in the extruder. Increasing temperature also increased WSI of cassava extrudates (Badrie and Mellowes, 2006), wheat-based extrudates (Ding *et al*., 2006) and corn extrudates (Mezreb *et al.,* 2002). The WSI is related to the quantity of soluble molecules, which is related to dextrinization. The rise in temperature increases the severity of thermal treatment in the extruder, which consequently raises WSI. These observations are similar to starch-based extrudates obtained either using single or twin screw extrusion (Ding *et al*., 2006; Gujska *et al*., 1990; Kadan *et al*., 2003).

1.1.2 Feed moisture

Moisture content of the feed mixture is a critical factor affecting the extrusion temperature, pressure and product texture. During the extrusion process,

the dough viscosity, elastic swell effect and bubble growth effect contribute to the structure change of the extrusion mix. Moisture content of 13 to 14% is generally recommended for produced extrudates (Matz, 1991). As feed moisture increases, extrusion temperature drops and less expansion occurs in the extrudate. Pores in the product become large and walls of the pores become thicker. Similarly result was observed by Ding *et al*. (2006) who reported that the degree of starch gelatinization and extrudate expansion was found to be reduced as the feed moisture increased. This would be caued by the water acts as a plasticizer to the starch-based material reducing its viscosity and the mechanical energy dissipation in the extruder thus the product becomes dense and bubble growth is compressed. Ding *et al*. (2006) reported that the extrudate density was found to be most dependent on feed moisture. High feed moisture results in a dense and hard product due to incomplete gelatinization of the starch. As feed moisture is reduced, extrusion temperature rises and the extrudate more expands.

Extrusion temperature and moisture content are known to affect gelatinization during extrusion, and consequently the WAI. Hagenimana *et al*. (2006) reported that high feed moisture during extrusion cooking of rice flour caused higher values of WAI of the extrudates due to reduced amounts of degradation of starch granules. In high moisture soy meat analog, WAI increased with increase in extrusion temperature and feed moisture (Lin *et al*., 2002). Similarly results were reported for corn starch extrudates, bean and chickpea extrudates (Gujska and Khan, 1990 and Singh *et al*., 2007). On the other hand, many researchers were repoted that increased feed moisture content decreased values of WSI (Ding *et al*., 2005; Hernandez-Diaz *et al*., 2007). This possibly caused by dextrinization occurred during extrusion with the low feed moisture content (14-22%), along with limitation of gelatinization. Similarly result was observed by Gomez and Aguilera (1983) who mentioned that dextrinization is the predominant mechanism of starch degradation during low moisture extrusion.

1.1.3 Screw speed

The basic functions of the screw in the extruder are transport the feed matreial from the feed hopper to the extruder outlet and carry out any mixing required during the extrusion process. Screw speed is the frequency of a screw rotation. The unit of screw speed is a revolution per minute (rpm). It annotates the number of full rotations completed in one minute around a fixed axis. The extruder screw configureuration affects the degree of mixing, shear forces introduced. Amount of heat generated by friction and the residence time distribution. All these factors affect the degree of cooking, the melt rheology and product quality and uniformity. An increase in screw speed may be expected to lower the melt viscosity of the feed mixture resulting in a less dense, softer extrudate (Ding *et al*., 2005; Ding *et al*., 2006; Badrie and Mellowes, 2006). Furthermore, increasing screw speeds at constant feed rate reduce pressure at the die and decreased motor torque of extruder (Pansawat *et al*., 2008).

Increasing screw speed caused an increase in values of WSI but decreased in values of WAI with the results in corn meal, corn-wheat extrudates and barley–tomato pomace extrudates (Mezreb *et al*., 2003; Altan *et al*., 2008). Additionally, Mezreb *et al*. (2003) reported that the increase of screw speed induced a sharp increase of specific mechanical energy, the high mechanical shear degraded macromolecules, and thus the molecular weight of starch granules decreased and hence increased WSI.

1.2 Effect of extrusion processing on nutritional quality

Extrusion of snack foods demands close control of many variables such as feed moisture, feed composition, feed particle size, feed rate, barrel temperature, screw speed, screw configuration, and die geometry. These material and process variables determine the extent of macromolecular transformations during extrusion, which in turn influence the rheological properties of the food melt in the extruder and, consequently, the product characteristics of extrudates (Singh *et al*., 2006). Effects of

extrusion cooking on nutritional quality are divided into two grops of macronutrients (i.e., carbohydrates, protein, lipids and dietary fiber) and micronutrients (vitamins and minerals) (Camire *et al*., 1990).

1.2.1 Macronutrients

a) Carbohydrates

Humans and other monogastric species cannot easily digest ungelatinized starch. Extrusion cooking is somewhat unique because gelatinization occurs at much lower moisture levels (12-22%) than is necessary in other food operations. Processing conditions that increase temperature, shear, and pressure tend to increase the rate of gelatinization. The presence of other food compounds, particularly lipids, sucrose, dietary fiber and salts, also affects gelatinization. In a sense, extrusion may pre-digest starch. Branches on amylopectin molecular are easily sheared off in the barrel. Reduction in molecule weight for both amylase and amylopectin molecules have been documented. Extrusion cooking of rice led to the degradation of high molecular weigh fraction of the starch, the extent of degradation increasing with increasing severity of extrusion conditions (Guha and Ali, 2002).

Extrusion of starchy food results in gelatinization, partial or complete destruction of the crystalline structure and molecular fragmentation of starch polymers and formation of complexes between starch and lipids (Colonna and Mercier, 1983). Amylose-lipid complex formation can also reduce starch digestibility. Monoglycerides and free fatty acid are more likely to form complexes than are triglycerides when added to high-amylose starch (Bhatnagar and Hanna, 1994). Steric acid mixed with normal corn starch with 25% amylase and extruded at 19% feed moisture and 110-140°C barrel temperature contained the most starch-lipid complex (Bhatnagar and Hanna, 1994). Conditions of high viscosity and longer residence time favored complex formation in small extruders.

b) Proteins and amino acids

Extrusion improves protein digestibility via denaturation, which exposes enzyme-access sites. Most proteins such as enzymes and enzyme inhibitors lose activity due to denaturation. The extent of denaturation is typically assessed as change in protein solubility in water or aqueous solutions. These changes are more pronounced under high shear extrusion conditions (Della *et al*., 1994), although mass temperature and moisture are also important influences. For example, wheat protein solubility is reduced even at the relatively low process temperatures used in pasta making (Ummadi *et al*., 1995). The chemical reaction between a reducing sugar and a free amino group on an amino acid, usually the epsilon-amino group of lysine, has important nutritional and functional consequences. This reaction, known as non-enzymatic browning, is actually a series of reactions providing a wide variety of compounds as a result (Feather, 1985). Furthermore, lysine loss has been related to extrusion process parameters such as raw material, feed moisture, screw speed, extrusion temperature, die diameter, feed rate, screw compression ratio, torque and pressure, energy input and pH (Asp and Bjorck, 1989; Camire *et al*., 1990). Proteins processed under conditions of alkaline pH and heat may develop amino acid residues that are not found in nature. Alanine is first converted to dehydroalanine, which may then react with lysine to form lysinoalanine, with cysteine to form lanthionine, or with ornithine to form ornithoalanine (Cheftel *et al*., 1985). These compounds are not well utilized, thus protein nutritive quality is reduced. Since these cross-links may develop between peptides or within a peptide, the resulting change in conformation may also affect the physical characteristics of the food containing them.

c) Lipids

Most extruded cereal foods contain less than 6-7 % lipids immediately after extrusion, because high lipid levels prevent expansion. In contrast, small lipid levels (~5%) facilitate steady extrusion and improve the texture. Expanded extrudates can thus be considered as low calorie foods. The nutritional value of lipids could be affected during extrusion as a result of isomerization or polymerization,

oxidation, hydrogenation,. According to Maga (1978), the extent of hydrogenation and cis to trans isomerization of fatty acids that takes place during extrusion is too small to be nutritionally significant. The inactivation of hydrolytic enzymes is possible with extrusion processing. A high temperature in extruder reduced the lipase activity and moisture level of extrudates. Thus this condition could decrease the factors favouring free fatty acid development and oxidation of fatty acids (Rao and Artz, 1989; Guzman *et al*., 1992). High moisture levels and enzyme activity rapidly deteriorate the food quality of rice bran, and even mild extrusion conditions had favorable effects on the stability of rice bran stored for 6 weeks (Sayre and Nayyar, 1985). However, the expanded, porous nature of extrudates causes them to be susceptible to the development of oxidation during storage. Screw wear is a concern as metals can act as pro-oxidants (Singh *et al*., 2006).

d) Dietary fiber

Cereals are an important source of dietary fiber. Modifications in particle size, solubility and chemical structure of the various fiber components could occur and cause changes in bacterial degradation in the intestine and in physiological properties. According to the solubility in water, total dietary fiber (TDF) can be categorized into two groups, namely soluble (SDF) and insoluble (IDF) dietary fiber. IDF components have nutritional value as bulking agents while SDF is more chemically reactive and may bind cholesterol in the intestine (Dreher, 1987). SDF represents a good substrate for some lactic bacteria and Bifidobacteria strains, which fare beneficial for gut health (prebiotic action) (Grizard and Barthomeuf, 1999), it is able to control glycemic index (Tudorica *et al*., 2002), and it reduces plasmatic cholesterol (Brown *et al.,* 1999). The thermomechanical nature of extrusion cooking has the added potential of causing a redistribution of soluble and insoluble components of fiber in favor of the former. This would tend to improve the hypocholesterolemic properties of fiber, and therefore enhance or improve the dietary fiber profile (Ohtsubo *et al*., 2004).

Effect of extrusion cooking on TDF, SDF and IDF have been reported. Esposito *et al.* (2005) studied the dietary fiber in durum wheat bran by-product extrudates. The SDF content of the durum wheat by-product ranged between 0.9% and 4.1%; while that of IDF was 21% and 64%. The water holding capacity is strictly related to the amount of IDF and to the granulometry of the by-products. Cooking-extrusion process does not affect the amount of SDF; by contrast, a significant increase of the IDF was detected. Extrusion cooking increased the TDF of barley flours. The TDF increase in waxy barley was the result of an increase in SDF (Vasantha *et al*., 2002). The change in dietary fiber profile during extrusion of barley flour may be attributed, primarily, to a shift from IDF to SDF, and the formation of resistant starch and enzyme-resistant indigestible glucans formed by transglycosidation. In contrast with results were reported by Onyango *et al*. (2004) who studied the application of a single-screw laboratory extruder to produce ready-to-eat from maize-finger millet blend. They reported that extrusion reduce TDF by 39-68 %, redistributed soluble to IDF ratios and had a negligible effect on the formation of resistant starch (less than 1 g/100g). Gajula *et al.* (2008) also observed that precooking bran-enriched wheat flour by extrusion significantly increased SDF in flours (by 22% to 73%); although in most cases it also led to a significant decrease in TDF. Larrea *et al.* (2005) modified the properties of fiber components in orange pulps using extrusion technology. They reported that the TDF of orange pulp extrudates was decreased after extrusion. The extrusion process decreased 39.06% IDF and SDF was increased by 80%. Ruiz-Ruiz *et al*. (2008) observed that extrusion conditions decreased dietary fiber content by 38% at 155 °C and 44 % at 170 °C. TDF and IDF decrease in the extrudates, but SDF increased. Increasing SDF can be caused by release of the soluble fraction from hemicelluloses as a result of heating.

1.2.2 Micronutrients (vitamins and minerals)

As vitamins differ greatly in chemical structure and composition, their stability during extrusion is also variable. The extent of degradation depends on various parameters during food processing and storage, e.g. moisture, temperature, light, oxygen, time and pH. Several summaries of the impact of food processing on

micronutrients have been published. Suknark *et al*. (2001) reported the reduction of tocopherols and retinyl palmitate in snack extrudates. Anderson and Sunderland (2002) reported that, α-tocopheryl acetate, is the form of vitamin E commonly added to fish feed, and ascorbyl-2-monophosphate (vitamin C) were loss during extrusion with discharge moisture and dryer processing temperature. In general, lower dryer processing temperatures improved vitamin and carotenoid retention in extruded fish diets. Chaovanalikit *et al*. (2003) reported that the extrusion (225 rpm screw speed, 4 kg h^{-1} feed moisture) decreased anthocyanins in blueberry cereals; ascorbic acid was retained better in cereals containing blueberry concentrate than in the sweetened corn cereals.

Cereal and cereal based products are also rich source of vitamins (Tiwari and Cummins, 2009). Like other bioactive compounds extrusion cooking have profound effect on the stability of vitamins in extruded snack food, for example higher barrel temperatures and low feed moistures favour ascorbic acid degradation during extrusion (Killeit, 1994). Stability of vitamins during extrusion is extensively reviewed (Camire *et al.,* 1990; Killeit, 1994; Riaz *et al*., 2009). Athar *et al*. (2006) studied the effect of extrusion processing conditions on the stability of vitamins. They observed that extrudates obtained from short barrel (90 mm) extruders had a higher retention rate of B vitamin (44-62%) compared to 20% for long barrel extruders. Anuonye *et al*. (2010) studied the stability of vitamins during extrusion of Acha (digitaria exilis)/soy bean blend and observed a 6% decrease in riboflavin (B2), a 86.36% decrease in pyridoxine (B), and no significant change in ascorbic acid content. In summary, the retention of vitamins in extrusion cooking decreases with increasing temperature, screw speed and specific energy input. It also decreases with decreasing moisture, feed rate and die diameter. Depending on the vitamin concerned, considerable degradation can occur, especially in products with high sensory application (Singh *et al*., 2007).

Minerals are heat stable and unlikely to become lost in the steam distillate at the die. Extrusion can improve the absorption of minerals by reducing other factors that inhibit absorption. Phytate may form insoluble complexes

with minerals and eventually affect mineral absorption adversely (Alonso *et al*., 2001). Extrusion hydrolyses phytate to release phosphate molecules. Extrusion of peas and kidney beans resulted in phytate hydrolysis, which explains the higher availability of minerals after processing (high temperature extrusion) (Alonso *et al*., 2001). Extrusion does not significantly affect mineral composition of pea and kidney bean seeds, except for iron. Iron content of the flours is increased after processing and it is most likely to the result of the wear of metallic pieces, mainly screws, of the extruder (Alonso *et al*., 2001). The incorporation of wheat bran in broken rice flour in extrusion (300 rpm of screw speed, 27 kg h^{-1} of feed rate, 5/32 inches die size, 93-97 °C outlet temperature) increases the content of calcium, phosphorus, iron and copper, which might be attributed to the addition of these minerals through water used during extrusion and also from the extruder barrel (Singh *et al*., 2000).

1.2.3 Antioxidant activity of some plant extrudates

Antioxidant activity of extruded products is dependent not only on the level of bioactive compounds but also on the composition of bioactive compounds. Extrusion conditions have the ability to produce both positive and negative influences on the bioactive compounds of the extrudates. Several studies have shown that extrusion processing significantly reduces measureable bioactive compounds in food products. Korus *et al*. (2007) investigated the effect of extrusion on polyphenol content and antioxidant activity of common bean. They observed a significant decrease in polyphenol content and antioxidant activity. Similarly, Delgado-Licon *et al.* (2009) observed a significant decrease in the total polyphenols and antioxidant activity during extrusion of bean/corn mixture. They observed that the decrease in bioactive compounds was dependent on process condition.

Shih *et al.* (2009) observed a significant decrease in β-carotene and anthocyanin for both yellow and orange sweet potatoes after extrusion. Repo-Carrasco-Valencia *et al*., 2009 showed a decrease up to 80.3% in the level of total phenolic acid after extrusion of kiwicha (*Amaranthus caudatus*). This decrease may be due to decarboxylation of phenolic acids during extrusion. Yagci and Gogus

(2010) also observed that both feed moisture content and barrel temperature caused a significant decrease in total phenolic content. Phenolic compounds during extrusion may undergo decarboxylation due to high barrel temperature may promote polymerisation of phenols and tannins leading to reduced extractability and antioxidant activity (Repo-Carrasco-Valencia *et al*., 2009; Dlamini, *et al*., 2007). However, in some cases the level of bioactive compounds in extruded products may increase in extrudates for example ferulic acid content was reported to increase by three times in extruded cereal grains (Zielinski *et al*., 2001). A similar increase in total phenolic compounds was reported after extrusion cooking (115 °C of barrel temperature, 120 rpm of screw speed, and 5.53 kg/h of feed moisture) of sweet potato (Shih *et al*., 2009) and cereals in combination with vegetables (Stojceska *et al*., 2008). The increase in the levels of phenolic acids in extruded products is generally due to the release from the cell wall matrix. Table 1 lists some examples showing the effect of extrusion on bioactive compounds. Yagci and Gogus (2009) reported increasing partially defatted hazelnut flour and fruit waste content caused increase in total phenolic content and antioxidant activity of the extruded samples. However, both feed moisture content and barrel temperature caused significant decrease in total phenolic content. Phenolic compounds during extrusion may undergo decarboxylation due to high barrel temperature and high moisture content may promote polymerisation of phenols and tannins leading to reduced extractability and antioxidant activity.

White *et al.* (2010) observed an increase in oxygen radical absorbance capacity (ORAC) values (16-30%) with an increase in barrel temperature. The increase in ORAC values might be due to the products formed during Maillard reaction. Yilmaz and Toledo (2005) showed that Maillard reaction products obtained from heated histidine and glucose had peroxyl radical scavenging activity and it relates strongly with ORAC assay. Hence, it is always advisable to obtain antioxidant activity by at least two procedures (e.g., Trolox equivalent antioxidant capacity, and ferric reducing antioxidant power) and level of polyphenols should have positive correlation with the antioxidant assay values. Khanal *et al*. (2009) revealed that extrusion of blueberry pomace with decorticated white sorghum flour at a ratio of 30 : 70 and 45% moisture content increased procyanidin monomer and dimers contents at

Table 1 Effect of extrusion process on bioactive compounds in some extrudates.

Raw materials	Extrusion condition*	Bioactive compounds	Reference
Wheat flour (8 -20%), defatted hazelnut flour (5-15%), fruit waste blend (3-7%) and rice grits	FM: 12-18% T: 150-175 °C SS: 200-280 rpm	Phenolic compounds decreased with an increased in barrel temperature and screw speed.	Yagci and Gogus (2009)
Blueberry pomace (30%) and white sorghum flour (70%)	FM: 45% T: 160, 170 and 180 °C SS: 100, 150 and 200 rpm	Temperature and screw speed had no significant effect on total anthocyanin	Khanal *et al*. (2009)
Cranberry pomace and corn starch (30:70, 40:60,50:50)	T: 150, 170 and 190°C SS: 150 and 200 rpm	Flavonols increased with an increase in barrel temperature. Total anthocyanin decreased with an increased in barrel temperature	White *et al*. (2010)
Corn, rice or wheat (80%) and tomato paste (20%) and skin powder (20%)	T: 140, 160 and 180°C SS: 350 rpm	Temperature and screw speed had no significant effect on lycopene content	Dehghan-Shoar *et al*. (2010)

* FM = feed moisture content, T = temperature, SS = screw speed

both temperature (160 and 180 ° C) and screw speeds (150 and 200 rpm). Unextruded pomace had the highest total anthocyanin content, which was significantly higher than the samples extruded across all conditions. However, temperature, screw speed, or their interaction had no significant effect on total anthocyanin content. Dehghan-

Shoar *et al*. (2010) studied the crisp extruded snacks made from corn, wheat and rice, with or without dried tomato skin or paste powder extruded at temperatures of 140, 160 or 180 °C. The result showed that increases in the processing temperature from 140 to 180 ° C had no significant effect on lycopene retention.

2. Germinated brown rice

Brown rice is unpolished whole grain rice that is produced by removing only the hull or husk using a motor and pestle or rubber rolls. The health benefits of brown rice are immeasurable. Brown rice is a whole grain meaning it contains a large amount of fiber. This is due to the fact that the whole grain contains all three components: bran, germ and endosperm (Figure 1). It contains more nutritional components, such as dietary fibers, E and B vitamins and gamma-aminobutyric acid (GABA), than the ordinary milled rice grains. These bio-functional components exist mainly in the germ and bran layers which are removed by polishing or milling (Standard tables of food composition in Japan, 2000; Champagne *et al*., 2004).

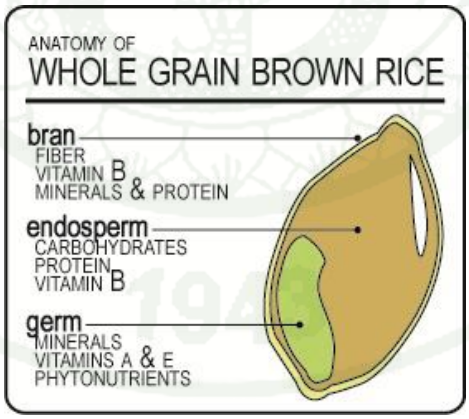

Figure 1 Anatomy of whole grain brown rice

Source: Lundberg Family Farms (2013)

It has been also reported that brown rice contains high phytic acid (antioxidant, anti-cancer); it decreases serum cholesterol (prevent cardio-vascular

diseases); and it is considered a low glycemic index food (low starch, high complex carbohydrates which decreases risk to type 2 diabetes) (Dinesh *et al*., 2009).

Germinated brown rice (GBR) is also call as "sprout brown rice" that considered as innovative rice by preserving all the nutrients in the rice grain for consumption (Patil and Khan, 2011). GBR is different from normal brown rice in that it has undergone the process of germination; more specifically, the rice embryo is sprouted under suitable environmental conditions. Generally, brown rice can be germinated by soaking it in warm water of 35–40 °C for about 10–12 h, draining water and keeping in moist condition for 20–24 h, and during soaking period, changing the water every 3–4 h to prevent fermentation (which usually produces undesirable odour) and to maintain consistent water temperature. The result yields a 0.5–1 mm long sprout from the brown rice grain; at this stage nutrient accumulation in the grain is maximum (Patil and Khan, 2011). During germination, hydrolytic enzymes are activated and decompose starch, non-starch, fibers and proteins, resulting in generating physiologically active intermediates (Komatsuzaki *et al*., 2007). Moreover, the germination improves the sensory quality of brown rice because the enzymatic hydrolysis of the polymeric materials softens the rice kernels and often improves its flavor (Hunt *et al*., 2002; Tian *et al*., 2004).

2.1 Health benefits of germinated brown rice

Kayahara *et al*. (2001) showed that not only existing nutrients are increased but new components are also released from the inner change due to germination. The nutrients which have increased significantly include gamma - aminobutyric acid (GABA), lysine, vitamin E, dietary fiber, niacin, magnesium, vitamin B1, and vitamin B6. The other nutrients that increased in GBR were inositols, ferulic acid, phytic acid, tocotrienols, potassium, zinc, prolylendopeptidase inhibitor and oryzanol (Kayahara and Tsukahara 2000). In particular, the amount of GABA in GBR was noticed to be ten times more as compared to milled white rice and two times more than that of brown rice. Further, they found that GBR contains less calories and sugar than that in milled rice. Nutrition of germinated grains has been

studied since decades ago. Kayahara and Tsukahara (2000) concluded that continuous intake of GBRwas good for preventing headache, relieving constipation, preventing cancer of colon, regulating blood sugar level and preventing heart disease. Saikusa *et al*. (1994) found that GABA increased dramatically if brown rice was soaked in 40 ℃ water for 8–24 h. Okada *et al*. (2000) reported that intake of GABA suppressed blood pressure and improved sleeplessness, and autonomic disorder observed during the menopausal or presenile period. Mitaka (2000) gave a method to produce GBR, which was easier to absorb and digest than regular brown rice. GBR helps in preventing Alzheimer's disease, due to its increased GABA content. Go Grains E-News (2004) reported that GBR significantly improved levels of spatial learning in mice. Kayahara and

Tsukahara (2000) showed that the brown rice sprouts contained a potent inhibitor of an enzyme called protylendopetidase, which was implicated in Alzheimer's disease. Ito *et al.* (2005) reported that intake of GBR instead of white rice was effective for the control of postprandial blood glucose concentration without increasing the insulin secretion in subjects with hyperglycemia. Hiroshi (2005) showed that besides containing other useful components, GBR mainly contained two active components viz. GABA, a neurotransmitter which was abounding in brain and spinal cord, and dietary fiber which activates the peristalsis of intestine. It also contains considerable phytic acid with a powerful anticancer activity and a prolyl endopeptidase activity inhibitor related to the metabolism of peptide. Chikako *et al*. (2005) reported that the protease activity in GBR was increased 1.5 times after germination. They suggested that decrease in soluble proteins and allergens was induced in part by proteolytic degradation and two abundant allergens were degraded in a different manner and probably by different protease in the grains during gerrmination. Varanyanond *et al*. (2005) investigated that water soaking of rice grains could enrich GABA content which increased as the soaking time was prolonged.

2.2 Utilization of germinated brown rice and marketing of GABA snack

2.2.1 Utilization of germinated brown rice

GBR has been suggested to be utilized as a healthy ingredient in a variety of foods. In Japan, people have been eating soaked brown rice (Kayahara and Takamura 2003). Various GABA enriched rice products were available in the market even in Seven Eleven such as GABA enriched mochi, cracker, miso, and powder for drink (Zhang *et al.*, 2006). In Taiwan, GABA tea was produced on a commercial basis for people with hypertension (Wang *et al.*, 2006) and many products were produced from GABA enriched rice. However, there have a few researches studied on germinated brown rice products such as, bread (Watanabe *et al*., 2004; Morita, 2004; Charoenthaikij *et al*., 2010) and noodle (Chung *et al*., 2012).

The composite wheat-rice flour blends have been used for bread making. Some researchers indicated that substitution of wheat flour with rice flour up to 30% (Watanabe *et al*., 2004; Nakamura *et al*., 2009) in bread was acceptable without compromising the sensory quality. Additionally, flours from germinated cereals were reported to retard staling of bakery products, especially bread (Watanabe *et al*., 2004). Morita (2004) developed a technology to bake bread with GBR and still have a light loaf. To label bread as having GBR, it must have at least 30% GBR, but that much rice interferes with the gluten and carbon dioxide escapes, causing bread to shrink (Watanabe *et al*., 2004). Charoenthaikij *et al*. (2010) developed breads containing 30% germinated brown rice flour with lower specific volume and greater hardness than the wheat control bread. However, these breads were acceptable to consumers. GBR was tested as a partial replacement of wheat flour in noodle (Chung *et al*., 2012). The noodles were prepared from the mixture in which wheat flour was substituted by GBR flour (30-70 g/100 g, solids basis). Substitution of to wheat flour dramatically degrades the cooking and textural quality of cooked noodles, resulting in increased softness.

2.2.2 The marketing of GABA snack in Thailand

According to Mintel's Global New Products Database (GNPD) (2013) database, there are 40 products of GABA snack in the market around the world espeacialy in Asia pacific. Data from GNPD in 2013 showed that Thailand had launched the highest number of GABA snack products in the world (20 products) followed by Japan (15 products), Taiwan (2 products), Hong Kong (2 products) and China (1 products), respectively. There are 4 sub category of GABA snack in Thailand market such as cold cereals (10 products), hot cereals (6 products), rice snacks (3 products) and snack/energy bar (1 products). The Brand of new GABA snack products in Thailand are such as instant embryo rice with multi grain cereal (brand: Richie Healthy Buddy), organic sprouted muesli (brand: Xongdur Fruity) and Kim Chi flavoured rice snack (Brand CAL Nutri Crisp) (Appendix figure A1). There are 5 companies produced GABA snack products such as Thailand Nutrition groups (6 products), Bann Thanyatip (5 products), Xongdur Thai Organic Food (5 products), CAL Intertrade (3 products) and Richie Confectionary (1 products). The popular flavor of GABA snack was unflavour/plain (4 products) followed by chocolate (3 products), Seaweed (Nori) (1 products), Kimchi (1 products) and so forth. Top three lists of GABA snack products claim about Halal food (45%), no allergen (35%) and whole grains (35%). However, these products did not show GABA content on the nutrional facts. This information revealed that GABA snack in the world are still lacking and Thailand is the big market in Asia pacific. The researchers or companies can categorize their new product ideas and find the gap in the market from these databases. This is the way that can be added value to Thai agricultural products and create the new product innovation.

3. Gamma-amino butyric acid

Gamma-aminobutyric acid (GABA, NH_2-$(CH_2)_3$-COOH), a four-carbon non-protein amino acid found in virtually all prokaryotic and eukaryotic organisms as a significant component of the free amino acid pool. GABA is produced primarily by the enzyme glutamate decarboxylase (GAD) which catalyses the irreversible

decarboxylation of L-glutamate to GABA. (Zhang *et al.*, 2006). GABA acts as the chief inhibitory neurotransmitter in the mammalian central nervous system. In humans, GABA is also directly responsible for the regulation of muscle tone. In insect species, GABA acts only on excitatory nerve receptors. GABA has various physiological functions in animals and humans. For example, GABA improve the sleeplessness and inhibitory neurotransmitter in brain and spinal cord: sedative effect. In addition, GABA is known to be involved in the blood pressure lowering effect and tranquilizing affect on elderly people especially women suffering from a menopausal disorder (Okada *et al.*, 2000). GABA also stimulates the anterior pituitary, leading to higher levels of human growth hormone (HGH). HGH contributes significantly to muscle growth and also prevents the creation of fat cells. Moreover, HGH depletion is prevalent in adults over the age of 40 which may be responsible for sleep disturbances or interrupted sleeping patterns (Koula *et al*., 1980).

3.1 Source of GABA

GABA is available in nature such as a neurotransmitter in brain and spinal cord of mammal. GABA is present in vegetables (e.g., spinach, potato and broccoli), fruits (e.g., apple), cereals (e.g., rice, barley and maize) (Oh *et al*., 2003) and plants (e.g., tea leaves and radish leaves) (Jeng *et al*., 2007). The concentrations of GABA are generally low in nature. For example, Maisont and Narkrugsa (2010) revealed that the GABA content in plant tissue is quite low (ranging from 0.03 to 2.00 mmol fresh weight). Tomato had 0.03-2.00 μmol/g of GABA content observed by Fougere *et al.* (1991) whereas GABA content of tea leaves was 1270 μg/g (Jeng *et al*., 2007). For rice, Charoenthaikij *et al.* (2007) reported that Khao Dawk Mali 105 brown rice had 4.80 mg/100g of GABA content while the Red Jasmine brown rice had 6.05 mg/100 g (Wichamanee and Teerarat, 2012).

3.2 GABA synthesis

GABA synthesis from glutamate is controlled by glutamate decarboxylase (GAD), a Ca^{2+}/calmodulin-regulated enzyme in plants (Zik *et al.*,

1998). GABA is catabolized in mitochondria through the GABA shunt, a metabolic pathway that bypasses two successive steps of the tricarboxylic acid (TCA) cycle catalyzed by α-ketoglutarate dehydrogenase and succinyl-CoA synthetase (Figure 2).

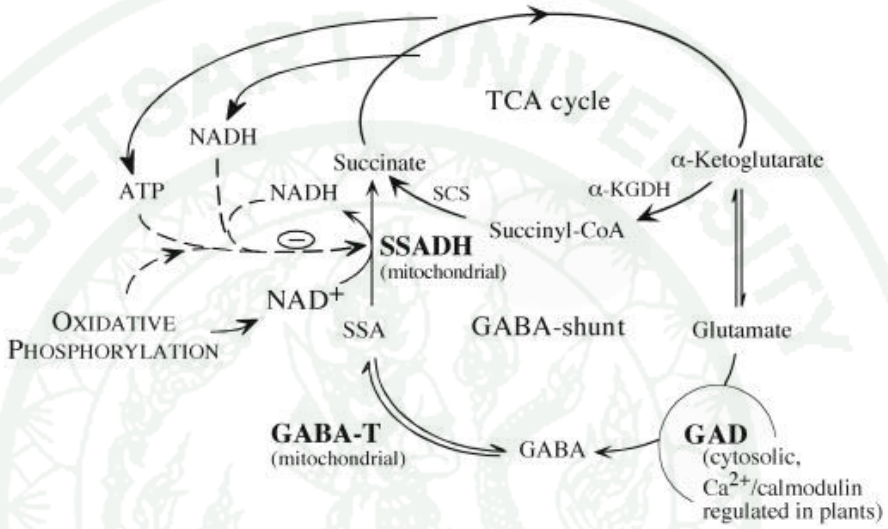

Figure 2 Schematic presentation of the GABA shunt metabolic pathway. The GABA shunt is composed of three enzymes (Depicted in boldface type): glutamate decarboxylase (GAD; EC 4.1.1.15), GABA transaminase (GABA-T; EC 2.6.1.19), and succinic-semialdehyde dehydrogenase (SSADH; EC1.2.1.16) TCA cycle, tricarboxylic acid cycle; SSA, succinic semialdehyde; SCS, succinyl- CoA synthetase; α-KGDH, α- ketoglutarate dehydrogenase; dashed lines, effectors; solid lines, substrates and products.

Source: Busch (2000)

The enzymes involved in GABA catabolism are GABA transaminase, which converts GABA to succinic semialdehyde, and succinic-semialdehyde dehydrogenase (SSADH), whichoxidizes succinic semialdehyde to succinate coupled with NADH production (Bouche *et al.*, 2003). Hence, GABA is a metabolite en route from glutamate to the TCA cycle, which provides succinate and NADH to the respiratory machinery. Two regulatory check points of the GABA shunt have been described in plants (Figure 2): positive regulation of GAD by Ca^{2+}/calmodulin in the cytosol and

negative regulation of SSADH by ATP and NADH in the mitochondrion (Busch, 2000). The former is considered to be a mechanism involved in the activation of the enzyme in response to stress, whereas the latter is thought to control the GABA shunt by mitochondrial energy charge and reducing potential.

3.3 Factors affecting GABA content in germinated brown rice

GABA levels in GBR are influenced by many factors, such as anoxia, cytosolic acidification, cold shock, mechanical stimulation, water stress and plant development (Bown and Shelp, 1997). Previous studies reported that increase in the GABA content in brown rice was correlated with the rice varieties (Charoenthaikij *et al.,* 2009; Karladee and Suriyong, 2012; Varanyanond *et al*., 2005), pH of soaking water (Banchune *et al*., 2010; Watchraparpaiboon *et al*., 2007; Charoenthaikij *et al.,* 2007), soaking time and temperature (Wichamanee and Teerarat, 2012; Chung *et al*., 2009; Watchraparpaiboon *et al*., 2010), substrate and enzyme addition (Bai *et al.,* 2009; Ohtsubo *et al.,* 2002; Zhang *et al.,* 2006). Therefore, many reports have attempted to increase the GABA content in germinated brown rice by various factors as described below.

3.3.1 Rice varieties

The different amounts of GABA content found among the rice varieties are mainly caused by their genetic constitution. Many germinated Thai rice varieties were selected to study GABA accumulation especially Khao Dawk Mali 105 variety. This is due to the fact that Khao Dawk Mali 105 variety is popular crop and consumed in Thailand which has the ability to synthesize GABA (Varanyanond *et al*., 2005). Charoenthaikij *et al.* (2009) reported that Khao Dawk Mali 105 brown rice had free GABA content of 2.11 mg/100g and 2.41 mg/100g (dry weight basis) in RD 6 glutinous brown rice. After germination process for 48 h, the result showed that germinated brown rice flour from Khao Dawk Mali 105 variety had free GABA content higher than that of RD6 variety. Although, Kaosa-ard and Songsermpong (2012) revealed that the GABA content of the fresh brown rice Khao Dawk Mali 105

(5.56 mg/100 g, db) was lower than Chainat 1 variety (7.22 mg/100 g, db). The levels of GABA content of germinated Khao Dawk Mali 105 and Chainat 1 obtained from soaking brown rice grains with tap water at 25 ^{o}C for 72 h were 73.05 and 92.42 mg/100 g, db, respectively. Moreover, Karladee and Suriyong (2012) studied GABA content compared between white rice and color rice varieties. Their result showed that GABA content of brown rice from white rice variety Khao Dawk Mali 105 and purple rice variety Kum Doi Saket were in the same range (4.04 mg/100g dry matter of Khao Dawk Mali 105 and 4.67 mg/100g dry matter of Kum Doi Saket). The GABA content of germinated brown rice grains soaked in water for 24 h were in the same range (23.48 mg/100g dry matter of Khao Dawk Mali 105 and 23.63 mg/100g dry matter of Kum Doi Saket). GABA content of germinated brown rice were complicated because of various factors including rice variety, rice quality and environment factors. Therefore, different rice varieties show opportunities in breeding for GABA accumulation that could be used as ingredients for introducing the new GABA food products.

3.3.2 pH of soaking water

Normally, germinated brown rice prepared from soaking brown rice grains in water (pH ~6.8) about one to two days. The increase in GABA content during water soaking is due to the activation of enzyme glutamate decarboxylase (GAD), which converts glutamate to GABA (Komatsuzaki *et al*., 2007). However, it is noticeable that GABA content in germinated brown rice increased when the rice was soaked in acid solution. This was probably due to the GABA synthesis in response to H+ is a pH-regulating mechcnism (Bown and Shelp, 1997). GAD was activated by the increase in the cystosolic levels of hydrogen ions (acidic solution) (Scott-Taggart *et al*., 1999) resulting in GABA accumulation. The pH optimum of the enzyme GAD was about 5.8 (Shelp *et al*., 1999).

Recently, many researchers produced germinated brown rice with high GABA by controlled pH of soaking water. For example, Choi *et al.* (2006) reported that soaking giant embryo brown rice in distill water (pH 6.8) for 4 h and

germinated in an incubator at 28-30^{o}C for 24 h, affected in GABA content. The result showed that GABA contents were increased clearly higher in germinated giant embryo brown rice (35.86 mg/100 g) compared to non-GBR (1.67 mg/100 g). The increased amount of these nutrients relative to those in the non-GBR was 7.97 times for GABA. Similar result was observed by Watchraparpaiboon *et al*. (2007) who reported that Khao Dawk Mali 105 brown rice soaked in water at pH 6 had the highest GABA content (16.50 mg/100g).

Charoenthaikij *et al*. (2007) found that soaking brown rice for 48 h at different pH solutions affected in GABA concentration. Decreasing the pH of steeping water (in the range of pH 3 to 7) caused an increase in free GABA content. The GABA content at pH 3, 5 and 7 was 67, 21.14 and 12.28 mg/100g, respectively. This similar result was found in brown rice variety Sangyod Muang Phatthalung Rice (SMPR) (Banchuen *et al.*, 2009). These studies indicated that GABA content of germinated brown rice from KDML 105 and SMPR had the highest amount when soaking at pH 3 for 48 h and pH 3 for 36 h, respectively compared with soaking at pHs 5, 6.8 and 7.

3.3.3 Soaking time and soaking temperature

As grains are being soaked at room temperature, the imbibition begins and respiration is accelerated. This further stimulates the metabolism of amino acids, resulting in the formation of enzyme systems. Amino acid such as GABA is also synthesized, and it can be detected at 12 h of soaking time (Karladee and Suriyong, 2012). Therefore, soaking time affected the GABA content in germinated brown rice. Many researches reveled that longer soaking time contributed to the higher GABA content. This agree with the study of Charoenthaikij *et al*. (2007) who reported that as the soaking time increased from 24 to 72 h, GABA increased. Similar result was observed from Kaosa-ard and Songsermpong (2012) who revealed that as the soaking time increased from 12 to 72 h, the GABA content increased. After soaking for 72 h, the GABA content of the germinated brown rice was about 13 times higher than brown rice, which was similar to Ohtsubo *et al*. (2005) who studied

germinated brown rice from *Koshihikari* varieties and found that GABA content increased during germination for 72 h. the GABA content of the germinated brown rice was 11.5 times higher than brown rice.

Soaking temperature is influenced GABA content in germinated brown rice. However, it depends on the other factors such as rice vatiety or types of cereal and soaking time. The optimal soaking temperature of rice grains was 35 ^{o}C (Watchararparpaiboon *et al*., 2010) because rice glutamate decarboxylase activity has an optimum temperature at 40 ^{o}C (Zhang *et al*., 2007), soaking at 35^{o}C temperature would enhance its activity and result in more GABA production. This supported by studied of Wichamanee and Teerarat (2012) who reported that GABA content in germinated Red Jasmine brown rice increased with higher temperatures and longer soaking time. The GABA content was the highest when soaking brown rice at 35^{o}C for 24 h. A similar result was observed by Watchararparpaiboon *et al*. (2010) who reported that GABA contents of brown rice (Khao Dawk Mali 105 and Chainat 1 variety) were the highest after soaking rice grains in water at temperature of 35^{o}C for 24 h. Chung *et al*. (2009) reported that low temperature (5^{o}C) was optimal temperature to produce GABA in barley grains, while Wichamanee *et al*. (2012) repoted that soaking at low temperature (5^{o}C) did not significantly increased GABA content in germinated Red Jasmine brown rice. This could be due to the difference in The **pH optimum** of the enzyme was about 8.0 grain types.

3.3.4 Substrate and enzyme addition

The content of produced GABA depends on various conditions such as the amount of precursor (i.e., substrate and enzyme). Bai *et al*. (2009) investigated the effects of glutamic acid (Glu), pyridoxal-5-phosphate (PLP) and calcium chloride ($CaCl_2$) in culture medium on glutamate decarboxylase (GAD) activity and GABA accumulation in foxtail millet (*Setaria italica* L.) during germination. Their results revealed that addition of Glu, PLP and $CaCl_2$ to culture medium influenced GAD activity and increased GABA content. These increases of GABA were probably caused by increases of the substrate in the vicinity of the

cytosolic GAD, which suggested that GAD activity and GABA content were regulated by glutamate addition.

Ohtsubo *et al*. (2002) has developed an enzymatic production method for GABA in rice germ by adding exogeneous glutamic acid. However, this method results in a large quantity of exogeneous glutamic acid remaining in the final products. It is not suitable because the use of a large amount of glutamic is expensive. Therefore, Zhang *et al.* (2006) attempted to establish a simple and effective technique for producing the high GABA content in rice germ without adding exogeneous glutamic acid. They studied the different protease enzyme such as trypsin, protamex, neutease and alcalase added in rice germ. The result demonstrated that the GABA yield in rice germ adding trypsin increased about 6 times compared with the simple water soaking method. Trypsin as a protease to hydrolyze the germ protein and produce glutamic acid for GABA accumulation.

4. Xanthones

Xanthones are unique chemical compounds found in nature, composed of a tricyclic aromatic system with a variety of phenolic, methoxy, and isoprene substituents, giving rise to several derivatives. Xanthones are the most characteristic secondary metabolite constituents of *G. mangostana*, and more than 80 compounds of this type have been isolated and characterized from the various parts of this plant (Chin *et al*., 2008). Several compounds in mangosteen appear to be active, particularly xanthones. Some of these xanthones include mangostin, mangostenol, mangostenone A, mangostenone B, trapezifolixanthone, tovophyllin B, α- and γ-mangostins, garcinone B, mangostinone, mangostanol, mangosharin, and the flavonoid epicatechin (Ray, 2009). The two most beneficial xanthones found in the mangosteen have been named α-mangostin and γ-mangostin. When isolated and throughtly tested by researchers, these two xanthones have been found to carry a host of benefits. Jung *et al*. (2006) reported the supportive of the use of the mangosteen pericarp as an antioxidant botanical dietary supplement. It is worth nothing that two of the active isolates obtained in the present investigation, α-mangostin and *γ*-mangostin,

were found to be major components of the CH_2C_{l2}-soluble extract of the pericarp of *G. mangostana*. Therefore, these two compounds may be used as marker components for quality control of this botanical dietary supplement.

4.1 Source of xanthone

Xanthones are natural constituents of plants in the families Bonnetiaceae and Clusiaceae and are found in some species in the family Podostemaceae. Many of these xanthones are found in the pericarp of the mangosteen fruit, which can be found in the region of Southeast Asia. In addition, Schieber *et al*. (2003) have demonstrated that mango peels of the cultivar Tommy Atkins contain a large number of flavonol *O*- and xanthone *C*-glycosides. Hwang *et al.* (2007) found prenylated xanthones from the root bark of *Cudrania tricuspidata*. The genus *Cudrania* belongs to the Moraceae family and is widely distributed in Korea, China, and Japan. Isoprenylated xanthones and flavonoids are the major compound classes in *C. tricuspidata*. Furthemore, xanthones are also found in higher plant families, fungi, and lichens (Cardona *et al*., 1990).

4.2 Types of xanthones

Xanthone is an organic compound with the molecular formula $C_{13}H_8O_2$. Xanthones or xanthen-9H-ones are secondary metabolites found in some higher plant families, fungi and lichens (Vieira and Kijjoa, 2005), and comprise an important class of oxygenated heterocycles. The xanthone nucleus is known as 9-xanthenone or dibenzo-c-pyrone and it is symmetric (Figure 3). Xanthones have been classified in five groups: (a) simple oxygenated xanthones, (b) xanthone glycosides, (c) prenylated xanthones, (d) xanthonolignoids and (e) miscellaneous xanthones (Jiang *et al.*, 2004). From 20 higher plant families (122 species in 44 genus), 19 fungi species and 3 lichens species, 278 new xanthones were identified between 2000 and 2004 (Vieira and Kijjoa, 2005). Currently, approximately 1000 different xanthones have been described (Souza and Pinto, 2005). The biological activities of this class of

compounds are associated with their tricyclic scaffold but vary depending on the nature and/or position of the different substituents (Souza and Pinto, 2005).

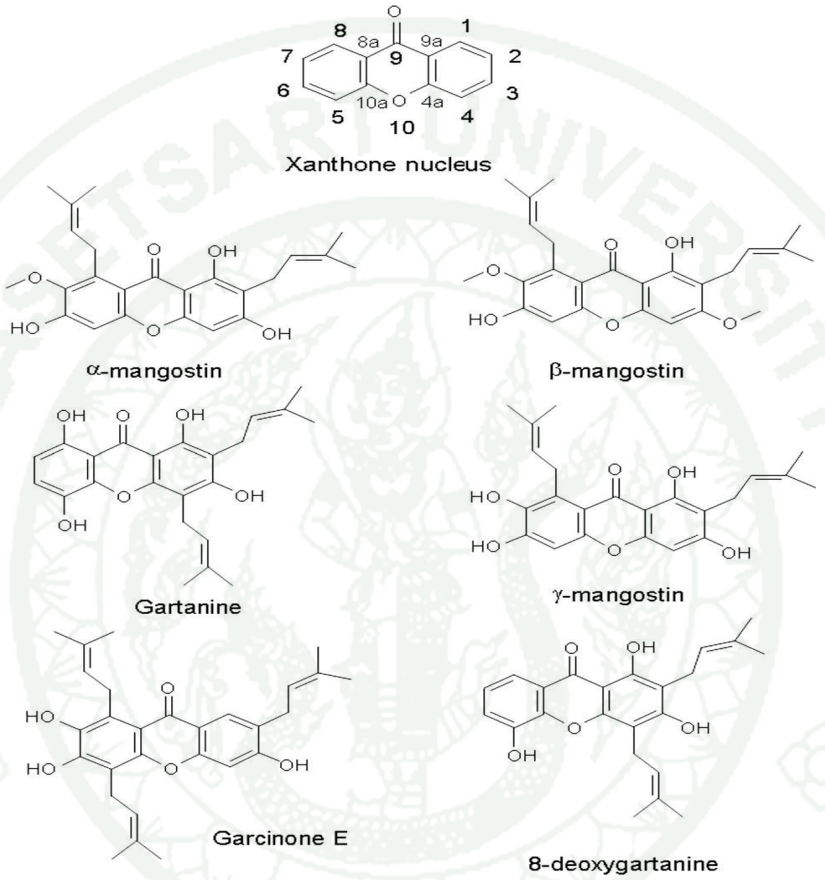

Figure 3 Xanthone nucleus with IUPAC numbers of carbons and chemical structure of the most studied xanthones.

Source: Vieira and Kijjoa (2005)

4.3 Health benefit of xanthones

Xanthones are well known for their numerous and varied pharmacological effects, including having antioxidant, antimicrobial, central nervous system (CNS) depressant or stimulant, antihypertensive, antidiabetic, anticancer, anti-inflammatory, hepatoprotective, and/or immunomodulation properties. Owing to their activity as potent antioxidants some mangosteen-based botanical products are

standardized to contain high levels of xanthones such as α- and γ-mangostin (Balunas *et al.*, 2008).

4.3.1 Antioxidant benefit

Xanthones and their derivatives have been reported to have high antioxidant activity (Jung *et al.*, 2006; Okonogi *et al.*, 2007; Tachakittirungrod *et al.*, 2007). An extract of *G. mangostana* was reported to have very good antioxidant action in the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay (Chomnawang *et al.*, 2007). Miwako *et al.* (2009) investigated the absorption and antioxidant effects of a xanthone-rich mangosteen liquid in healthy human volunteers after the acute consumption of 59 mL of the supplement. The liquid contained mangosteen, aloe vera, green tea, and multivitamins. Results indicated that α-mangostin and vitamins B(2) and B(5) were bioavailable. The antioxidant capacity measured with the oxygen radical absorbance capacity (ORAC) assay was increased with a maximum (18%) of human plasma antioxidant capacity after 2 h of oral admistration of a xanthone-rich mangosteen liquid, and the increased antioxidant level lasted at least 4 h. Overall, this study demonstrated the bioavailability of antioxidants from a xanthone-rich mangosteen product and its in vivo antioxidant effects.

4.3.2 Cancer protection

Several studies have been designed to examine the anticancer activities of xanthones isolated from mangosteen-fruit pericarp. Ho *et al.* (2002) found that garcinone E has a potent cytotoxic effect on hepatocellular carcinoma cell lines. They studied the cytotoxic effect of six xanthones isolated from mangosteen-fruit pericarp and found that garcinone E was the most toxic. Garcinone E exhibited a very broad spectrum of dose and time dependent cytotoxic effects against various cancer cell lines were killed. Moongkarndi *et al.* (2004) showed the extract obtained from *G. mangostana* against SKBR3 human breast adenocarcinoma cell line. The potent activity of the extract from *G. mangostana* with the half maximal inhibitory concentration (IC_{50}) value of 15.45 ± 0.5 lg/mL. Matsumoto *et al.* (2003) reported that

six xanthones (α-, β- and γ-mangostins, mangostinone, garcinone E and 2-isoprenyl-1,7-dihydroxy-3-methoxy xanthone) isolated from mangosteen fruit pericarp on the cell growth inhibited the human leukemia cell line HL60. The most abundant compound in the extract was α-mangostin, and it showed the highest inhibitory activity (IC50 =10 μM).

4.3.3 Antimicrobial properties

Selected xanthones as well as extracts of *G. mangostana* have relatively strong antifungal and antibacterial activities. The antifungal activity of naturally occurring xanthones from mangosteen fruit against three phytopathogenic fungi, *Fusarium oxysporum vasinfectum*, *Alternaria tenius* and *Dreschlera oryzae*, was tested and a correlation between their structures and biological effects was established (Gopalakrishnan *et al.*, 1997). It was suggested that phenolic hydroxyls in rings A and B as well as isoprenyl groups play important roles in the antifungal mechanism of action of xanthones as the difference in the presence and position of these functional groups influenced the inhibition. Among the compounds tested γ-mangostin was the most active against all the test fungi.

Sakagami *et al.* (2005) investigated the antibacterial activity of α-mangostin against vancomycin resistant *Enterococci* (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA). The α-mangostin was found to be active against five strains of VRE and nine strains of MRSA with minimal inhibitory concentration (MIC) values of 6.25 μg/mL and from 6.25 μg/mL to 12.5 μg/mL, respectively. Additionally, synergism between α-mangostin and commercially available antibiotics was investigated. It was suggested that α-mangostin alone or in combination with gentamicin against VRE, and incombination with vancomycin hydrochloride against MRSA, might be useful in controlling VRE and MRSA infections.

5. Antioxidants

The word “antioxidant” is increasingly popular in modern society as it gains publicity through mass media coverage of its health benefits. An antioxidant may be defined as any substance that when present at low concentrations, compared with those of the oxidizable substrate, significantly delays or inhibits oxidation of that substrate (Micheal *et al*., 2002). In food science, antioxidants have a broader scope, in that they include components that prevent fats in food from becoming rancid as well as dietary antioxidants. A substance in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiological function in humans. The autoxidation is caused primarily by radical chain reactions between oxygen and the substrates. Effective antioxidants are radical scavengers that break down radical chain reactions. Sterically hindered phenols and amines are often used as antioxidants in the rubber and plastic industries. Therefore, a dietary antioxidant can (sacrificeally) scavenge reactive oxygen/nitrogen species (ROS/RNS) to stop radical chain reactions, or it can inhibit the reactive oxidants from being formed in the first place (preventive) (Huang *et al*., 2005). Dietary antioxidants often broadly include radical chain reaction inhibitors, metal chelators, oxidative enzyme inhibitors, and antioxidant enzyme cofactors.

5.1 Antioxidant assays commonly used for food constituents

In general, the methods for determining the antioxidant capacity of food components can deactivate radicals by two major mechanisms and were divided into two major groups: assays based on the sigle electron transfer (SET) reaction and assays based on a hydrogen atom transfer (HAT). The end result is the same, regardless mechanism, but kinetics and potential for side reactions different. SET-based methods detect the ability of potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyls and radicals. SET displayed through a change in colour as the oxidant reduced (Huang *et al*., 2005). HAT-based methods measure the classical ability of antioxidant to quench free radicals by hydrogen donation HAT reactions are solvent and pH independent and usually quite rapid, typically completed in seconds minutes. The presence of reducing agents,

including metals, is a complication in HAT assays and can lead erroneously high apparent reactivity (Prior *et al.*, 2005). The HAT reaction-based methods, the antioxidant is able to quench free radicals by hydrogen donation. Methods based on the HAT reaction include the following methods (Huang *et al*., 2005): 1) Oxygen radical absorbance capacity (ORAC), 2) Total radical-trapping antioxidant parameter (TRAP), 3) Inhibition of induced LDL oxidation, 4) Total oxyradical scavenging capacity assay (TOSCA), 5) Crocin-bleaching assays and 6) Chemiluminescent assay

SET-based methods measure the ability of a potential antioxidant. These methods transfer one electron to reduce any compound, including metal ions, carbonyls and radicals (Wright *et al*., 2001). SET and HAT mechanisms almost always occur together in all samples, with the balance determined by antioxidant structure and pH. SET based methods detect the ability of an antioxidant to transfer one electron to reduce any compound, including metals, carbonyl groups and radicals. SET reactions are usually slow and can require long times to reach completion, so antioxidant capacity calculations are based on percent decrease in product rather than kinetics. SET methods are very sensitive to ascorbic acid and uric acid, which are important in maintaining plasma redox tone, and reducing polyphenols are also detected. Importantly, trace components and contaminants such as metals interfere with SET methods and can account for high variability and poor reproducibility and consistency of results (Ou *et al*., 2005). Relative reactivity of the SET method is based on deprotonation and in ionization potential of the reactive functional group (Wright *et al*., 2001; Lemanska *et al*., 2001; Prior *et al*., 2005). Therefore, SET methods are pH dependent. Generally, ionization potential decreases with increasing pH values, which reflects a higher electrondonating capacity with deprotonation. The reactions based on the electron transfer are usually slow, and calculations are based on product percentage decrease more than in kinetic terms (Prior *et al*., 2005). The SET-based methods include following assays: 1) Total phenolics assay by Folin-Ciocalteu reagent assay, 2) Trolox equivalence antioxidant capacity (TEAC) assay, 3) Ferric ion reducing antioxidant power (FRAP) assay, 4) Total antioxidant potential assay, using a Cu-complex as an oxidant, 5) 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) scavenging, 6) 2,2-Azinobis 3-ethylbenzthiazoline-6-sulphonic acid radical (ABTS)

scavenging assay, 7) N,N-dimethyl-p-phenylenediamine radical (DMPD) scavenging assay and 8) Cupric ions (Cu^{2+}) reducing antioxidant power (CUPRAC) assay. The advantage and disadvantage of some antioxidant methods are presented in Table 2.

Table 2 Advantage and disadvantage of some antioxidant methods.

Methods	Advantage	Disadvantage
1) Oxygen radical absorbance capacity (ORAC)	It implies equally well for both antioxidants that exhibit distinct lag phase and those that have no lag phases. ORAC assay has been broadly applied in academy and in the food and dietary supplement industries as a method of choice to quantify antioxidant capacity (Caldwell, 2001).	ORAC is limited to measurement of hydrophilic chain but ignores lipophilic antioxidants. It requires fluorometers, which may not be routinely available in analytical laboratories. Temperature control decreases reproducibility (Caldwell, 2001).
2) Ferric-reducing antioxidant power (FRAP) assay	It is simple, speedy, inexpensive, and robust does not required specialized equipment. It can be performed using automated, semiautomated, or manual methods (Benzie and Strain, 1999).	FRAP cannot detect species that act by radical quenching (H transfer), particularly SH group containing antioxidants like thiols, such as glutathione and proteins (Prior and Cao, 2000; Huang *et al*., 2005).
3) Cupric ions (Cu^{2+}) reducing antioxidant power (CUPRAC) assay	It requires sophisticated instrumentation. As a more convenient and less costly alternative. (Badarinath *et al*., 2010).	Sophisticated instruments are required which are more expensive (Badarinath *et al*., 2010).

Table 2 (Continued)

Methods	Advantage	Disadvantage
4) 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging	The DPPH assay is commonly used because it is technically simple and gives accurate and repeatable results. The assay is valid to quantify samples with hydrophilic or lipophilic antioxidants (Prior *et al*., 2005).	DPPH is a stable, long-lived nitrogen radical unlike radicals present in living organisms and has no similarity to the highly reactive and transient peroxyl radicals that are involved in lipid peroxidation. Thus, antioxidants that react quickly with peroxyl radicals may react slowly or may be inert to the DPPH radical (Prior *et al*., 2005).
5) 2,2-Azinobis 3-ethylbenzthiazoline-6-sulphonic acid radical (ABTS) scavenging assay	ABTS reacts rapidly with antioxidants, typically within 30 min. It can be used over a wide pH range. ABTS is soluble in both aqueous and organic solvents and is not affected by ionic strength, so can be used in multiple media to determine both hydrophilic and lipophilic compounds in test sample.(Badarinath *et al.*, 2010).	It is not the inhibition of the oxidative process but the capability of the sample to interact with ABTS. Moreover, depending on the sample used can take a long time and false values can be read before the reaction has ended (Badarinath *et al*., 2010).

5.2 Some antioxidant assays based on the sigle electron transfer methods

5.2.1 Total phenols assay by Folin-Ciocalteu Reagent (FCR)

The FCR-based assay gained popularity and is commonly known as the total phenols (or phenolic) assay. Numerous publications applied the total phenols assay by FCR and an SET-based antioxidant capacity assay and often found excellent linear correlations between the total phenolic profiles and the antioxidant activity. The FCR is typically made by first boiling (for 10 h) the mixture of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$, 100 g), sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$, 25 g), concentrated hydrochloric acid (100 mL), 85% phosphoric acid (50 mL), and water (700 mL). After boiling, lithium sulfate ($Li_2SO_4 \cdot 4H_2O$, 150 g) is added to the mixture to give an intense yellow solution the FC reagent. Contamination of reductants leads to a green color, and the addition of oxidants such as bromine can restore the desired yellow color. The exact chemical nature of the FC reagent is not known, but it is believed to contain heteropolyphosphotunstates-molybdates. Sequences of reversible one- or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})^{4-}$. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs on the reduction of Mo (VI) to Mo (V).

Obviously, the FC reagent is nonspecific to phenolic compounds as it can be reduced by many nonphenolic compounds (e.g., vitamin C, Cu (I), etc.). Phenolic compounds react with FCR only under basic conditions (adjusted by a sodium carbonate solution to pH ~10). Dissociation of a phenolic proton leads to a phenolate anion, which is capable of reducing FCR. This supports the notion that the reaction occurs through electrontransfer mechanism. The blue compounds formed between phenolate and FCR are independent of the structure of phenolic compounds, therefore ruling out the possibility of coordination complexes formed between the metal center and the phenolic compounds. Despite the undefined chemical nature of FCR, the total phenols assay by FCR is convenient, simple, and reproducible. As a result, a large body of data has been accumulated, and it has become a routine assay in studying phenolic antioxidants.

5.2.2 Ferric Ion Reducing Antioxidant Power (FRAP) Assay

The FRAP assay measures the ability of antioxidants to reduce the ferric 2,4,6-tripyridyl-s-triazine complex $[Fe^{3+}\text{-}TPTZ_2]^{3+}$ to the intensely blue coloured ferrous complex $[Fe^{3+}\text{-}TPTZ_2]^{2+}$ in acidic medium (Benzie and Strain, 1999). This method measures reducing power in plasma, but the assay subsequently has also been adapted and used for the assay of antioxidants in different food or beverages (Pellegrini *et al*. 2003), tea (Benzie and Szeto1999), juice (Gil 2000), spices (Gulcin *et al*., 2005) vegetables (Ou *et al*., 2002), fruits (Proteggente *et al*., 2002; Gulcin *et al*., 2011) and cereals (Sreeramulu *et al*., 2009). As seen in Figure 4, the reaction measures reductionof ferric 2,4,6-tripyridyl-s-triazine (TPTZ) to a coloured product (Benzie and Strain 1996).

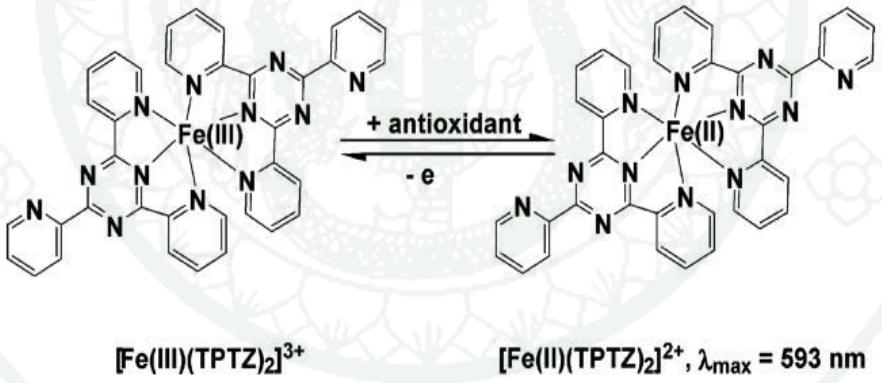

Figure 4 $[Fe^{3+}\text{-}TPTZ_2]^{3+}$ - $[Fe^{3+}\text{-}TPTZ_2]^{2+}$ reduction reaction of FRAP assay

Source: Gulcin (2012)

The FRAP assay is conducted at acidic pH 3.6 to maintain iron solubility. Reaction at low pH decreases the ionization potential that drives electron transfer and increases the redox potential, causing a shift in the dominant reaction mechanism. It has been argued that the ability to reduce iron has little relationship to the radical quenching processes (H transfer) mediated by most antioxidants. However, oxidation or reduction of radicals to ions still stops radical chains, and reducing power

reflects the ability of compounds to modulate redox tone in plasma and tissues. The mechanism of FRAP assay is totally electron transfer rather than mixed SET and HAT, so in combination with other methods can be very useful in distinguishing dominant mechanisms with different antioxidants (Prior *et al*., 2005).

FRAP values are calculated by measuring the absorbance increase at 593 nm and relating it to a ferrous ions standard solution or to an antioxidant standard solution. The change in absorbance is proportional to the combined FRAP value of the antioxidants in the sample (Ou *et al*., 2002). The absorption (λ_{593}) slowly increased for polyphenols such as caffeic acid, tannic acid, ferulic acid, ascorbic acid and quercetin, even after several hours of reaction time. Thus, a single-point absorption endpoint may not represent a completed reaction (Prior *et al*., 2005). Concerning its limitations, any compound with redox potential lower than that of the redox pair Fe^{3+}/Fe^{2+} can theoretically reduce Fe^{3+} to Fe^{2+}, contributing to the FRAP value and inducing falsely high results. On the other hand, not all antioxidants reduce Fe^{3+} at a rate fast enough to allow its measurement within the observation time. FRAP assay develop from assay that rely on the hypothesis that the redox reaction proceed so rapidly that all reactions are complete within 4 and 6 min, respectively, but in fact this is not always true. FRAP results can vary tremendously depending on the time scale of analysis. Fast-reacting phenols that bind the iron or break down to compounds with lower or different reactivity are best analysed with short reaction times. However, some polyphenols react more slowly and require longer reaction times for detection. Pulido *et al*. (2003) observed that dietary polyphenols react more slowly and require longer reaction times for total quantification, and depending on the analysis time, the order of their reactivity is changed. The polyphenols with such behaviour include caffeic acid, ferulic acid, quercetin and tannic acid. The FRAP assay does not measure thiol antioxidants, such as glutathione. FRAP actually measures only the reducing capability based upon the ferric ion, which is not relevant to antioxidant activity mechanistically and physiologically. However, in contrast to other tests, the FRAP assay is simple, speedy, inexpensive and robust and does not require specialized equipment.

5.2.3 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay

DPPH is one of a few stable and commercially to express the antioxidant capacity of a certain antioxidant. Besides the mechanistic difference from the HAT reaction that normally occurs between antioxidants and peroxyl radicals, DPPH is long lived nitrogen radical, which bears no similarity to the highly reactive and transient peroxyl radicals involved in lipid peroxidation. Many antioxidants that react quickly with peroxyl radicals may react slowly or may even be inert to DPPH. In DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellowcoloured diphenyl-picrylhydrazine. This method is based on the reduction of DPPH in alcoholic solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H in the reaction. This assay is based on the measurement of the reducing ability of antioxidants toward DPPH.

DPPH is usually used as reagent to evaluate free-radical scavenging activity antioxidants (Elmastas *et al*., 2006). It is a stable free radical showing a maximum absorbance at 517 nm. When DPPH radicals encounter a proton-donor substrate such an antioxidant, the radicals would be scavenged and the absorbance is reduced (Gulcin *et al*., 2009). In this assay, as seen in Figure 5, the purple chromogen radical (DPPH) is reduced by radical scavenging compounds (AH) to the corresponding pale yellow hydrazine (DPPH-H) (Elmastas *et al*., 2006). The test is simple and rapid and needs only a UV-VIS spectrophotometer to perform, which probably explains its widespread use in antioxidant screening.

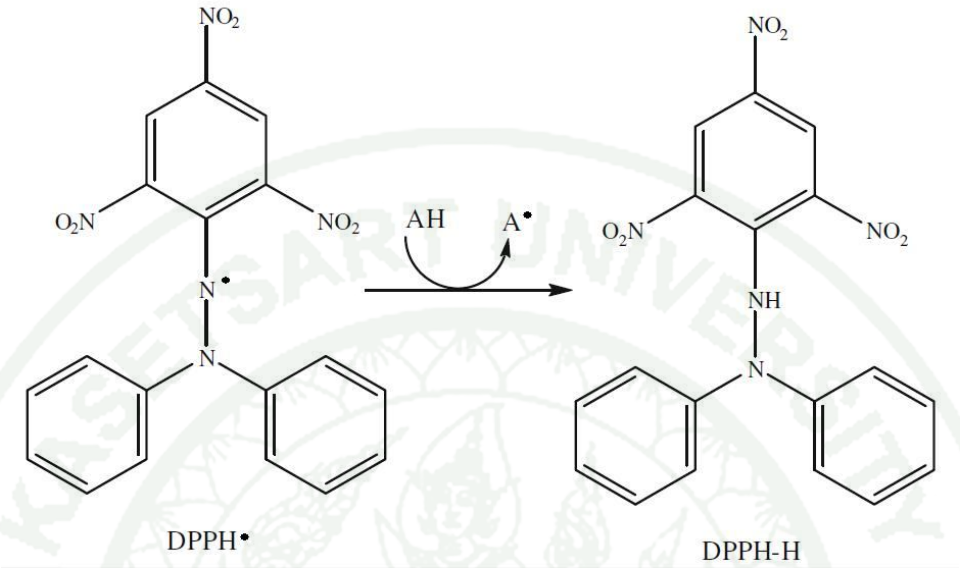

Figure 5 DPPH radical scavenging effect by an antioxidant (AH)

Source: Gulcin (2012)

5.3 Effect of some food processes on antioxidant activity

Food processing operations such as extrusion and drying are necessary steps in production of convenience foods, but they could affect the retention of antioxidants in food products (Kader *et al*., 2002; Wu *et al*., 2004).

5.3.1 Extrusion cooking

Extrusion cooking has the ability to increase or decrease the antioxidant activity of the extrudates. Total phenolic compound and the antioxidant activity were generally reduced by extrusion cooking due to thermal degradation (Ondo and Ryu, 2013; Nayak *et al*., 2011). However, several studies have shown that total phenolic compound and the antioxidant activity of extrudates were increased after extrusion cooking (Shama *et al*., 2012; Shin *et al*., 2009). This was probably due to extrusion cooking caused more free phenolic to be released from cell walls leading to higher total phenolic content and antioxidant activity compared to raw material.

Moreover, during extrusion of a food material the environment inside the extruder is ideal for Maillard reaction products to form. Maillard reaction products (particularly melanoidins) are extensively known to have antioxidant activity (Manzocco *et al*., 2005). Therefore, content of total phenolic compound and antioxidant activity of extrudates is highly dependant on the level and composition of bioactive compound in raw material and extrusion conditions.

Ondo and Ryu (2013) studied the effect of melting temperature ranging from 130 to 150^{o}C to produce expanded extrudates from the mixtures of natural cocoa powder (NCP) (0-20%) and corn meal (CM) in a twin screw extruder. The antioxidant capacity was determined using the DPPH free radical scavenging activity and the ferric reducing antioxidant power (FRAP) assays. The antioxidant activity extruded CM with higher NCP content had higher antioxidant activity than that of the extruded without NCP, whereas increasing melting temperature resulted in lower antioxidant activity in extrudates. Low antioxidant activity observed with increasing in melting temperature was probably due to the effect of heat on breaking complex polyphenols into low molecular weight phenolic compounds with scavenging activity.

Shama *et al*. (2012) reported that extrusion cooking exhibited a significant effect on the antioxidant properties of barley extrudates. Total phenolic content (TPC) and total flavonoid content decreased and DPPH radical scavenging activity increased upon extrusion. The thermal processing is also known to alter the antioxidant profile and generate more antioxidants that contribute in antioxidant activity. Increase in antioxidant activity due to thermal processing has been widely reported (Dewanto *et al*., 2002; Nicoli *et al*., 1997). Similarly result was observed by Shih *et al*. (2009) who reported that the extrusion process increased the DPPH radical scavenging activity in the sweet potato extrudates. This was due to the release of total phenolic acid and its derivatives from the cell walls of the plant matter resulted in high yield of antioxidant activity.

5.3.2 Drying process

The term drying refers generally to the removal of moisture from a substance. It is the most common and most energy consuming food preservation process (Ratti, 2001). There are many drying methods such as hot air drying, drum drying, freeze drying, spray drying, vacuum drying, microwave and low-pressure superheated steam drying (LPSSD). The main factors influenced the antioxidant activity in dried product was drying method and temperature.

Yang *et al*. (2010) studied the effect of drying methods (hot-air drying and microwave drying) on the antioxidant activity in sweet potato tubers (*Ipomoea batatas* L. Lam.). They found that dried sweet potatoes in microwave drying (95-105°C) possessed the highest antioxidant activity, while the lowest activity was observed in hot-air (65°C) dried samples. The highest content of antioxidant activity in microwave dried samples could be associated with the release of more bound phenolics from breakdown of cellular constituents during drying process at high temperature. Similar result was observed by Del Caro *et al*. (2004) who indicated that dried prunes using air dryer at the higher temperatures (85°C) had higher content of polyphenols and antioxidant activity than those of the dried prunes at lower temperature (60°C).

García *et al*. (2010) studied the drying temperature influenced on the loss of antioxidant capacity (measured by DPPH radical scaventing activity) in dried tomatoes. Riped pear tomatoes (*Lycopersicon esculentum* Mill) were air dried at different temperatures between 50 and 90º C during 18 to 120 h. Drying at 90º C caused antioxidant capacity losses of 25% higher than at 50º C. However, lycopene was detected in higher concentrations when the drying temperatures were higher, due to the rupture of the tomato cell membranes and the elevated thermal resistance of this compound.

6. Principal Components Analysis

Principal component analysis (PCA) is a technique for forming new variables which are linear composites of the original variables. The maximum number of new variables that can be formed is equal to the number of original variables, and the new variables are uncorrelated among themselves (Sharma, 1996). PCA is a statistical analytical tool that is used to explore, sort and group data. PCA take a large number of correlated (interrelated) variables and transform this data into a smaller number of uncorrelated variables (principal components) while retaining maximal amount of variation, thus making it easier to operate the data and make predictions. PCA is a way of identifying patterns in data, and expressing the data in such a way as to highlight their similarities and differences (Smith, 2002).

6.1 Advantages and disadvantages of principal component analysis

6.1.1 Benefits of principal component analysis

A primary benefit of PCA arises from quantifying the importance of each dimension for describing the variability of a data set (Shlens, 2009). PCA can also be used to compress the data, by reducing the number of dimensions, without much loss of information. When using PCA to analyze a data set, it is usually possible to explain a large percentage of the total variance with only a few components. PCA is completely nonparametric, any data set can be plugged in and the answer comes out, requiring no parameters to tweak and no regard for how the data was recorded.

6.1.2 Limitations of principal component analysis

PCA is not a statistical method from the viewpoint that there is no probability distribution specified for the observations. Therefore, it is important to keep in mind that PCA best serves to represent data in simpler, reduced form. The mission when using PCA is often to get rid of correlation and interdependence of variables. PCA succeeds in getting rid of second order dependences, but it has trouble

with higher-order dependencies. This problem might be solved by using kernel PCA or independent component analysis. The fact that PCA is agnostic to the source of the data is also a weakness (Shlens, 2009).

6.2 Applications of principal component analysis

Importance of PCA is manifested by its use in so many different fields of science and life. For example, in food science Charoenthaikij *et al.* (2010) studied the characteristics of bread formulations and physical and sensory acceptability.The result from the PCA biplot indicated that bread produced from wheat-germinated brown rice flour was different from bread produced from simple wheat flour, particularly in terms of hardness. Shaviklo *et al*. (2011) studied the quality and storage stability of extruded puffed corn-fish snacks during 6-month storage at ambient temperature. The result from the PCA biplot indicated that the corn snack products fortified with fish or fish proteins had good storage stability and did not differ from the control product for 5 months. The important characteristics of dried snacks during storage were crispness and rancidity; these attributes could seperated snack products stored for 6 months at the same region group on the PCA biplot.

7. Logistic Regression Analysis

Logistic regression analysis (LRA) is a type of regression analysis used for predicting the outcome of a categorical dependent variable based on one or more predictor variables. LRA measures the relationship between a categorical dependent variable and one or more independent variables, which are usually continuous, by using probability scores as the predicted values of the dependent variable (Allison, 1999). The simple logistic model as shown in equation 1.

$$\text{Logit (Y)} = \text{natural log (odds)} \ln [P/1\text{-}P] = \alpha+\beta x \quad (1)$$

In essence, the logistic model predicts the logit of Y from X. The logit is the natural of logarithm (ln) of odds of Y, and odds are ratios of probabilities (P) of Y

happening to probabilities (1-*P*) of Y not happening. Taking the antilog of equation on both sides, one derivers an equation to predict the probability of the occourrence of outcome of interest as shown in equation 2:

$$P = \frac{e^{\alpha+\beta x}}{1+ e^{\alpha+\beta x}} \qquad (2)$$

The odds ratio is a measure of effect size, describing the strength of association or non-independence between two binary data values. The odds ratio is equal to *exp*(*B*), or sometimes written e^B. This means that the probability that *Y* equals 1 is twice as likely (2.12 times to be exact) as the value of X is increased one unit. An odds ratio of 1.0 indicates there is no relationship between X and Y.

7.1 Advantages and disadvantages of logistic regression analysis

7.1.1 Benefits of logistic regression analysis

Logistic regression has several advantages over discriminant analysis for example, it is more robust: the independent variables don't have to be normally distributed, or have equal variance in each group. It does not assume a linear relationship between the independent variables and dependent variables. It may handle nonlinear effects. There is no homogeneity of variance assumption (Allison, 1999).

7.1.2 Limitations of logistic regression analysis

Unfortunately, the advantages of logistic regression come at a cost: it requires much more data to achieve stable, meaningful results. For logistic regression, at least 50 data points per predictor is necessary to achieve stable results. Moreover, LRA relies on a goodness-of-fit of the model and it highly sensitive to multicollinearity among predictor variables.

7.2 Applications of logistic regression analysis

Logistic regression is used extensively in numerous disciplines, including the medical and social science fields. It is also used in marketing applications such as prediction of a customer's propensity to purchase a product. LRA is applied in consumer test to predict both product acceptability and purchase intent based on the odd ratio point estimate of consumer perception. Ares *et al*. (2008) studied the shelf life estimation of minimally precessed lettuce and reported that results from LRA could suggest consumers' consideration and decision making wheather to purchase or to consume the lettuce during the different storage condition. Charoenthaikij *et al*. (2010) studied the sensory attributes that influenced purchase intent of wheat-germinated brown rice bread during storage time. The results from LRA showed overall liking and overall flavor were attributes influencing purchase intent. LRA was used to identify sensory attributes that influenced overall acceptance and purchase intent of the butter cake products. The result of LRA demonstrated that the critical sensory attributes that influenced overall acceptance and purchase intent of the butter cake products was overall liking (Sae-Ew *et al*., 2007).

MATERIALS AND METHODS

Materials

1. Raw materials

1.1 Brown rice

Two cultivars of Thai rice: Khao Dawk Mali 105 (KM) (*Oryza sativa* L. cv. KDML105) and the Hom Nin (HN) (*Oryza sativa* L. cv. Hom Nin) were used. The KM paddy rice was harvested in November 2008 and 2009 used for analysis in study 1 and 2. The KM paddy rice harvested in November 2010 used for analysis in study 3 and 4. All KM paddy rice obtained from the Department of aAgriculture, Sakhonnakorn province, Thailand. The HN paddy rice was harvested in October 2009 and 2010 by the organic farming, Pathumthani province, Thailand used in study 1. The HN paddy rice was harvested in October 2010 by the organic farming, Chang-Mai province, Thailand used for analysis in study 2, 3 and 4. Afterwards the paddy rice was packed in plastic bags made of linear low-density polyethylene (LLDPE), placed in containers and stored at 8 ˚C prior to the experiment.

1.2 Ingredients for extruded snack

1.2.1 Soy protein isolate (Eurofins Gene Scan Incorporated, LA, U.S.A.)

1.2.2 $CaCO_3$ (Thai Food and Chemical Co. Ltd, Bangkok, Thailand)

1.2.3 Crude xanthone powder (Department of Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand)

1.3 Ingredients for syrup coating

1.3.1 Glucose syrup DE 42 (Fancy craft brand, Chareonworakit Co., Ltd, Bangkok, Thailand)

1.3.2 Sucrose (Wangkanai Corp., Ltd, Kanchanaburi, Thailand)

1.3.3 Paste sugar (Mitr Phon industry Corp., Ltd, Bangkok, Thailand)

1.3.4 Salt (Prung Thip Pure salt industry Co., Ltd, Nakhonratsima, Thailand)

1.3.5 Margarine (Best food Co., Ltd, Bangkok, Thailand)

1.3.6 Butter milk cream flavor (Winner's Brand, Greathill Co., Ltd, Bangkok, Thailand)

1.3.7 Cocoa powder (Cocoa Ducth Brand, Pongjit Co., Ltd, Bangkok, Thailand)

2. Analytical reagents

2.1 Reagent for GABA analysis (Acetonitrile-acetate buffer, Dabsyl-Cl acetonitile solution and sodiumhydrogencarbonate)

2.2 Reagent for reducing sugar analysis (Nelson reagent, Alkaline copper reagent, D-Glucose and ethanol)

2.3 Reagent for total phenolic analysis (Folin-Ciocalteau's reagent, sodium carbonate and gallic acid)

2.4 Reagent for antioxidant activity (2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), 2,4,6-tripyridyls-triazine (TPTZ), ferric chloride, hydrochloric acid, acetate buffer, absolute acetic acid and gallic acid)

2.5 Reagent for α-mangostin analysis (α-mangostin reference standard (purity exceeded 97 %), methanol and acetonitrile)

2.6 Buffer for germination (phosphate buffer and citrate buffer)

3. Equipments

3.1 Equipments for preparing germinated brown rice flour

3.1.1 Commercial incubator (Siam Incubtators System Co., Ltd, Thailand)

3.1.2 Commercial sieve shaker (Kluay Nam Thai Co., Ltd., Bangkok, Thailand)

3.1.3 Commercial tray drying oven (Model HA 200, K.D.L. Engineering Co., Ltd., Thailand)

3.1.4 Ultra centrifugal mill (Retsch model ZM100, Germany)

3.2 Equipments for extrusion process

3.2.1 A co-rotating twin-screw extruder (Hermann Berstorff Laboratory, ZE25 x 33D, Germany)

3.2.2 A volumetric feeder (K-Tronsoder AG 5702, type 20, Switzerland)

3.2.3 Tray Dryer (Model B.W.S., B.W.S. Trading, Thailand)

3.2.4 Fitz mill (Model M5, The Fitzpatrick Co., Ltd, U.S.A.)

3.2.5 Gear mixer (Model Ts 207, Kluay Num Thai Tow OP., Ltd., Thailand)

3.3 Equipments for syrup coating

3.3.1 Coating machine (K.S.L. Engineering Co., Ltd, Thailand)

3.3.2 Hot plate (IKA® C-MAG HS7, U&V holding (Thailand) Co., Ltd., Thailand)

3.3.3 Commercial tray drying oven (Model HA 200, K.D.L. Engineering Co., Ltd., Thailand)

3.4 Analytical equipments

3.4.1 Benchtop Centrifuge (Model 2-16, Sigma, Germany)

3.4.2 Hot air oven (Model FD115, WTB Binder, U.S.A.)

3.4.3 High performance liquid chromathography (HPLC) (Agilent 1100 Series, Agilent Technologies, U.S.A.)

3.4.4 Incubator (Yamto® IC 1800, Yamato scientific Co., Ltd., Japan)

3.4.5 Novasina (a_w TH 200, Novasina, Switzerland)

3.4.6 Protein analyzer (Leco model FP528, St Joseph, U.S.A.)

3.4.7 Rapid visco analyzer (RVA) (4D. Newport Scientific Pty. Ltd., Australia)

3.4.8 Spectrophotometer (UV-160A, Shimadzu Co., Japan)

3.4.9 Spectrophotometer (CM-3500d, Minolta Co., Ltd., Japan)

3.4.10 Texture Analyzer (TA-XT plus, Stable Micro System, Texture Technologies Crop, U.S.A.)

3.4.11 Water Bath (WB22, Memmert, Germany)

3.4.12 pH meter (CyberScan pH 510, Eutech instruments, Singapore)

4. Statistical software

4.1 SPSS software for window version 12 (SPSS Inc., Thailand)

4.2 STATISTICA software version 10 (Trial) (Statsoft Inc., U.S.A.)

4.3 XLstat 2007 software (Addinsoft, France)

Methods

1. The effect of pH conditions of steeping water and storage time of paddy rice on physicochemical properties of germinated brown rice flour

1.1 Preparation of brown rice

The Khao Dawk Mali 105 (*Oryza sativa* L. cv. KDML 105), KM, paddy rice was harvested in November 2008 (replication1) and 2009 (replication 2) by the Department of Agriculture, Sakhonnakorn province, Thailand. In this study, storage time started in January 2009 for replication 1 and January 2010 for replication 2. Paddy rice was removed the husk by the rice milling machine to produce brown rice for germinate and analyze sample every 2 months for the duration of 12 months.

The Hom Nin (*Oryza sativa* L. cv. Hom Nin), HN, paddy rice was harvested in October 2009 (replication1) and 2010 (replication2) by the organic farming, Phatumthani province, Thailand. In this study, storage time started in November 2009 for replication1 and November 2010 for replication 2. Paddy rice was removed the husk by the rice milling machine to produce brown rice for germinate and analyze sample every 2 months for the duration of 12 months.

Freshly harvested both paddy rice were stored at room temperature (30 °C) for 2 months to break dormancy before germination. Afterwards the paddy rice was packed in plastic bags made of linear low-density polyethylene (LLDPE), placed in containers and stored at 8 °C prior to the experiment.

1.2 Preparation of germinated brown rice flour (GBRF)

The experiment was carried out by steeping 100 g of brown rice grains (brow rice grains from KM (KMR) and HN (HNR)) in 500 mL of 1,000 ppm of peroxyacetic acid (Tsunami®100, Ecolab Inc., St. Paul, Minn., U.S.A.) for 15 min, washing with tap water, and rinsing with distilled water to neutral pH. Subsequently,

grains were soaked in steeping water with a grain-to-water ratio of 1:5 (w/v) at 35 ˚C. The steeping water was 50 mM citrate buffer (pH 3), or reverse osmosis water (RO, pH 6.8). The nongerminated brown rice flour was used as the control. The grains were dried at 55 ˚C after reaching the required germination period to obtain a final moisture content of 10±2% and finely ground with an ultra centrifugal mill (Retsch model ZM100, Haan, Germany) with a 0.20 mm sieve to produce uniform-size flour (100 mesh). The preparation of germinated brown rice flour as shown in Figure 6. Both GBRFs from KMR (GKMF) and HNR (GHNF) were packed in plastic bags made of LLDPE and stored at 8 ˚C until further analyses.

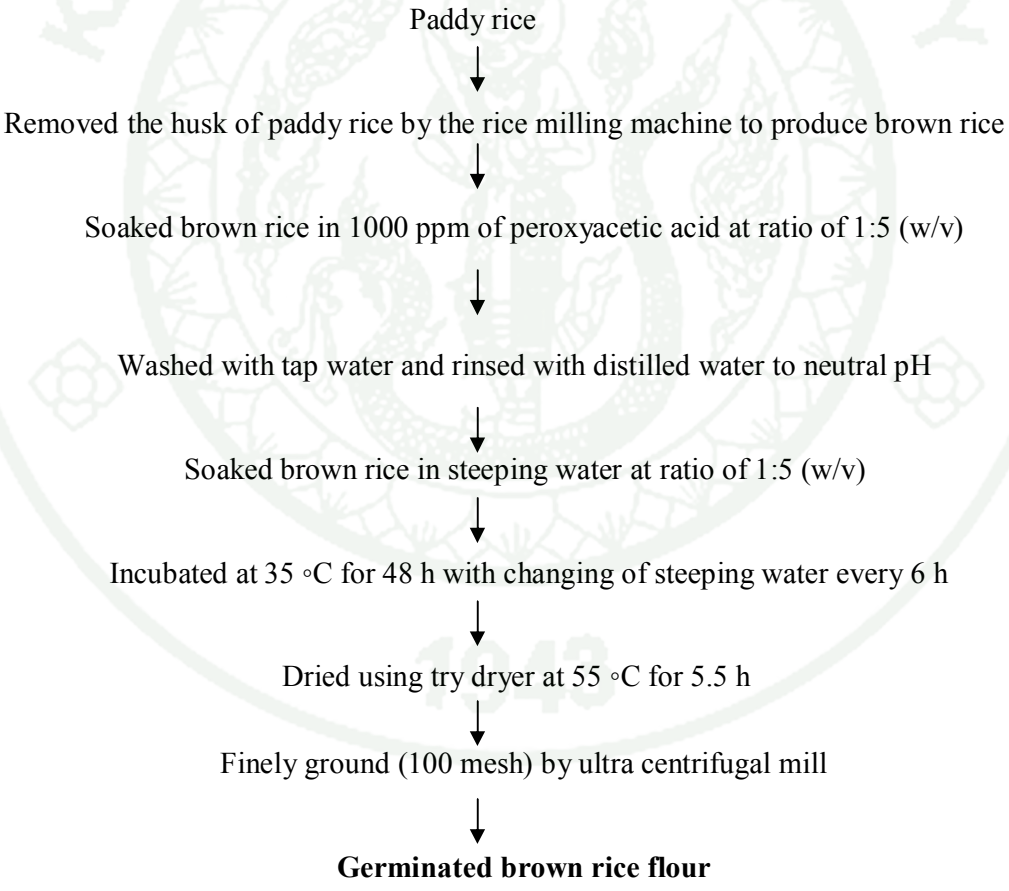

Figure 6 Preparation of germinated brown rice flour

1.3 Experimental design

A 6x2 factorial arrangement in completely randomized design (CRD) with 6 storage periods (2, 4, 6, 8, 10, and 12 months) and 2 pH levels of steeping condition (in a buffer solution (pH 3) or in reverse osmosis water (RO, pH 6.8)) for 48 h were investigated. The non-GBRF served as the control. Two separate batches of GBRFs for both rice varieties were prepared and measured the physicochemical properties as follows.

1.4 Quality determination

1.4.1 Physical measurements

Pasting profiles was analyzed in triplicate using a Rapid Visco Analyzer (RVA)(4D, Newport Scientific Pty. Ltd., Narrabeen, Australia) following AACC method 61-20 (AACC, 2000).

1.4.2 Chemical measurements

a) Moisture content was determined in triplicate according to the AOAC method 945.14 (AOAC, 1990).

b) Crude protein was determined in triplicate by a combustion method using a protein analyzer (Leco model FP528, St. Joseph, Mich., U.S.A.). Percentage of protein was calculated by multiplying %N with a factor of 5.95 (Bulletin of the international dairy federation, 2006).

c) Free GABA content was determined in triplicate using high performance liquid chromathography (HPLC) according to method of Cohen and Michaud (1993). Analysesd by Institute of Food Reseach and Product Development, Kasetsart University. Two hundred to five hundred milligrams (200-500 mg) of GBRF or BRF was weighed into the plastic tube and 2 mL of deionized water was

added. The mixture was centrifuged at 2,264 x *g* for 10 min. One mL of supernatant was pipetted and added with 200 µL of 0.4 M $NaHCO_3$ and 400 µL of 6 mM Dabsyl-Cl acetonitrile solution. The reaction was performed at 70 ℃ for 20 min. After derivatization, the sample was filtered into a vial and injected into HPLC (Agilent 1100 Series, Agilent Technologies, Calif., USA) equipped with column (Supelcosil LC-DABS 4.6 mm I.D. x 150 mm, USA). Acetonitrile-acetate buffer pH 6.8 (20:80, v/v) was used as the mobile phase with a flow rate of 1.0 mL/min and injection volume of 10.0 µL. The column temperature was 40 ℃ and the ultraviolet detector was set at 315 nm.

d) α-amylase activity was analyzed in triplicate using a rapid visco analyzer (RVA) (4D, Newport Scientific Pty. Ltd., Narrabeen, Australia) following AACC method 22-08 (AACC, 2000). The results were reported as the stirring number (SN) (Appendix B).

e) Reducing sugar from GBRF was extracted by using aqueous ethanol (50%, v/v). Reducing sugar content was then determined in triplicate according to the Nelson–Somogyi's method (Somogyi, 1951) (Appendix B).

1.4.3 Statistical analysis

Physiochemical data obtained from each condition were analyzed using the analysis of variance (ANOVA). Duncan's New Multiple Range Test (DMRT) was performed for post-hoc multiple comparison. Statistical significant difference was established at $P \leq 0.05$. Principle component analysis (PCA) was carried by using XLstat 2007 software (Addinsoft, Paris, France).

2. The study of extrusion conditions on the qualities of extruded snack from germinated brown rice flour

2.1 Preparation germinated brown rice flour

The KM paddy rice was harvested in November 2009 by the Department of Agriculture, Sakhonnakorn province, Thailand. The HN paddy rice was harvested in October 2010 by the organic farming, Chang-Mai province, Thailand. Both paddy rice varities stored for 4 months used to study in this part due to the high value of free GABA content and still containing effective dose of GABA. The amount of GABA content on relaxation and immunity during stress in humans were in the range of 20-30 mg/100g flour (Nakamura *et al*., 2009).

Preparations of both GBRFs were the same procedure as described in the previous method section 1.2 using the optimum pH from the result of study 1 to produce high free GABA extruded snack. After 48 h of germination period, the GBR were dried at 55°C for 5.5 h and finely ground with Fitz mill (80 mesh). The GBRF (90.5%), soy protein isolate (SPI) (9.05%) and $CaCO_3$ (0.45%) were mixed using gear mixture for 10 min and packed in LLDPE plastic bag at 25 °C before extrusion.

2.2 Extrusion process

A co-rotating twin-screw extruder (Hermann Berstorff Laboratory, ZE25 x 33D, Germany) comprised a barrel with seven parts (section 1 to section 7) ending in 24.5 mm thick die plate and one circular die hole (diameter 2.5 mm) was used. The barrel length-to-diameter ratio (L/D) of the extruder was 870:25. The mixed flour was fed into the extruder with a volumetric feeder (K-Tron soder AG 5702, type 20, Switzerland). Water was pumped into the first barrel section of the extruder to maintain the required moisture contents. The barrel temperature profile was 35°C (section 1), 45 °C (section 2), 55 °C (section 3), 95 °C (section 4), 125 °C (section 5), 140 °C (section 6), 130 °C (section 7) and 120 °C (Die), respectively. The feed rate was controlled at 3.6 kg/h. After extrusion, the extrudates from GKMF (GKME) and

extrudates from GHNF (GHNE) were collected and dried in an air oven at 80°C for 10 min, and then the dried extrudate samples were packaged in LLDPE plastic bags and stored at 25 °C until further analyses. The processing of extruded snack from germinated brown rice flour is shown in Figure 7.

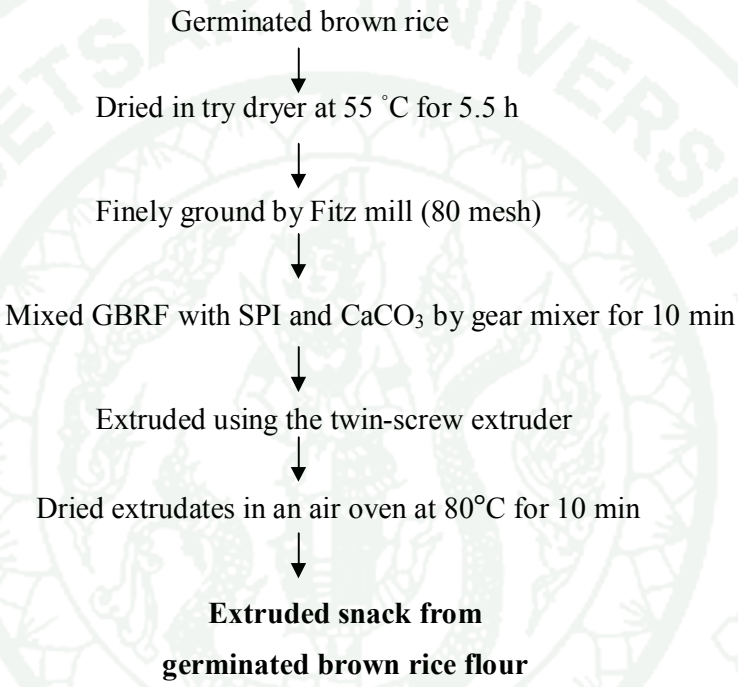

Figure 7 The processing of extruded snack from germinated brown rice flour

2.3 Experimental design

A 3x3 factorial arrangement in completely randomized design (CRD) with three levels of feed moisture (14, 18 and 22%) and three levels of screw speed (300, 350 and 400 rpm) was used. The GBRF before extrusion served as control. Two separate batches of extrudates from both rice varieties were prepared and measured in the physical, chemical and sensory properties. Response surface methodology (RSM) was used in this experiment. The STATISTICA software version 10 (trial) was used to generate the experimental design, analysis and contour plot. The measured responses functions (Y_i) were free GABA and overall liking. These values were

related to the coded variables (X_1= feed moisture and X_2 = screw speed) by a second degree polynomial using the equation below (equation 3).

$$Y_i = b_0+b_1X_1+b_2X_2+b_{11}X_1^2+b_{22}X_2^2+b_{12}X_1X_2 \quad (3)$$

The coefficients of the polynomial were represented by b_0 (constant term), b_1, b_2 (linear effects), b_{11} and b_{22} (quadratic effects) and b_{12} (interaction effects).

2.4 Quality determination

2.4.1 Physical measurements

a) Color of extruded snacks was measured with a Minolta spectrophotometer CM-3500d equipped with a D65 illuminant (Minolta Co., Ltd, Osaka, Japan). This procedure was repeated with two other samples. The average value of ten measurements was reported. Color readings were displayed as L*(color lightness), a*(redness) and b*(yellowness) values.

b) Bulk density was measured according to the method of Bhatnagar and Hanna (1995). Bulk density of the extrudates was determined using a seed displacement method. The 200 mL sesame seeds were used as the displacement medium. The seeds were poured into a graduated cylinder (250 mL). The cylinder was tapped soundly 20 times. The weight of each treatment was weighed and bulk density (g/cm^3) was calculated by dividing the weight of the extrudates by the volume displaced. Each value was an average of ten independent measurements. Bulk density was expressed on a dry basis.

c) Expansion ratio of the extrudate was determined by applying a vernier caliper to measure the diameter of extrudate (ten randomly chosen pieces of extrudate from each test run) and calculated as the ratio of the cross section diameter of the extrudate to the die hole (Alvarez-Martinez *et al.,* 1988) as shown in equation 4:

Expansion ratio = Diameter of extrudate / Diameter of die hole (4)

d) The textural properties of the extrudates were measured with a TA-XT plus Texture Analyzer (Stable Micro System, Texture Technologies Crop, NY., U.S.A) using a 36 mm cylinder probe. A 5 mm/sec pre-test speed, 5 mm/sec test speed, 10 mm/sec post-test speed and compression of 50% strain were used to compress one piece of sample until it broke (Ding *et al*., 2005; Altan *et al.,* 2008). Hardness was measured by the mean maximum peak force. The results were expressed as the average of ten measurements.

2.4.2 Chemical measurements

a) Free GABA content measurement was the same procedure as described in the previous method section 1.4.2 c).

b) Total phenolic content (TPC) of extracts was determined according to the Folin and Ciocalteau's method (1927). Extract was prepared from ground extrudate weighed (2.0 g) accurately and extracted at a room temperature with methanol under agitation using a magnetic stirrer for 30 min. The mixtures were centrifuged at 2500 x *g* for 10 min and the supernatants were collected. The residues were reextracted twice under the same conditions, resulting finally in 50 mL extract in methanol. All extracts were analyzed for total phenolic acid and antioxidant activity. Gallic acid was used as a standard. Mixed 0.5 mL of each sample with 2.5 mL of a ten-fold diluted Folin-Ciocalteau's reagent and 2 mL of 7.5% sodium carbonate. The mixture was allowed to stand in the dark for 30 min at a room temperature before the absorbance was read at 760 nm with a UV-visible spectrophotometer (UV-160A, Shimadzu Co., Japan). All determinations were performed in triplicates. The total phenolic content was expressed as mg gallic equivalents per 100 g extrudate (Appendix C).

c) 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The method was based on the reduction of methanolic DPPH in the presence of a hydrogen-donating antioxidant, DPPH solution, an intense violet color, which showed absorption band at 517 nm, were measured in terms of electron transfer reaction using the stable free radical DPPH methods. The radical scavenging activity of the phenolics extracts on the DPPH radical was measured according to the method described by Ozgen *et al*. (2006) with some modifications. The extract of extrudates (3 mL) (the same procedure as described in the previous method section 2.4.2 b) was mixed with 2 mL of 0.2 mmol DPPH solution. The mixture was vigorously shaken and left to stand in the dark for 30 min. The absorbance was measured at 517 nm with a UV-visible spectrophotometer (UV-160A, Shimadzu Co., Japan). All determinations were performed in triplicates. The percentage of free radical scavenging activity was calculated as equation 5. Then, DPPH radical scavenging activity was converted to mg gallic equivalents per 100 g extrudate (Appendix C).

$$\text{DPPH radical scavenging activity (\%)} = (1 - (A_{sample} - A_{control})) \times 100 \quad (5)$$

where $A_{control}$ is the absorbance of the control (DPPH solution without test sample), A_{sample} is the absorbance of the test sample (DPPH solution with test sample).

d) Ferric reducing antioxidant power (FRAP)

The FRAP of the extracts from extrudates (the same procedure as described in the previous method section 2.4.2 b) was measured according to the method of Benzie and Strain (1996), with slight modifications. The FRAP reagent consisted of 10 $mmolL^{-1}$ 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 $mmolL^{-1}$ HCl, 20 $mmolL^{-1}$ $FeCl_3.6H_2O$ and 0.1 $mmolL^{-1}$ sodium acetate buffer, at pH 3.6 in the ratio of 1:1:10. The extracts (0.1 mL) were added to 3 mL of FRAP reagent and mixed thoroughly. After standing in the dark for 30 min at a room temperature,

the absorbance was measured at 595 nm with a UV-visible spectrophotometer (UV-160A, Shimadzu Co., Japan). All determinations were performed in triplicates. FRAP values, expressed as mg of $FeSO_4$ equivalents per 100 g extrudate (Appendix C).

2.4.3 Sensory evaluation

Untrain panellist (n=30) were recruited from Kasetsart University, Bangkok, Thailand. Criteria for recruitment were that they were regular snack consumers and not allergic to ingredients used in snacks. The test was conducted in a sensory laboratory room with a partitioned booth for an individual consumer at a controlled temperature at 25°C. Each consumer was presented with 9 coded of the extruded samples without coat flavor (3.25 g/serving). Consumers evaluated two sets of samples, first set contained four samples and second set contained five samples. Between samples, consumers rinsed their mouths with water. They were also subjected to a 5 min break between the sample sets. They were instructed to (1) visually evaluate acceptability for shape of product, (2) bite and masticate the product before scoring acceptability for hardness, taste and overall liking, on a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely) (Peryam and Pilgrim, 1957), and the paper ballot was used. Consumers were provided with room-temperature water to rinse their mouth before testing and between samples.

2.5 Statistical analysis

Physiochemical and sensory data obtained from each condition were analyzed using the analysis of variance (ANOVA). Duncan's New Multiple Range Test (DMRT) was performed for post-hoc multiple comparison. Statistical significant difference was established at $P \leq 0.05$. Response surface methodology (RSM) was used to determine the effects of screw speed and feed moisture levels on chemical and sensory properties by regression model. Contour plots were analyzed by STATISTICA software version 10 (trial). The regression equation and detail of RSM were decribed in the previous method section 2.3. The correlation coefficient (r) and

the root mean square error (RMSE) between the observed and predicted values of extruded snack from selected conditions for verification of optimized region.

The correlation coefficient (r) often measured as a correlation coefficient indicates the strength and direction of a linear relationship between two variables (for example model output and observed values). The mathematical formular for computing r shown in equation 6:

$$r = \frac{n\sum xy - \left(\sum x\right)\left(\sum y\right)}{\sqrt{n\left(\sum x^2\right) - \left(\sum x\right)^2}\sqrt{n\left(\sum y^2\right) - \left(\sum y\right)^2}} \quad (6)$$

where n is the number of pairs of data

The value of r is such that $-1 \leq r \leq +1$. The + and – signs are used for positive linear correlations and negative linear correlations, respectively. If x and y have a strong positive linear correlation, r is close to +1. An r value of exactly +1 indicates a perfect positive fit. Positive values indicate a relationship between x and y variables such that as values for x increases, values for y also increase. If x and y have a strong negative linear correlation, r is close to -1. An r value of exactly -1 indicates a perfect negative fit. Negative values indicate a relationship between x and y such that as values for x increase, values for y decrease. If there is no linear correlation or a weak linear correlation, r is close to 0. A value near zero means that there is a random, nonlinear relationship between the two variables.

The root mean square error (RMSE) is a frequently used measure of the difference between values predicted by a model and the values actually observed from the environment that is being modelled. These individual differences are also called residuals, and the RMSE serves to aggregate them into a single measure of predictive power. The RMSE of a model prediction with respect to the estimated variable X_{model} is defined as the square root of the mean squared error (equation 7):

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(X_{obs,i} - X_{model,i})^2}{n}} \quad (7)$$

where X_{obs} is observed values and X_{model} is modelled values at time/place i.

The RMSE values can be used to distinguish model performance in a calibration period with that of a validation period as well as to compare the individual model performance to that of other predictive models.

3. The study of barrel temperature on physicochemical properties of extruded snack made from germinated brown rice flour fortified with crude xanthone powder

3.1 Preparation of crude xanthone powder

Crude xantone powder was obtained from Department of Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand. The preparation of crude xanthone powder start with collected mangosteen fruit from Chantaburi province, Thailand in 2007. The air-dried fruit hulls were then ground to powder, and extracted with 95% ethyl alcohol at room temperature for 48 h. Solvent was evaporated to dryness under reduced pressure at 50 °C to yield a brownish residue. Water was then added to the residue, the mixture was stirred occasionally and the resulting solid was filtered and then dried in vacuo to give crude xanthone powder as yellow solid (45.2 g). Crude xanthone powder contained more than 46% of α-mangostin (major xanthone), pH in the range of 2.5-4.0 and moisture content was less than or equal to 5% w/w. The crude xanthone powder was separately kept in air tight laminated aluminium foil and protected from light until used. Crude xanthone powder as shown in Figure 8.

Figure 8 Crude xanthone powder

3.2 Extrusion process

In this study, the paddy rice of KM was harvested in November 2010 and HN was harvested in October 2010. Both GBRFs prepared from paddy rice stored for 4 month and steeped under the optimum pH from the result of study 1. GBRF (90.17 %) was thoroughly mixed with 0.36% of crude xanthone powder, 9.02 % of soy protein isolate and 0.45% of $CaCO_3$ by a gear mixer for 10 min before feeding into the twin screw extruder. The preparation of germinated brown rice flour mixed with crude xanthone powder as shown in Figure 9. One circular die hole (diameter 2.5 mm) was used. The feed rate was controlled at 3.6 kg/h. The feed moisture and screw speed of both rice varieties were selected fom the result of study 2. After extrusion, the extrudates from both rice varieties were collected and dried in an air oven at 80°C for 10 min, and then the dried extrudate samples were packaged in LLDPE plastic bags and stored at 25 °C until further analyses. Two separate batches of extrudates from both rice varieties were prepared and measured the physical and chemical properties.

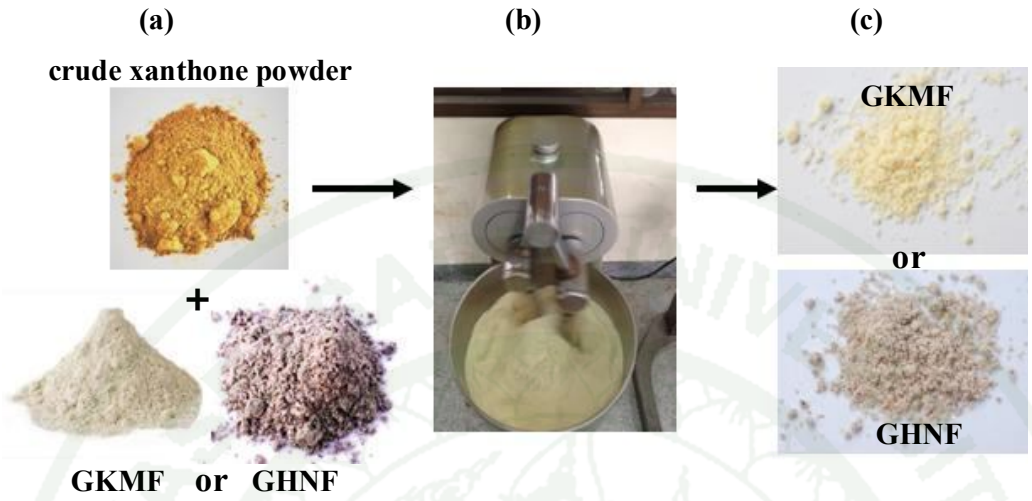

Figure 9 Preparation of germinated brown rice flour (GKMF or GHNF) mixed with crude xanthone powder (a) using gear mixer for 10 min (b) and the germinated brown rice flour fortified with crude xanthone powder before extrusion process.

3.3 Experimental design

The experiment was conducted in a completely randomized design (CRD) with three levels of barrel temperature (120, 140 and 180°C) at section 6. The barrel temperature profiles of extrusion process are presented in Table 3. The GBRF fortified with crude xanthone powder before extrusion served as a control. Two separate batches of extrudates fortified with crude xanthone powder from both rice varieties were prepared and measured the physical and chemical qualities.

Table 3 Barrel temperature profile (°C) of extrusion process

Barrel temperature profile (°C)							
section 1	section 2	section 3	section 4	section 5	section 6	section 7	Die
35	45	55	80	110	120	120	110
35	45	55	95	125	140	130	120
35	45	55	110	140	160	140	130

3.4 Quality determination

3.4.1 Physical measurements

Water activity (a_w), color, bulk density, expansion ratio and textural properties (hardness) measurement were the same procedures as described in the previous method section 2.4.1.

3.4.2 Chemical measurements

a) Free GABA content measurement was the same procedure as described in the previous method section 1.4.2 c).

b) Total phenolic content measurement was the same procedure as described in the previous method section 2.4.2 b).

c) Antioxidant activity measurement was the same procedure as described in the previous method section 2.4.2 c) and d).

d) α-mangostin content analysed by Department of Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand using the method as described.

(1) Extraction of the extrudates

One hundred milligrams of ground extrudates were weighed and added with 10 mL of MeOH (AR grade), the mixture was sonicated for 10 min and the solvent was filtered through a Whatman no.1. Afterward, the solvent was evaporated and each sample was done in triplicate.

(2) Preparation of Standard Solutions

A stock solution of α-mangostin reference standard (purity exceeded 97 %) was prepared by dissolving an accurately weighed 5 mg of α-mangostin in 10 mL of water-acetonitrile in a volumetric flask. Various concentrations of the standard solution were diluted to obtain final concentrations at 20-140 μg/mL with acetonitrile. The α-mangostin was detected at 254 nm and confirmed by retention time at 28.773 min.

(3) HPLC analysis of α-mangostin content

The α-mangostin content for all samples were determined by HPLC Thermo (Thermo Finnigan, USA) according to the method of Chaivisuthangkura *et al*, (2009). The equipment comprises a degasser, thermo separation product, quaternary gradient spectra system P400, UV detector spectra system 2000 and Chrom Quest software. Compounds were separated on a 150 mm x 4.6 mm, 4μm particle, Synergi Hydro column. The mobile phase was a gradient prepared from acetonitrile (component A), 2% (v/v) acetic acid in water (component B), and *n*-butanol (component C). The gradient program was A:B:C from 30:70:0 to 45:45:10 in 2 min, from 45:45:10 to 80:15:5 in 23 min, from 80:15:5 to 80:20:0 in 2 min, from 80:20:0 to 95:5:0 in 8 min, and isocratic at 95:5:0 for 25 min. The flow rate was 0.5 mL min-118 and the total separation time was 60 min. Chromatography was performed at ambient temperature. The contents of α-mangostin were expressed as mg per 100 g extrudate. Each determination was carried out in triplicate.

4. Develop formulation of syrup coating for extrudrd snack from germinated brown rice flour

In order to satisfy consumer acceptance for extruded snacks from germinated brown rice flour, taste of extruded snack are very important. Therefore, the study of flavor coating was conducted. Basic flavor coating formulations were consisted of 8.77% of glucose syrup, 35.09% of sucrose, 21.05 % of paste sugar, 0.50% of salt, 2.50% of margarine, 15% of water and 2% of flavor from Institute of Food Reseach and Product Development (2010). Procedure of coating extruded snacks with syrup flavor as shown in Figure 10. Glucose syrup, sugar, salt and water were put in a pot, stirred and heated using hot plate at 220 °C until the mixture was dissolved and then added paste sugar and stired well until obtain 75 °Brix of the mixture. Margarine was added in the mixture and stirring constantly until the margarine was melt.

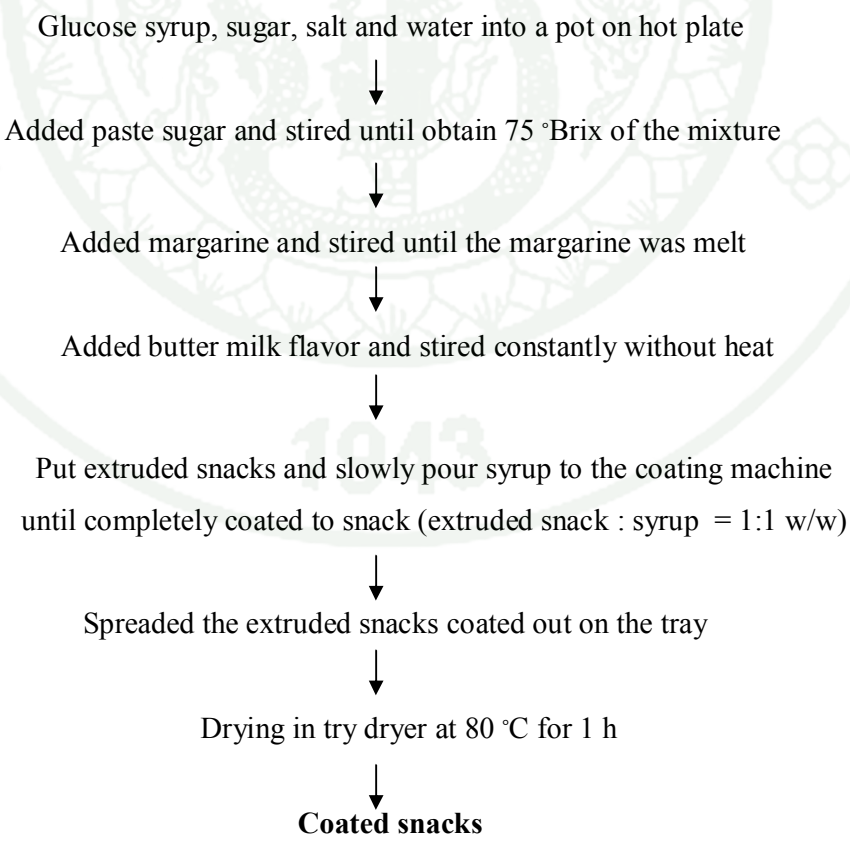

Figure 10 Procedure of coated snacks with syrup flavor

Subsequently, the butter milk flavor was dropped and stired without heat to obtain the syrup flavor. Extruded snack was put in the coating machine and syrup was slowly pour until completely coated (extruded snack-to-syrup ratio of 1:1 w/w) to snack. The coated extruded snacks were spread out on the tray. The drying process was carried out at 80 ◦C for 1 h using a try dryer. After the drying process was done, the coated extruded snacks were stand at room temperature for 10 min to let them cool. The coated snack were packed in an aluminum foil and stored at room temperature before further analyses.

After coating process, the coated extruded snacks were subjected to sensory evaluation.Untrain panellist (n=30) were recruited from Kasetsart University, Bangkok, Thailand. The test was conducted in a sensory laboratory room with a partitioned booth for an individual consumer at a controlled temperature at 25◦C. Each consumer was presented with 2 coded of coated extruded snacks (3.25 g/serving of GKME and GHNE). The 9-point hedonic scale (1 = dislike extremely and 9 = like extremely) (Peryam and Pilgrim, 1957) and the 3-point Just About Right (JAR) scale, where 1= not enough, 2= about right, and 3= too strong (Stone and Sidel, 1993) was used for acceptability ratings.

5. Quality evaluation of developed snack from germinated brown rice flour

5.1 Quality determination of developed product

5.1.1 Physical measurements

Color, bulk density, expansion ratio and hardness were the same procedures as described in the previous method section 2.4.1.

Water absorption index (WAI) and water solubility index (WSI) were determined according to the method of Anderson *et al.* (1969) (Appendix C).

5.1.2 Chemical measurements

a) Free GABA content was the same procedure as described in the previous method section 1.4.2 c).

b) Total phenolic content was the same procedure as described in the previous method section 2.4.2 b).

c) DPPH radical scavenging activity was the same procedure as described in the previous method section 2.4.2 c).

d) FRAP was the same procedure as described in the previous method section 2.4.2 d).

e) Proximate qualities of developed snack were analysed in triplicate moisture content was determined in triplicate according to the AOAC method 945.14 (AOAC, 2000). Crude protein was determined by the Kjeldahl method according to the AOAC method 976.06 (AOAC, 1995). Crude fat was determined by the ether-extraction method using a soxtec system according to the AOAC method 920.39 (AOAC, 1995). Ash was determined by the muffle furnace method according to the AOAC method 930.22 (AOAC, 2000). Crude fiber was measured using a fiber analyzer according to the AOAC method 962.09 (AOAC 2002). Carbohydrate content (%) obtained from the calculation of 100 minus the sum of protein, crude fat, crude fiber and ash based on dry basis.

5.1.3 Microbiological measurements

Total plate count and yeast and molds were determined according to Bacteriological Analytical Manual (BAM, 2000).

5.1.4 Sensory evaluation

To evaluate the preference to developed extruded snack from both GBRFs, the preference test will be conducted in a laboratory by untrained panelist (n=30). The participants will be recruited and asked to rate each attribute of the selected sample on the ballot using 9-point hedonic scale.

6. The study of the consumer acceptance of developed snack from germinated brown rice flour

6.1 Consumer acceptance and purchase intent

Two consumer groups (Thai and US. population) were selected for this study. The central location test (CLT) for consumer acceptance was used. In Thailand, consumers (n=200) were recruited from Kasetsart University, Bangkok, Thailand. Thai consumer acceptance was conducted at the canteen in Kasetsart University. While conducting consumer acceptance test in the US., the consumer test protocol was approved by the Louisiana State University (LSU) Agricultural Center (AgCenter) Institutional Review Board. American consumers (n=100) were recruited from Baton Rouge, La. The US consumer acceptance was conducted at the dairy store in LSU AgCenter. Both Thai and US. consumers were prescreened for potential food allergies to rice flour and all the ingredients used in extruded snack made from GBRFs. Questions (in Thai) in the ballot used for Thai consumers were the same questions as of questionnaires used for US. consumers.

Consumers were asked to provide demographic information, including age and gender questionnaire shown in Appendix D. They were briefed about the questionnaire, particularly the sensory attributes and their meanings. They were instructed to (1) visually evaluate acceptability for shape and color of the product, (2) sniff to evaluate aroma acceptability and (3) bite and masticate the product before scoring acceptability for hardness, taste and overall liking, on a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely), and the

paper ballot was used. Consumers were provided with room-temperature water to rinse their mouth before testing and between samples. Additional samples were given upon request. To avoid biases, consumers did not receive any monetary incentive for participation.

6.2 Statistical analysis

Data were subjected to analysis of variance (ANOVA). Duncan's New Multiple Range Test (DMRT) was performed for post-hoc multiple comparison. Logistic regression analysis (LRA) was performed to identify sensory attributes influencing overall acceptance and purchase intent. Statistical significant difference was established at $P \leq 0.05$.

RESULTS AND DISCUSSION

1. Effect of pH conditions of steeping water and storage time of paddy rice on physicochemical properties of germinated brown rice flour

After obtaining the GBRF from each month during storage period, GBRFs from both rice varities were subjected to subsequence analysis.

1.1 Chemical properties

1.1.1 Moisture and crude protein content

Steeping brown rice grains under different conditions led to change in selected physicochemical properties of both GBRFs. The moisture content of GKMF and GHNF was ranged from 6.15% to 9.18% and from 6.10% to 8.80%, respectively (data not shown). Table 4 and 5 shows the changes in chemical properties of GKMF and GHNF, respectively compared to those of the control (non-GBRF). In present experiment, protein content of GKMF steeping at pH 3, pH 6.8 and control was ranged from 5.49 to 7.00 %, 7.01 to 8.79 %, and 6.24 to 8.87% based on a dry weight basis, respectively. The protein content of GHNF steeping at pH 3, pH 6.8 and control was ranged from 6.9 to 8.00 %, 7.20 to 8.70 %, and 7.80 to 9.20 % based on a dry weight basis, respectively. In fact, rice properties are known to be dependent on the variety of rice, methods of cultivation, processing and cooking conditions (Roy *et al*., 2011). In addition, the results indicated that GBRF from both rice varieties obtained under pH 3 had significantly lower ($P \leq 0.05$) protein content than those of GBRF obtained under pH 6.8. The reduction of protein occurred probably due to its solubility into the soaking solution. Our observations agreed with the study of Ohishi *et al*. (2003) who observed that the amounts of rice proteins in acetic acid solution

Table 4 Crude protein, free GABA, reducing sugar, and stirring number (SN) of GKMF by various steeping condition and storage time[A].

Germination condition		Crude protein	Free GABA	Reducing sugar	SN
Steeping condition	Storage time (months)	(%,dry basis)	(mg/100 g flour, dry basis)	(mg/100 g flour, dry basis)	(RVU)
pH 6.8	2	7.90±0.10^{cd}	30.70±0.60^{f}	166.80±1.50^{g}	156.70±0.20^{a}
pH 3	2	6.10±0.20^{gh}	29.80±0.90^{f}	574.40±2.10^{e}	154.40±0.10^{ab}
Control	2	7.60±0.01^{cde}	1.80±0.10^{i}	66.50±0.80^{j}	156.70±0.80^{a}
pH 6.8	4	7.40±0.01^{de}	30.40±0.80^{f}	145.90±1.20^{h}	156.80±1.50^{a}
pH 3	4	5.50±0.00^{i}	28.70±1.00^{f}	718.30±3.90^{d}	118.80±0.10^{e}
Control	4	7.70±0.00^{cd}	2.20±0.20^{i}	67.30 ±0.40^{j}	152.50±1.50^{b}
pH 6.8	6	7.50±0.30^{cde}	37.70±2.20^{e}	237.60 ±2.70^{f}	46.40±0.90^{f}
pH 3	6	5.90±0.20^{hi}	83.80±1.80^{b}	11825.60±8.40^{c}	15.70±0.10^{h}
Control	6	7.30±0.30^{def}	1.60±0.10^{i}	68.00±0.70^{j}	147.30±0.30^{c}
pH 6.8	8	8.80±0.50^{a}	54.20±3.00^{c}	257.70±3.20^{f}	10.90±1.10^{i}
pH 3	8	7.00±0.10^{ef}	99.80±0.40^{a}	21846.60±11.40^{a}	6.20±0.40^{j}
Control	8	8.90±0.40^{a}	2.10±0.10^{i}	35.20±0.10^{k}	144.90±1.70^{cd}
pH 6.8	10	8.70±0.00^{a}	44.40±2.10^{d}	200.30±2.00^{fg}	10.90±1.60^{i}
pH 3	10	6.70±0.10^{fg}	86.30±3.30^{b}	20822.30±10.90^{b}	10.80±0.80^{i}
Control	10	8.50±0.30^{ab}	3.20±0.30^{i}	29.90±0.50^{l}	143.10±1.70^{cd}
pH 6.8	12	7.00±0.20^{ef}	27.20 ±0.70^{fg}	83.00±0.80^{i}	40.40±0.00^{f}
pH 3	12	5.60±0.20^{i}	23.70±0.70^{gh}	558.30±2.60^{e}	26.40±3.10^{g}
Control	12	6.20±0.20^{gh}	2.30±0.60^{i}	15.80±0.60^{m}	155.30±5.00^{ab}

[A]Means ± SD of triplicate measurements. Means with different superscript letters in each column are significantly different ($P \leq 0.05$).

Nongerminated brown rice flour served as the control.

Table 5 Crude protein, free GABA and reducing sugar of GHNF by various steeping condition and storage time[A].

Germination condition		Crude protein	Free GABA	Reducing sugar
Steeping condition	Storage time (months)	(%, dry basis)	(mg/100 g flour, dry basis)	(mg/100 g flour, dry basis)
pH 6.8	2	8.10±0.10^{c}	24.20±0.60^{fg}	225.11±0.01^{h}
pH 3	2	7.00±0.10^{ef}	23.60±1.00^{g}	243.84±0.20^{d}
Control	2	8.40±0.10^{bc}	11.48±0.40^{j}	7.60±0.00^{cde}
pH 6.8	4	7.80±0.00^{cd}	35.70±2.20^{b}	291.50±0.30^{g}
pH 3	4	7.00±0.20^{ef}	29.70±2.00^{de}	424.90±0.90^{de}
Control	4	8.50±0.10^{bc}	11.80±0.60^{h}	7.70±0.00^{cd}
pH 6.8	6	8.00±0.30^{c}	32.40±4.00^{bcd}	318.60±0.40^{fg}
pH 3	6	7.60±0.20^{cde}	30.70±0.60^{cde}	451.20±1.00^{d}
Control	6	8.80±0.30^{ab}	10.70±0.20^{h}	7.30±0.30^{def}
pH 6.8	8	8.60±0.20^{bc}	33.90±1.00^{bc}	405.00±0.70^{e}
pH 3	8	8.00±0.10^{c}	42.50±0.30^{a}	504.40±1.00^{c}
Control	8	9.20±0.30^{a}	11.80±0.50^{h}	8.90±0.40^{a}
pH 6.8	10	8.80±0.10^{ab}	30.80±0.80^{cde}	656.80±2.00^{b}
pH 3	10	7.70±0.10^{cd}	35.80±0.90^{b}	1231.80±10.90^{a}
Control	10	9.00±0.20^{a}	11.50±0.30^{h}	8.50±0.30^{ab}
pH 6.8	12	7.20±0.20^{e}	26.00±0.80^{f}	27.30±0.50^{ef}
pH 3	12	6.90±0.30^{g}	28.10±6.60^{e}	489.20±0.60^{d}
Control	12	7.80±0.20^{cd}	9.50±0.20^{ij}	6.20±0.20^{gh}

[A]Means ± SD of triplicate measurements. Means with different superscript letters in each column are significantly different ($P \leq 0.05$).
Nongerminated brown rice flour served as the control.

(pH 2.7) were greater than those without acetic acid (pH 6.8).The main effect of acetic acid on the dissolution of rice proteins was enhancement of the solubility of albumin, globulin and glutelin, while the effect of proteases was minor. This may be due to the effect of acidic soaking which led to a leach of acid soluble protein. However, the effect of protease enzyme on protein content of GBRF should be

interested because the optimum pH of protease was 3.5 (Li *et al.*, 2011). Protease can digest protein and becomes peptides and amino acids, more particularly glutamic acid, which is the principle amino acid in rice (Zhang *et al.*, 2006). Glutamic acid is the substrate in the production of GABA, consistent with the increased amount of free GABA as shown in Table 4 and 5. Moreover, in germinated rice grains, hydrolytic enzymes such as amylase and protease are activated by absorbed water and then decompose starch, non-starch polysaccharides or proteins (Ohtsubo *et al.*, 2005).

1.1.2 Free GABA content

Germination and storage time induce changes in the bio-functional composition of germinated cereals. Free GABA contents of both GBRFs were significantly ($P \leq 0.05$) higher than those of control (Table 4 and 5). This result agreed with the previous studies of GABA content in rice (Komatsuzaki *et al.*, 2007; Charoenthaikij *et al.*, 2009). GABA is produced primarily by the glutamate decarboxylase, GAD, which catalyses the irreversible decarboxylation of L-glutamate to GABA (Shelp *et al.*, 1999). Free GABA levels in GBRF are influenced by many factors, including the duration of storage period of paddy rice. Genetic differences among rice variety in their ability to synthesize GABA in grains are also worth evaluating, as genetic diversity is a basic prerequisite for successful exploitation of desirable traits through breeding (Karladee and Suriyong, 2012). Free GABA content of GBRFs from both rice varieties under different conditions significantly increased ($P \leq 0.05$) as the paddy rice was stored for 8 months and then significantly decreased ($P \leq 0.05$). That would be probably caused by the reduction of the substances, including glutamic acid, in the paddy rice during storage through the metabolic pathway (Songtip *et al.*, 2012).

Both GBRFs steeping brown rice grains at pH 3 and pH 6.8 provided the highest amount of free GABA (99.80 and 54.20 mg/100 g flour, respectively for GKMF and 42.50 and 33.90 mg/100 g flour, respectively for GHNF) when the paddy rice was kept for 8 months. After 8 months of storage time free GABA content, of rice steep at the above mentioned conditions, decreased. Both

GBRFs obtained from paddy rice stored for 8 months and germinated at pH 3 yielded the highest free GABA content (99.8 and 42.5 mg/100 g flour prepared from GKMF and GHNF, respectively). However, the free GABA content during 6-10 months of storage were high and ranged from 37.73-99.84 mg/100g flour for GKMF and 30.70-42.5 mg/100g flour for GHNF. The different pH conditions of steeping water had an effect on free GABA content. In the present study, during 6 to 10 months of storage times, steeping both varieties of brown rice grain at pH 3 had significantly higher ($P \leq 0.05$) free GABA content than those of pH 6.8, while free GABA content from each condition during 2 to 4 months of storage times was low and non significant. A similar result was observed by Charoenthaikij *et al.* (2009) who reported that GABA content of brown Khao Dawk Mali 105 rice stored for 8 months had the highest amount when steeping at pH 3 for 48 h compared with steeping at pH 5, distilled water at pH 6.8, and pH 7. This was perhaps due to the fact that GABA synthesis in response to H^+ is a pH-regulating mechanism (Bown and shelp, 1997). Glutamate decarboxylase (GAD) was activated by the increase in the cystosolic levels of hydrogen ions (acidic solution) (Scott-Taggart *et al*., 1999) resulting in GABA accumulation.

1.1.3 α-amylase activity

***α*-amylase** is of prime importance in the initial stages of starch degradation and seed germination (Muralikrishna and Nirmala, 2005). During cereal seed germination, ***α*-amylase** in the aleurone layer plays an important role in hydrolyzing the endosperm starch into metabolizable sugars, which provide the energy for the growth of roots and shoots (Sugimoto *et al*., 1998). The stirring number (SN) is defined as the apparent viscosity in rapid visco unit at the 180^{th} sec of stirring a hot aqueous flour suspension undergoing liquefaction. By the action of the hydrolytic *α*-amylase enzyme increases, viscosity of GBRF decreases but SN increases. Enzyme activity is also an indication of sprouting of grains (AACC, 2000). In this study, *α*-amylase activity (SN value) of non-GBRF from KMR during storage were in the range of 143-156 RVU, even though the data was significantly different

($P \leq 0.05$). This could be due to the hydrolytic enzyme still had some activity during storage of paddy rice because of the respiration of seed. Normally, hydrolytic enzymes are activated when water is absorbed for several hours or a day. The study reported by Joseph (1990) who indicated that protease and amylase enzymes were not deactivated or destroyed during storage even higher storage temperatures (up to 37°C) or longer storage times (up to 18 months). Activity of α-amylase was absent in dry seeds of rice, wheat, and barley, but it was present and rapidly increased in all three seed types, as the process of germination occurred (Lorenzo *et al*., 1995).

In this study, the α-amylase activity of GKMF soaked in pH 3 and pH 6.8 significantly ($P \leq 0.05$) increased during 8 months of storage and then decreased (Table 4 and 5). The decrease in enzyme activity in the seed lowers its respiratory potential, which in turn lowers both the energy (ATP) and food supply to the germinating seed (Lawrence and Miller, 2001). In addition, steeping KM brown rice grains at pH 3 resulted in significantly lower ($P \leq 0.05$) SN than that of other treatments for each month during storage. However, the effect of the acid used, in germination under pH 3, on the viscosity reduction of GKMF should be of concern. The SN values of GHNF did not show because the α-amylase activity of GHNF is not clear due to the high validation of Hom Nin rice variety (Appendix B).

1.1.4 Reducing sugar

Starch is destructed to become sugar in a saccharification process during germination (Charoenthaikij *et al.*, 2009). In this study, steeping conditions remarkably affected reducing sugar content of GBRF compared to those of the control (Table 4 and 5). Previous experiments have also indicated that germination markedly increased the sugar content of sprouting grains, such as brown rice (Charoenthaikij *et al.*, 2009; Ohtsubo *et al*., 2005). Paul *et al*. (1972) established a positive correlation between respiration and germination percentages, whereas a negative correlation was found between the germination percentage and storage periods. GKMF from steeping brown rice grain at pH 3 and pH 6.8 contained the highest amount of reducing sugar content (257.75 and 21846.56 mg/100g flour,

respectively) when stored paddy rice for 8 months. Both steeping conditions of GHNF had the highest amount of reducing sugar content (1231.80 mg/100g flour at pH 3 and 656.80 mg/100g flour at pH 6.8) when stored paddy rice for 10 months. The different pH conditions of steeping water affected reducing sugar content. In the present study, steeping brown rice grain at pH 3 had reducing sugar content significantly ($P \leq 0.05$) higher than those steeped at pH 6.8. The increase in reducing sugar was mainly due to amylase activity resulting in the formation of sugars and hydrolysis of starch from acidic solution (pH 3). A similar result was observed by Charoenthaikij *et al.* (2009) who reported that the maximum reducing sugar content was observed at pH 3 for 72 h (3130.63 mg/100 g flour) of brown rice (KDML 105).

1.2 Pasting properties of GBRF

Among the important practical properties of GBRF are their pasting characteristics. The results showed that the storage time of paddy rice and steeping water condition remarkably affected pasting profiles of GBRF compared to those of the control (Figure 11). Storage time had no significant effect on peak viscosity of non-GBRF from KMR (243.69-252.34 RVU). The set back of starch-based samples was quite marked. Setback has been correlated with texture of various products and also associated with syneresis (Newport Scientific, 1998). The result showed that setback value of GKMF were significantly ($P \leq 0.05$) lower than that of non-GBRF during storage period (Table 6). Steeping conditions remarkably affected the pasting profiles of GBRF compared to those of the control. Pasting temperature from GKMF obtained at pH 6.8 and pH 3 was in the same range compared to that of the control (73.20-76.00 °C). For other characteristic value of RVA viscogram, there were significant ($P \leq 0.05$) differences among GBRF prepared under varying steeping water. The peak viscosity of GKMF obtained at pH 6.8 (22.82-68.51 RVU) and pH 3 (12.65-114.58 RVU) was significantly lower than that of their controls (211.68 and 252.34 RVU, respectively) during storage times. Additionally, GKMF obtained at pH 3 stored for 8 months showed the markedly decreased in peak viscosity (12.65 RVU) compared to that of other conditions. The reduction in the viscosity caused by α-amylase activity and acid solution degraded the starch granule. Our observations

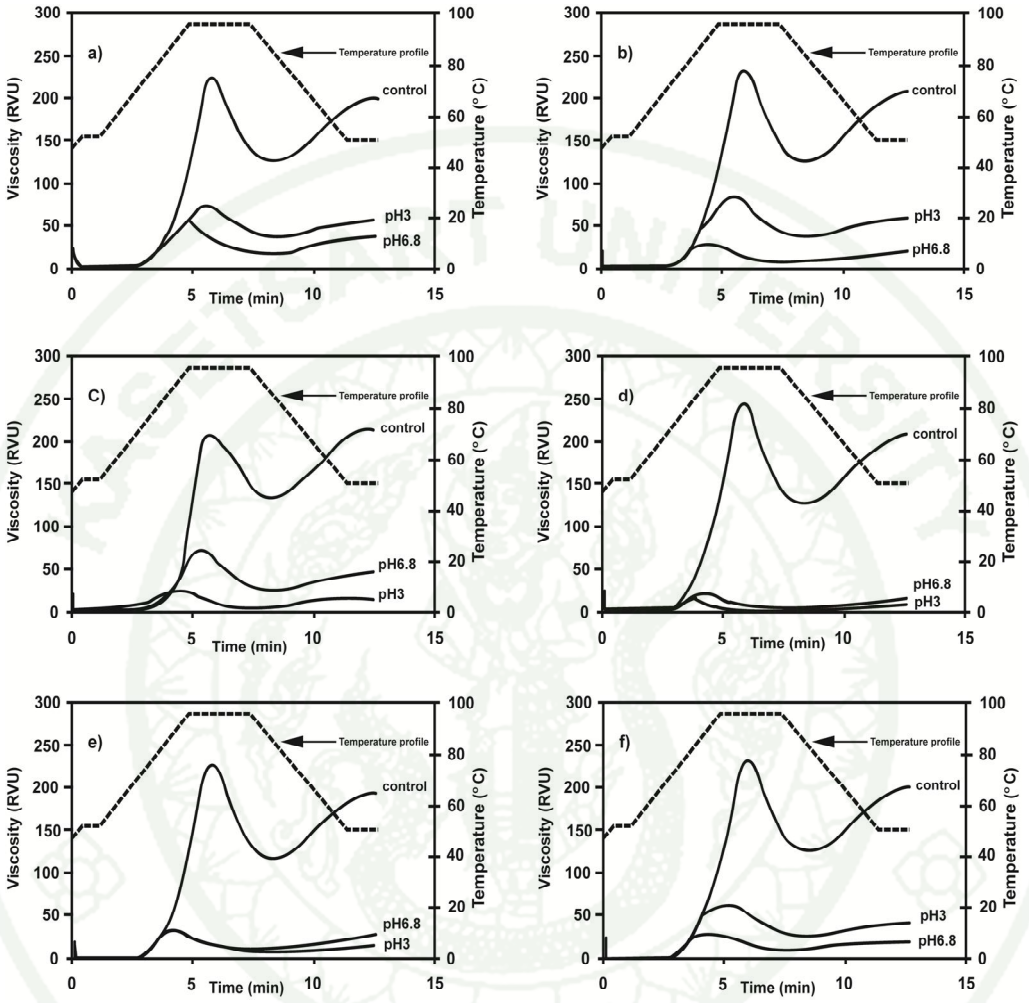

Figure 11 Pasting profile of GKMF during storage periods: 2 months (a), 4 months (b), 6 months (c), 8 months (d), 10 months (e), and 12 months (f) obtained from steeping at 35°C for 48 h in buffer solution (pH 3) and reverse osmosis water (RO, pH 6.8) compared to that of non-GBRF (control) measure by RVA.

Table 6 Pasting characteristics of GKMF as affected by various steeping condition and storage time[A].

Germination condition		Viscosity (RVU)					
Steeping condition	Storage time (months)	Pasting temperature (°C)	Peak Viscosity	Trough	Breakdown	Final viscosity	Set back from through
pH 6.8	2	71.20±2.00^{k}	68.50±1.50^{e}	18.20±0.40^{g}	34.40±0.80^{g}	41.70±0.40^{g}	23.50±0.90^{d}
pH 3	2	72.30±0.40^{ijk}	114.60 ±4.20^{c}	58.40±1.20^{d}	56.10±3.30^{e}	87.60±0.80^{e}	29.10±3.40^{d}
Control	2	73.80±0.5^{fghi}	249.60±3.70^{a}	132.80±2.10^{ab}	106.80 ±2.10^{c}	213.00±2.10^{b}	80.20±1.90^{b}
pH 6.8	4	74.60±0.50^{defg}	52.60±0.70^{f}	7.40±0.40^{ij}	21.30±1.80^{hi}	19.20 ±1.10^{ij}	11.8±1.20^{e}
pH 3	4	73.60±0.70^{ghij}	85.80±9.50^{d}	37.20±6.90^{e}	48.60±2.70^{f}	60.10±9.60^{f}	22.90±0.30^{d}
Control	4	73.20±0.50^{hij}	245.20±11.60^{a}	128.50±1.90^{b}	114.70±9.80^{b}	220.10 ±0.30^{a}	91.60±4.30^{a}
pH 6.8	6	77.00±0.60^{a}	28.70±2.30^{gh}	6.80±1.20^{ij}	17.30±1.10^{ij}	44.40±1.50^{g}	45.40±3.00^{c}
pH 3	6	73.00±0.60^{ghij}	17.00±1.50^{jk}	22.90 ±1.20^{f}	45.80±1.10^{f}	9.80±1.50^{k}	13.10±3.00^{e}
Control	6	76.00±0.60^{abcd}	243.70±1.50^{a}	129.00 ±1.20^{b}	115.50±1.10^{b}	124.70±1.50^{d}	82.6±3.00^{ab}
pH 6.8	8	74.10±0.50^{efghi}	22.80±1.00^{hij}	6.30±0.60^{ij}	16.50±0.90^{ij}	16.40 ±0.70^{jk}	10.0±0.80^{e}
pH 3	8	75.20±0.80^{abcde}	12.60±0.30^{k}	1.00±0.10^{k}	11.60 ±0.40^{j}	2.60±0.20^{l}	1.50±0.50^{f}
Control	8	75.60±0.60^{bcde}	249.60±1.30^{a}	130.80±3.30^{b}	118.80±4.60^{ab}	216.70±1.80^{ab}	85.90±3.10^{ab}
pH 6.8	10	74.90±1.00^{cdef}	34.50±0.30^{g}	8.80±0.50^{hi}	25.70±0.10^{h}	23.90±0.60^{i}	15.10±2.90^{e}
pH 3	10	76.80±0.01^{a}	26.90±1.80^{ghi}	5.80±0.50^{ij}	21.10±1.30^{hi}	12.20±1.00^{jk}	6.40±0.80^{f}
Control	10	75.60±0.70^{bcde}	246.70±0.80^{a}	119.60±1.60^{c}	117.00±1.70^{ab}	202.90 ±3.00^{c}	83.30±3.00^{ab}
pH 6.8	12	76.30±0.50^{abc}	62.80±5.70^{e}	24.00±3.60^{f}	19.60±1.20^{hi}	20.60±0.80^{ij}	12.70±1.50^{e}
pH 3	12	76.50±0.50^{ab}	27.50±1.20^{ghi}	8.00±0.70^{i}	38.80±2.40^{g}	40.50±5.20^{g}	16.50±1.30^{e}
Control	12	75.20±1.20^{bcde}	252.30±0.60^{a}	133.70±7.10^{ab}	118.70±6.50^{ab}	213.50±4.20^{b}	79.90±3.60^{b}

[A]Means ± SD with different superscript letters in each column are significantly different ($P \leq 0.05$). Nongerminated brown rice flour served as the control.

agreed with those reported by Charoenthaikij *et al.* (2009) who observed that the viscosity of GBRF obtained at pH 3 exhibited extreme decrease after 24 h of steeping time. The results of pasting properties showed a negative relationship between viscosity and ***α***-amylase activity. Furthermore, hydrolysis of starch from acidic solution (pH 3) lowered paste viscosities. The result did not show the data of pasting profile from GHNF. The α-amylase activity of GHNF is not clear due to the high validation of Hom Nin rice variety. The α-amylase activity related to the pasting properties of the flour (Appendix B).

1.3 Principal component analysis (PCA)

The PCA plots provide visualization among physicochemical properties of GBRF of each storage time under different steeping conditions compared to the control. The results of PCA from GKMF showed that the first 2 components were explained by 77.78% of total variance (65.26% and 12.52%, respectively). The first principal component (PC1) was closely related to reducing sugar, free GABA, SN, peak viscosity, trough, breakdown, final viscosity, while the second principal component (PC2) associate with pasting temperature. All conditions of GKMF were significantly different from their controls as shown in Figure 12. The bi-plot indicated that controls under different storage times were load on PC1 in the positive direction, which presented the highest pasting profiles and SN, but they had the lowest contents of free GABA and reducing sugar. The PCA plot revealed that the free GABA and reducing sugar contents were negatively related to the SN and pasting profiles (Figure 11). At different storage times, GKMF produced from paddy rice stored for 6 to 10 months provided high free GABA and reducing sugar contents but low values of pasting profiles.

The results of PCA from GHNF showed that the first 2 components were explained by 90.44% of total variance (65.86% and 24.57%, respectively) (Figure 13). The first principal component (PC1) was closely related to reducing sugar and free GABA while the second principal (PC2) component associate with crude protein. The bi-plot indicated that controls under different storage times were different from

GBRFs, which presented the highest crude protein, but they had the lowest contents of free GABA and reducing sugar. At different storage times, GHNF produced from paddy rice stored for 8 to 10 months provided high free GABA and reducing sugar contents but low values of crude protein.

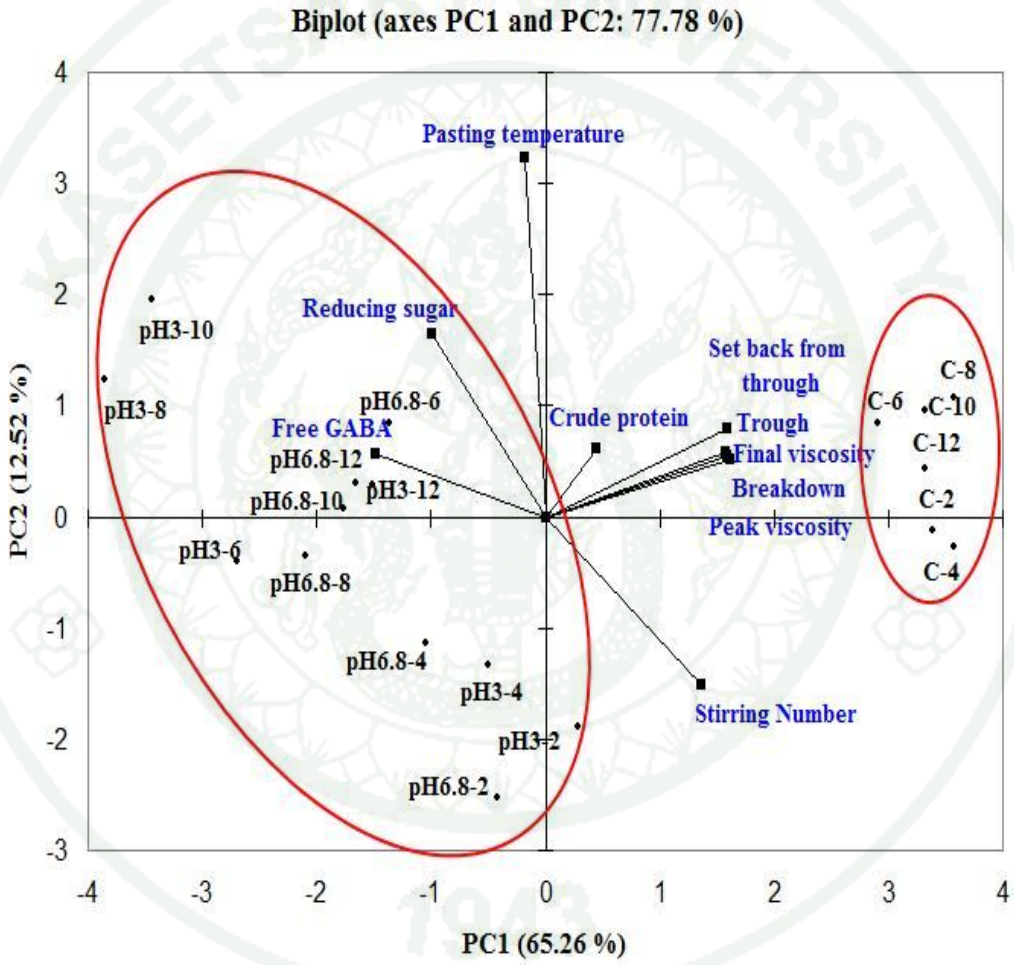

Figure 12 Principal component analysis (PCA): a PC score plot of the first principal component (PC1) and the second principal component (PC2) visualizing among GKMF and physicochemical properties. C = control; pH 6.8 = pH level of reverse osmosis water; pH3 = pH level of citrate buffer solution. The numbers 2 to 12 are different storage time of paddy rice (2 to 12 months).

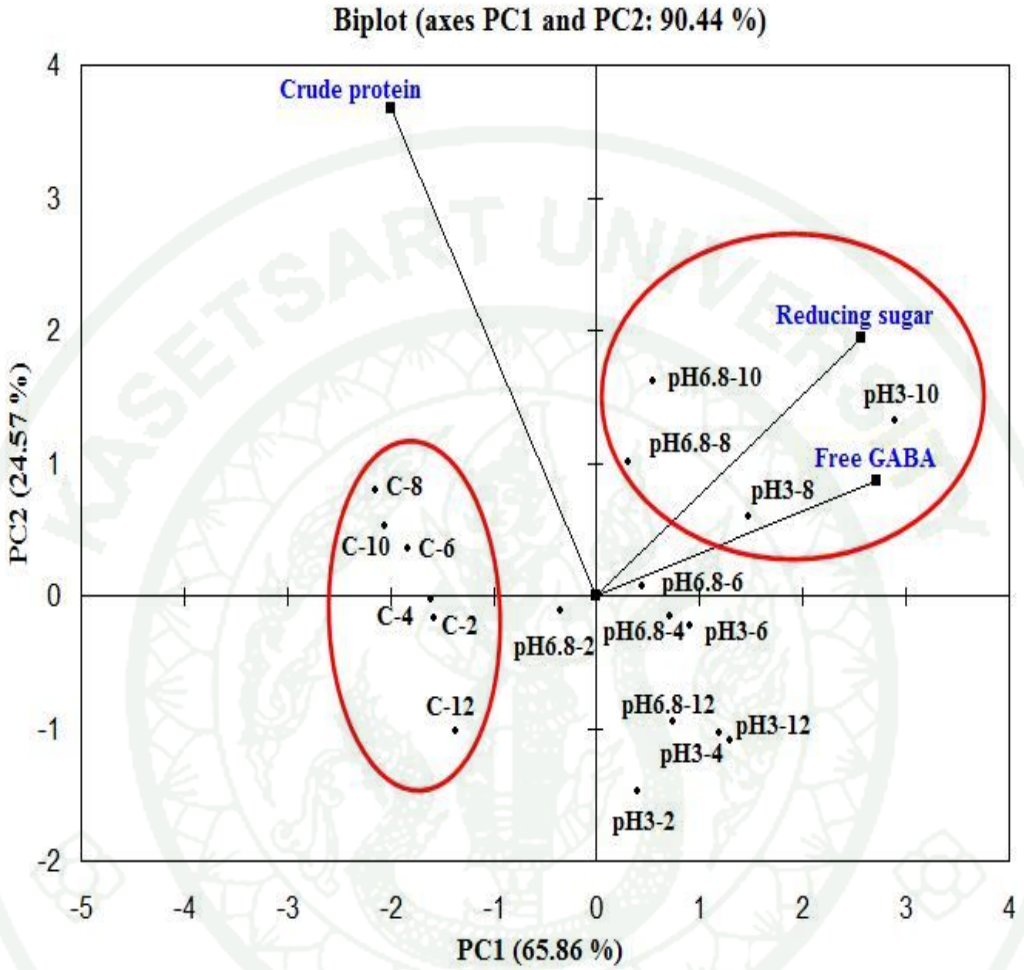

Figure 13 Principal component analysis (PCA): a PC score plot of the first principal component (PC1) and the second principal component (PC2) visualizing among GHNF and physicochemical properties. C = control; pH 6.8 = pH level of reverse osmosis water; pH3 = pH level of citrate buffer solution. The numbers 2 to 12 are different storage time of paddy rice (2 to 12 months).

2. Effect of extrusion conditions on the qualities of extruded snack from germinated brown rice flour

In this experiment, the conditions of germination were selected from the result of the study 1. The resulted showed that both GBRFs produced from paddy rice stored for 6-10 months and prepared under pH 3 had high free GABA content. However, paddy rice stored for 4 months and steeped brown rice grains at pH 3 for 48 h was used for produced extrudates. This condition still had high value of free GABA content (38.28 mg/100g flour for GKMF and 68.77mg/100g flour for GHNF) and they still contained effective dose of GABA on relaxation and immunity during stress in humans (20-30 mg/100g flour) (Nakamura *et al*., 2009). The pasting profile of GBRF before extrusion is shown in Figure 14. The peak viscosity of KN brown rice was higher than that of HN rice. This was due to the different in their genetic structure of each rice variety. The peak viscosity of both GBRFs obtained at pH 3 was lower than that of their control. This was probably due to the degradation of starch by enzyme activity during the germination process. After extrusion process the extrudates from both rice varieties were subjected to subsequence analysis.

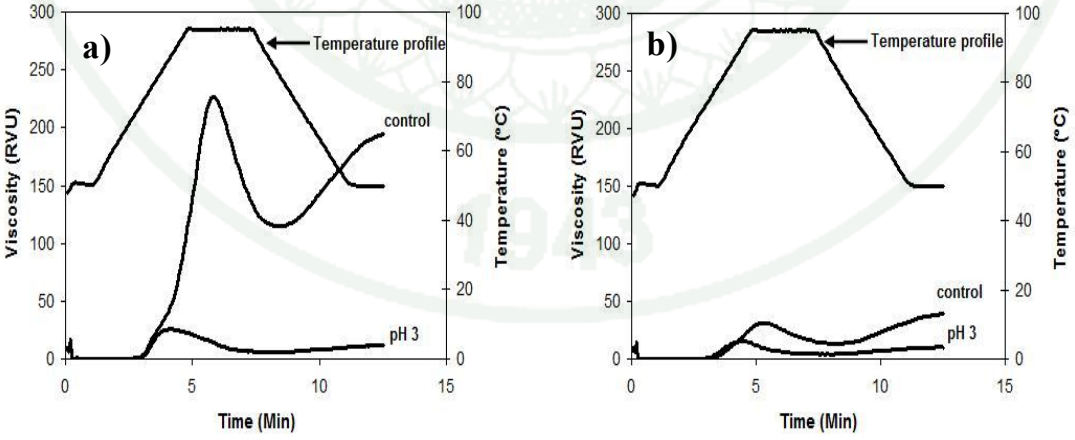

Figure 14 Pasting profile of GKMF (a) and GHNF (b) prepared from paddy rice stored for 4 months and steeped brown rice grains at pH 3 for 48 h compared with non-germinated brown rice flour.

2.1 Physical properties

2.1.1 Color

Color and dimension of GKME and GHNE by various feed moisture and screw speed levels were presented in Figure 15 and 16, respectively. The results showed that feed moisture had a significant effect on the color of the products. Increasing feed moisture content increased L* but decreased a* and b* value of extrudates from GKMF (Table 7). Decreasing feed moisture content reduced the lightness resulted in the dark yellow color of the extrudates.

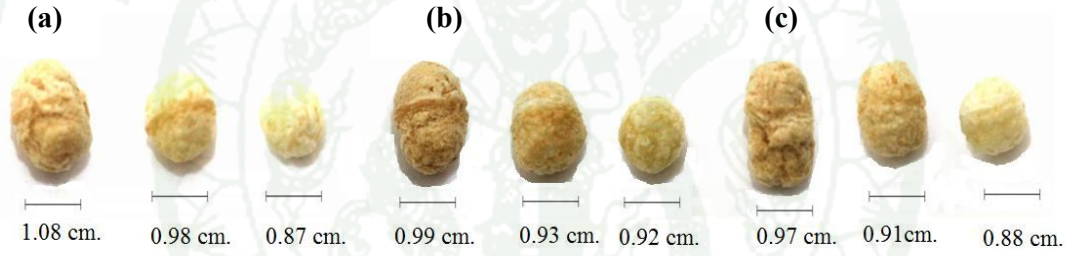

Figure 15 Color and dimension of GKME by various feed moisture (14%, 18%, and 22% respectively) and screw speed levels 300 rpm (a), 350 rpm (b), and 400 rpm (c).

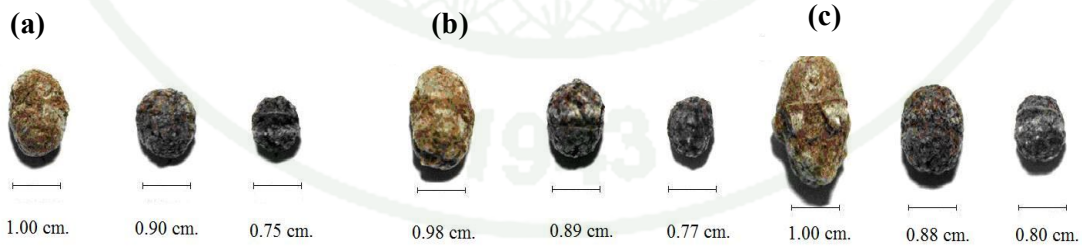

Figure 16 Color and Dimension of GHNE by various feed moisture (14%, 18%, and 22% respectively) and screw speed levels 300 rpm (a), 350 rpm (b), and 400 rpm (c).

In the present study, the Maillard reaction occurred in extrudates because of the reaction between reducing sugars and free amino groups from soy protein isolate,

which raw material mixer. Therefore, the dark brown color that appeared in the extrudates from GKMF under low feed moisture condition. The lowest L* value (70.71) of the extrudates from GKMF was found at 14% of feed moisture and 400 rpm of screw speed. The Maillard reaction may produce colored or colorless reaction products depending on the stage of the reaction as well as other factors such as pH, temperature and water activity (Yilmaz and Toledo, 2005). On the other hand, a higher feed moisture content lead to decrease L* value of extrudates from GHNF (Table 8). It is the fact that extrudates from GHNF has based dark purple color (Figure 16), consequently at higher feed moisture produced the product with more dense, deep purple color and less lightness.

2.1.2 Bulk density and expansion ratio

Bulk density is an index of the extent of puffing. The result revealed that feed moisture content had a significant effect while the screw speed was observed to have a slight impact on the bulk density of extrudates. These values of extruded snacks from GKMF and GHNF were ranged from 0.05 to 0.20 g/cm^3 and 0.11 to 0.29 g/cm^3, respectively (Table 7 and 8). The higher moisture content of flour mixture produced the higher bulk density of the extruded product from both rice varieties. The bulk density increased with increasing feed moisture from 14 to 22 %. A similar observation was found in extrudate of rice flours reported by Anastase *et al*. (2006) and also found in defatted soy-rice blend extrudates (Garg and Singh, 2010). At higher moisture content, the specific mechanical energy input decrease resulting in a lower motor torque, a lower product temperature and die pressure (Ryu, 2004). Therefore, these conditions generated lower steam which produced less product puffing. In this study, both GKME and GHNE produced at the lowest feed moistuer and screw speed caused the lowest value of bulk density (0.05 g/cm^3 for GKME and 0.11 g/cm^3 for GHNE).

Table 7 Physical properties of GKME by various feed moisture and screw speed levels.

Feed moisture (%)	Screw speed (rpm)	Color			Bulk density (g/cm^3)	Expansion ratio	Hardness (N)
		L*	a*	b*			
14	300	72.82± 0.10^{d}	9.50± 0.00^{a}	26.72± 0.10^{b}	0.05± 0.01^{f}	4.30± 0.15^{a}	15.83± 1.00^{f}
18	300	78.32± 0.40^{b}	6.38± 0.20^{d}	24.11± 0.50^{d}	0.10± 0.02^{de}	3.94± 0.13^{bc}	19.72± 2.70^{e}
22	300	79.40± 0.30^{a}	3.51± 0.10^{g}	21.25± 0.40^{g}	0.20± 0.03^{a}	3.48± 0.13^{e}	24.95± 1.20^{a}
14	350	70.76± 0.10^{e}	9.84± 0.00^{a}	27.09± 0.10^{a}	0.07± 0.02^{ef}	3.96± 0.18^{bc}	16.76± 0.80^{f}
18	350	75.74± 0.10^{c}	8.23± 0.10^{b}	25.82± 0.20^{c}	0.11± 0.02^{de}	3.70± 0.30^{de}	21.15± 1.10^{cde}
22	350	78.59± 0.10^{b}	4.71± 0.00^{e}	23.87± 0.10^{e}	0.16± 0.03^{b}	3.67± 0.13^{e}	21.62± 2.40^{bcd}
14	400	70.71± 0.10^{e}	9.72± 0.10^{a}	27.08± 0.10^{a}	0.08± 0.02^{e}	3.88± 0.17^{cd}	20.31± 1.00^{de}
18	400	76.46± 0.10^{c}	7.76± 0.10^{c}	25.47± 0.30^{c}	0.12± 0.01^{d}	3.65± 0.30^{e}	22.21± 3.10^{bc}
22	400	79.34± 0.10^{a}	4.04± 0.10^{f}	22.30± 0.10^{f}	0.14± 0.03^{c}	3.51± 0.13^{e}	23.10± 1.00^{b}

Means ± SD of ten measurements with different superscript letters in each column are significantly different ($P \leq 0.05$).

Table 8 Physical properties of GHNE by various feed moisture and screw speed levels.

Feed moisture (%)	Screw speed (rpm)	Color			Bulk density (g/cm^3)	Expansion ratio	Hardness (N)
		L*	a*	b*			
14	300	52.15± 0.20^{a}	8.24± 0.10^{b}	12.77± 0.10^{b}	0.11± 0.01^{h}	3.95± 0.26^{b}	17.66± 0.70^{bcd}
18	300	46.92± 0.50^{b}	7.21± 0.10^{e}	7.04± 0.20^{d}	0.17± 0.02^{f}	3.60± 0.13^{d}	18.39± 1.00^{bc}
22	300	40.64± 0.40^{d}	7.31± 0.10^{d}	5.56± 0.20^{g}	0.29± 0.04^{a}	3.08± 0.17^{f}	20.47± 2.70^{a}
14	350	51.55± 0.20^{a}	8.30± 0.00^{b}	12.79± 0.10^{b}	0.14± 0.05^{g}	3.90± 0.28^{bc}	14.38± 1.50^{f}
18	350	45.14± 0.30^{b}	7.32± 0.10^{d}	6.69± 0.10^{e}	0.19± 0.03^{e}	3.53± 0.14^{d}	18.23± 2.20^{bc}
22	350	39.20± 0.30^{d}	7.22± 0.10^{e}	5.46± 0.10^{g}	0.26± 0.04^{b}	3.09± 0.17^{f}	19.36± 3.20^{ab}
14	400	52.20± 0.10^{a}	8.42± 0.00^{a}	13.71± 0.10^{a}	0.17± 0.03^{f}	4.14± 0.46^{a}	15.32± 1.00^{ef}
18	400	47.89± 0.20^{b}	7.44± 0.10^{c}	7.56± 0.20^{c}	0.20± 0.01^{d}	3.55± 0.12^{d}	16.28± 0.80^{de}
22	400	42.44± 0.20^{c}	7.39± 0.10^{cd}	5.95± 0.20^{f}	0.24± 0.06^{c}	3.19± 0.23^{e}	17.00± 2.40^{cde}

Means ± SD of ten measurements with different superscript letters in each column are significantly different ($P \leq 0.05$).

Screw speed generally has a negative effect on extrudate density. It was probably due to the increase in shear, and thus decrease in melt viscosity induced by high screw speeds (Ding *et al*., 2005; Rodríguez-Miranda *et al*., 2011). Expansion ratio is an important characteristic of extruded snacks. This index describes the degree of puffing undergone by the sample as it exits the die of the extruder. From the experiment, the expansion ratio for extruded snacks from GKMF and GHNF were ranged from 3.51 to

4.30 and 3.08 to 4.14, respectively (Table 7 and 8). Feed moisture has been found to be the main factor affecting extrudate expansion and, which is consistent with this work. As the feed moisture increased (14 to 22 %), the expansion ratio of extruded snacks from both varieties decreased. Low moisture contents of the starch may restrict the material flow inside the extruder barrel, increasing the shear rate and residence time, which would perhaps increase the degree of starch gelatinization, and thus, the expansion.

Hagenimana *et al*. (2006) found that expansion ratio decreased with increasing feed moisture from 16% to 19% and 22% when rice flour was extruded in a twin-screw extruder. This agrees with an observation by Ding *et al*. (2006) who reported that increasing feed moisture content resulted in rice extrudates with a lower expansion ratio and a higher density. The water acts as a plasticizer to the starch-based material reducing its viscosity and the mechanical energy dissipation in the extruder and thus the product becomes dense and bubble growth is compressed, leading to a less expanded product (Ding *et al*., 2005).

2.1.3 Texture hardness

Product hardness from GKME and GHNE was in the range of 15.83 to 24.95 N and 14.38 to 20.47 N, respectively. Increasing of feed moisture content caused an increase in hardness. The data showed that hardness was the highest (24.95 N and 20.47 N from GKME and GHNE, respectively) at 22% of feed moisture content and 300 rpm of screw speed (Table 7 and 8). In fact, extrudates from dough with higher moisture contents were harder after cooling than those with lower moistures (characterized by porous, expanded and sponge like structures) (Hagenimana *et al*., 2006). A vapor pressure is low when extruded under high feed moisture, probably due to a reduced barrel temperature, resulting in a lower flashing of moisture and finally in a reduced expansion ratio and increased hardness of the extrudate. The increase in hardness may have been a consequence of the decreased expansion and increased bulk density at the higher feed moisture content. A parallel effect was found in expanded rice products (Ding *et al*., 2005; Chanlat *et al*., 2011).

The screw speed had only significant effect ($P \leq 0.05$) on the hardness of GHNE. An increase in screw speed resulted in a decrease in hardness. An increase in screw speed may be expected to lower the melt viscosity of the raw material mixture resulting in a less dense, softer extrudate.

2.2 Chemical properties of extrudates

2.2.1 Free gamma-aminobutyric acid (GABA) content

The result showed that the free GABA content of extrudates was significantly affected by feed moisture content. The free GABA content from both of GBRFs before extrusion were significantly ($P \leq 0.05$) higher than those of extrudates (Table 9 and 10). Through this study, reduction of free GABA content for GKMF and GHNF were on the range of 21-59% and 14-41%, respectively compared to GBRFs from each rice verity before extrusion. The reduction in the free GABA content is probably caused by the high temperature at low feed moisture extrusion conditions resulting in the destruction of the compounds. Different free GABA content from both rice varieties was dependent on the variety of rice and methods of cultivation. Free GABA content of extruded snacks from GKMF and GHNF were ranged from 15.68 to 38.28 mg/100g extrudate and 40.08 to 58.07 mg/100g extrudate, respectively. This study found that increasing the feed moisture increased retention of free GABA content (Table 9 and 10). This may be attributed to the higher moisture content of feed, which could reduce destruction of the free GABA compound during extrusion. Moreover, the presence of a greater amount of moisture in the raw material mixture would lead to a gentler processing in the extruder barrel (Ozer *et al.*, 2006).

Table 9 Chemical properties of GKME by various feed moisture and screw speed levels.

Feed moisture (%)	Screw Speed (rpm)	Free GABA (mg/100g extrudate)	TPC (mgGAE/100g extrudate)	DPPH (mg GAE/100g extrudate)	FRAP (mg $FeSO_4$ /100g extrudate)
14	300	23.27±1.10^{c}	34.47±0.70^{b}	4.78±0.01^{c}	1040.14±3.60^{b}
18	300	26.25±2.30^{b}	13.80±2.10^{e}	2.23±0.02^{f}	500.76±6.30^{g}
22	300	30.30±4.70^{a}	4.32±0.60^{g}	0.83±0.00^{h}	413.03±2.10^{i}
14	350	15.68±0.90^{e}	35.90±0.10^{b}	6.57±0.00^{b}	1274.78±8.40^{a}
18	350	22.35±0.40^{c}	10.26±0.00^{f}	3.05±0.00^{e}	595.50±4.20^{c}
22	350	27.75±0.80^{b}	5.07±0.20^{g}	1.52±0.00^{g}	559.42±3.70^{f}
14	400	19.33±1.40^{d}	42.59±0.30^{a}	7.31±0.00^{a}	695.05±7.50^{c}
18	400	25.71±1.30^{bc}	20.94±2.50^{c}	3.95±0.00^{d}	623.85±4.20^{d}
22	400	22.62±1.30^{c}	17.33±0.10^{d}	1.62±0.00^{g}	430.70±5.60^{h}
*Control		38.28±0.50	90.33±3.40	8.03±0.00	1363.49±4.20

Means ± SD of triplicate measurements with different superscript letters in each column are significantly different ($P \leq 0.05$).

*Germinated brown rice flour before extrusion served as the control.

The highest free GABA content in the extrudate samples obtained from production parameter of 22% feed moisture and 300 rpm of screw speed for GKMF and 22% feed moisture and 350 rpm of screw speed for GHNF, under the lowest severe condition. However, Chanlat *et al.* (2011) found that feed moisture content (15.6-22.3%) and screw speed (264-434 rpm) at 130°C of barrel temperature had no significant effect on the GABA content of extruded snack made from pre-germinated brown rice. This is in agreement with Ohtsubo *et al.* (2005) who reported that GABA content of extruded germinated brown rice (30.01 mg/100g) did not

Table 10 Chemical properties of GHNE by various feed moisture and screw speed levels.

Feed moisture (%)	Screw Speed (rpm)	Free GABA (mg/100g extrudate)	TPC (mgGAE/100g extrudate)	DPPH (mg GAE/100g extrudate)	FRAP (mg $FeSO_4$ /100g extrudate)
14	300	41.34±0.50^{d}	49.65±0.10^{b}	8.28±0.20^{b}	963.76±7.40^{b}
18	300	54.99±4.20^{ab}	45.29±0.60^{c}	4.37±0.00^{g}	675.59±5.70^{c}
22	300	55.94±4.70^{ab}	11.69±0.30^{g}	3.68±0.00^{h}	615.58±4.20^{d}
14	350	41.88±1.20^{d}	48.83±0.00^{b}	7.99±0.01^{c}	978.53±7.10^{b}
18	350	54.45±0.10^{ab}	31.61±0.10^{d}	4.89±0.00^{e}	575.10±2.08^{e}
22	350	58.07±1.60^{a}	25.15±0.00^{e}	2.53±0.00^{i}	361.54±2.10^{g}
14	400	40.08±1.00^{d}	69.13±0.80^{a}	8.80±0.00^{a}	1183.47±9.40^{a}
18	400	52.00±2.80^{c}	49.58±2.50^{b}	5.38±0.01^{d}	621.63±7.20^{d}
22	400	57.43±3.40^{a}	21.39±0.50^{f}	4.47±0.01^{f}	469.72±9.70^{f}
*Control		67.88±5.80	149.27±0.50	8.85±0.00	1272.98±18.40

Means ± SD of triplicate measurements with different superscript letters in each column are significantly different ($P \leq 0.05$).
*Germinated brown rice flour before extrude served as the control.

decreased compared with unprocessed germinated brown rice (32.05 mg/100g) by twin screw extruder condition (150 °C of barrel temperature, 100g/min of feed rate and 150 rpm of screw speed). The screw speed had significant effect ($P \leq 0.05$) on the free GABA content of GKME while GHNE had no significant effect.

The free GABA content decreased with increasing screw speed from 300 to 400 rpm. It was attributed to the higher screw speed produced shear to obtain high temperature during the extrusion process. This result showed that GKME produced at severe condition (14% of feed moisture and 400 rpm of screw speed) obtained the extrudate with the lowest free GABA content (40.08 mg/100g flour).

Severe extrusion conditions can cause nutritional destruction. Generally, high extrusion temperature (≥ 200 °C) and low moisture content (≤ 15%) should be avoided to maintain nutritional quality (Singh *et al*., 2006).

2.2.2 Total phenolic compound (TPC)

Plant phenolics are a major group of compounds performing as primary antioxidants or free radical scavengers. Anton *et al.* (2009) reported that extrusion process resulted in the losses of phenolics, tocopherols, carotenoids, anthocyanins, flavonoids, tannin, etc., which were considered natural antioxidants in raw material and processed products. This research also agreed with the result of this study showing that extrusion cooking caused degradation of TPC. Significant differences ($P \leq 0.05$) of TPC values were found between GBRF before extrusion and extrudate (Table 9 and 10). In this study, reduction of TPC content for GKME and GHNE were on the range of 49.86-95.22% and 53.69-92.17%, respectively compared to GBRFs from each rice verity before extrusion. On the other hand, Zielinski *et al*. (2001) found that free phenolic acid (i.e., ferulic acid) of rye extrudates increased under extrusion cooking (process temperature of 120°C, 160°C and 200 °C) compared to rye before extrusion. This could be due to the fact that cereal grains contain phenolic acids and glycosides, in both free and bound forms. Free phenolic acids are found in outer layer of the pericarp and bound pheneolic acids are esterified to the cell walls (Ganil *et al*., 2012). Accordingly, the liberated free phenolic acids and their derivatives may contribute higher TPC and antioxidant activity of the extrudates.

Both GKME and GHNE obtained from production parameter of the highest feed moisture content (22%) at all screw speed levels had the lowest TPC content compared to other feed moisture content at the same screw speed level. Phenolic compounds during extrusion may undergo decarboxylation due to high barrel temperature (Dlamini *et al*., 2007). Moreover, at higher moisture content probably promote polymerization of phenols leading to reduce extractability (Remy *et al*., 2000). The highest TPC in both GKME and GHNE obtained from production parameter of 14% feed moisture and 400 rpm of screw speed, under the severe

condition compared to other conditions. The increase in total phenolic content could be explained, at least partially, by the formation of Maillard reaction products with phenolic type structure during extrusion process (Sahin *et al*., 2009).

Between rice varieties the result showed that extrudates from GHNF contained TPC higer than that of GKMF. The type and concentration of polyphenols in the rice grain vary among genotypes and are related mainly to the pericarp color. Normally, grains with red and black pericarp color have a higher concentration of phenolic compounds compared to those with a light brown pericarp color (Tian *et al*., 2004). It can be concluded that the TPC depends both on the rice varity and on the process conditions.

2.2.3 Antioxidant activity

The antioxidant activity of most food is caused typically by phenolic compounds (Singh *et al*., 2006). There are many methods for total antioxidant determination, and every one has its limitations (Yu *et al*., 2002). Some of these antioxidant assays give different antioxidant activity trends (Ou *et al*., 2002). Therefore, in order to receive reliable results, two other complemented assays for the determination of the total antioxidant activity were used. It can be measured by DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP), based on the reaction of the reagent with antioxidant compounds that are electron-donating or produce hydrogen radicals (Huang *et al*., 2005).The antioxidant activity was also significantly affected by extrusion process, which reduced DPPH radical scavenging activity and FRAP of extrudates made from both GBRFs. Significant differences ($P \leq 0.05$) of DPPH radical scavenging activity and FRAP values were found between GBRF before extrusion and extrudate (Table 9 and 10). Due to the loss of original antioxidants by heating impact, extrusion was reported to consequently reduce the antioxidant activities in the extrudate products (Anton *et al*., 2009; Repo-Carrasco-Valencia *et al*., 2009). This might be a result of the reduction of TPC in extrudates during extrusion. Similar changes in the amounts of phenolic acids were observed.

All the analyzed samples of both rice extrudates obtained from the lowest feed moisture (14%) and the highest screw speed (400 rpm) contained higher antioxidant of DPPH radical scavenging activity and FRAP than extrudates processed at the other conditions (Table 9 and 10). The process conditions used in extrusion cooking-high temperatures in combination with low water content are known to favour the Maillard reaction (Singh *et al*., 2006). Moreover, the antioxidant properties of heated foods can be maintained or even enhanced by the development of new antioxidants, such as Maillard reaction products or non-enzymatic browning. (Pokorny and Schmidt, 2006). Maillard reaction product, especially melanoidins, have been reported to have antioxidant activity through scavenging oxygen radicals or chelating metals (Yilmaz and Toledo, 2005). In addition, extrudates made from GHNF had higher antioxidant activity than that of GKMF. Thus, it is prudent to conclude that the effect of extrusion on bioactive compounds is cultivar dependent. In this study, it was found that colored rice (HNR) contain more antioxidant activity than non-colored rice (KMR).

2.3 Sensory acceptability of extrudates

The statistical evaluation of sensory properties of the extrudate samples (uncoated flavor) made from GKMF and GHNF were presented in Table 11 and Table 12, respectively. Feed moisture content was the most important parameter affecting the sensory properties of extrudates. The samples with the lowest feed moisture content (14%) had lower liking scores of shape attribute than the higher feed moisture content samples (18 and 22%) due to its uneven shape and very blister (Figure 15 and 16).

Table 11 Sensory properties of extruded snack made from GKMF.

Feed moisture (%)	Screw speed (rpm)	Shape	Hardness	Taste	Overall liking
14	300	6.3±1.0^{a}	6.6±1.1^{a}	5.4±1.4^{ab}	6.0±1.0^{ab}
18	300	6.8±1.1^{a}	6.9±1.0^{a}	5.5±1.4^{ab}	6.6±0.8^{a}
22	300	4.5±1.0^{cd}	4.6±1.4^{c}	4.4±1.4^{d}	4.6±1.3^{cd}
14	350	5.4±0.9^{b}	6.5±0.9^{ab}	5.3±1.4^{abc}	5.6±1.2^{b}
18	350	6.7±1.3^{a}	6.5±1.2^{ab}	5.9±1.3^{a}	6.4±1.1^{a}
22	350	4.4±1.2^{d}	4.3±1.6^{c}	4.4±1.3^{d}	4.3±1.3^{d}
14	400	4.4±1.3^{d}	5.9±1.1^{b}	4.6±1.1^{cd}	4.9±0.9^{cd}
18	400	6.8±0.9^{a}	6.9±0.8^{a}	5.4±1.3^{ab}	6.2±0.9^{a}
22	400	5.0±1.0^{bc}	5.0±1.4^{c}	4.9±1.4^{b}	5.0±1.0^{c}

Means ± SD based on 30 consumer responses and on a 9-point hedonic scale, wherein 1 = dislike extremely, 5 neither dislike nor like and 9 = like extremely. Means with different superscript letters in each column are significantly different ($P \leq 0.05$).

The highest levels of feed moisture (22%) tended to obtain products with low liking scores of hardness; this was likely due to its small diameter (0.87-0.92 cm for GKME-105 and 0.77-0.80 cm for GHNE) and dense in texture. This confirmed by the previous reult in Table 7 and 8 showed that high feed moisture content increased the hardness of extrudates. An increase in hardness may have been a result of the decreased expansion at the higher feed moisture content. This was likely due to the pressure differential smaller for higher moisture extruded foods, leading to a less expanded product (Singh *et al*., 2007). The hardness of extrudates is a perception of the consumer and is related with the expansion and cell structure of the product (Ding *et al.,* 2005). The taste liking score of the uncoated extruded product from both rice varieties ranged from 4.4 (slightly dislike) to 6.0 (slightly like).

Table 12 Sensory properties of extruded snack made from GHNF.

Feed moisture (%)	Screw speed (rpm)	Shape	Hardness	Taste	Overall liking
14	300	5.2±1.6^{d}	6.0±1.8^{bc}	5.2±1.2^{bc}	5.3±1.2^{b}
18	300	7.5±1.0^{a}	7.2±1.4^{a}	6.0±1.0^{a}	6.7±1.1^{a}
22	300	6.0±1.5^{c}	4.2±1.5^{d}	4.5±1.2^{d}	4.7±1.3^{c}
14	350	4.9±1.3^{d}	6.1±1.6^{b}	4.9±1.5^{cd}	5.2±1.2^{b}
18	350	7.3±0.9^{ab}	7.4±1.0^{a}	6.0±1.3^{a}	6.5±1.0^{a}
22	350	7.0±1.0^{ab}	5.3±1.9^{c}	5.0±1.4^{cd}	5.1±1.5^{b}
14	400	4.2±1.4^{e}	6.0±1.4^{bc}	4.7±1.3^{cd}	4.7±1.1^{b}
18	400	6.8±1.0^{b}	7.3±1.0^{a}	5.8±1.3^{ab}	6.6±1.1^{a}
22	400	6.8±1.0^{b}	5.3±1.6^{e}	4.7±1.2^{cd}	5.2±1.4^{b}

Means ± SD based on 30 consumer responses and on a 9-point hedonic scale, wherein 1 = dislike extremely, 5 neither dislike nor like and 9 = like extremely. Means with different superscript letters in each column are significantly different ($P \leq 0.05$).

The acceptability scores for all sensory attributes (shape, hardness, taste and overall liking) f extrudates processed at 18% of feed moisture, regardless of screw speed, were higher than extrudates at other feed moisture levels. This product acceptability showed that extrusion process can produce nutritious extruded snack from GBRF with high free GABA and acceptable for product user. Thus, further studies on investigation of coating extruded snack with syrup and flavor are required to increase acceptable score.

2.4 Optimization and verification of extrusion condition of snacks made from germinated brown rice flour

To identify the optimum condition that suitable for producing high nutritious snack and acceptable to consumer, the interesting physicochemical properties of extrudates are free GABA content and antioxidant activity. According to free GABA is the main unique substance in GBRF and its function is good for health such as enhance brain function, prevent Alzheimer's disease and treat sleeplessness (Komatsuzaki *et al*., 2007; Hayakawa *et al*., 2002). Antioxidant activity in the product should be concerned as it can reduce risk of cancer. However, the result showed that high antioxidant activities of extrudates were found at the low levels of feed moisture in extrusion condition producing uneven shape and dark color that consumers were not acceptable. For this reason, free GABA content and overall liking scores were used to obtain the optimum region in this study.

Table 13 exhibits the regression equation describing the effect of extrusion variables on the free GABA and overall liking of both rice extrudates. Good predictive models with high R^2 values of free GABA and overall liking scores were 0.94 and 0.99 for GKME and GHNE, respectively. The coeffcients in the regression equation can be used to examine the significance of independent variables relative to each dependent variable. Statistical analysis showed that feed moisture (X_1) had a greater effect on the free GABA and overall liking values than the screw speed (X_2). The optimisations of physicochemical and sensory properties were done using response surface methodology (RSM) (Figure 17 and 18). The predetermined cut-off criterion was arbitrarily set at free GABA content from both rice varieties of $\geq$ 25 mg/100 g extrudate for the reason that effective dose of GABA on relaxation and immunity during stress has been investigated in humans were the range of 20-30 mg/100 g (Nakamura *et al.*, 2009); however, % daily value was not established.

Table 13 The predictive regression models for free GABA content and overall liking score of extruded snack made from two rice varieties (GKME and GHNE).

Properties	Predictive model [A]	[B]R^2
GKME		
Free GABA	$140.592+1.496\ X_1-0.769\ X_2-0.004\ X_1^2+0.001X_2^2-0.001X_1Xx_2$	0.94
Overall liking	$-20.0974+3.009\ X_1-0.0013X_2-0.0971X_1^2-3.3333*10^{-5}X_2^2+0.0014X_1X_2$	0.99
GHNE		
Free GABA	$-322.7872+10.8002\ X_1+1.510X_2-0.2664\ X_1^2-0.0023X_2^2+0.0034\ X_1X_2$	0.95
Overall liking	$-0.3299+2.264\ X_1-0.0688X_2-0.0849\ X_1^2+4.2667*10^{-5}X_2^2+0.002X_1X_2$	0.98

[A] Equation of regression : $Y_i = b_0+b_1X_1+b_2X_2+b_{11}X_1^2+b_{22}X_2^2+b_{12}\ X_1X_2$

The responses function (Y_i) measured were free GABA and overall liking.

The coded variables were X_1= feed moisture and X_2 = screw speed.

The coefficients of the polynomial were represented by b_0 (constant term), b_1 and b_2 (linear effects), b_{11} and b_{22} (quadratic effects) and b_{12} (interaction effects),

[B]R^2 = correlation of determination.

Overall liking scores from both rice varieties were rated as ≥ 6.0 (slightly like on a 9-point hedonic scale) considered to be acceptable. Based on these constrains, the optimum ranges of variables obtained from the contours plot of GKMF were 16-19% of feed moisture levels, and 300-310 rpm of screw speed (shown by the shaded area in Figure 17). Within this optimum range, the characteristics for GBRF extrudates were free GABA content of 25.0-30.0 mg/100g and overall liking score was “slightly like” to “moderately like” (6.0-6.7).

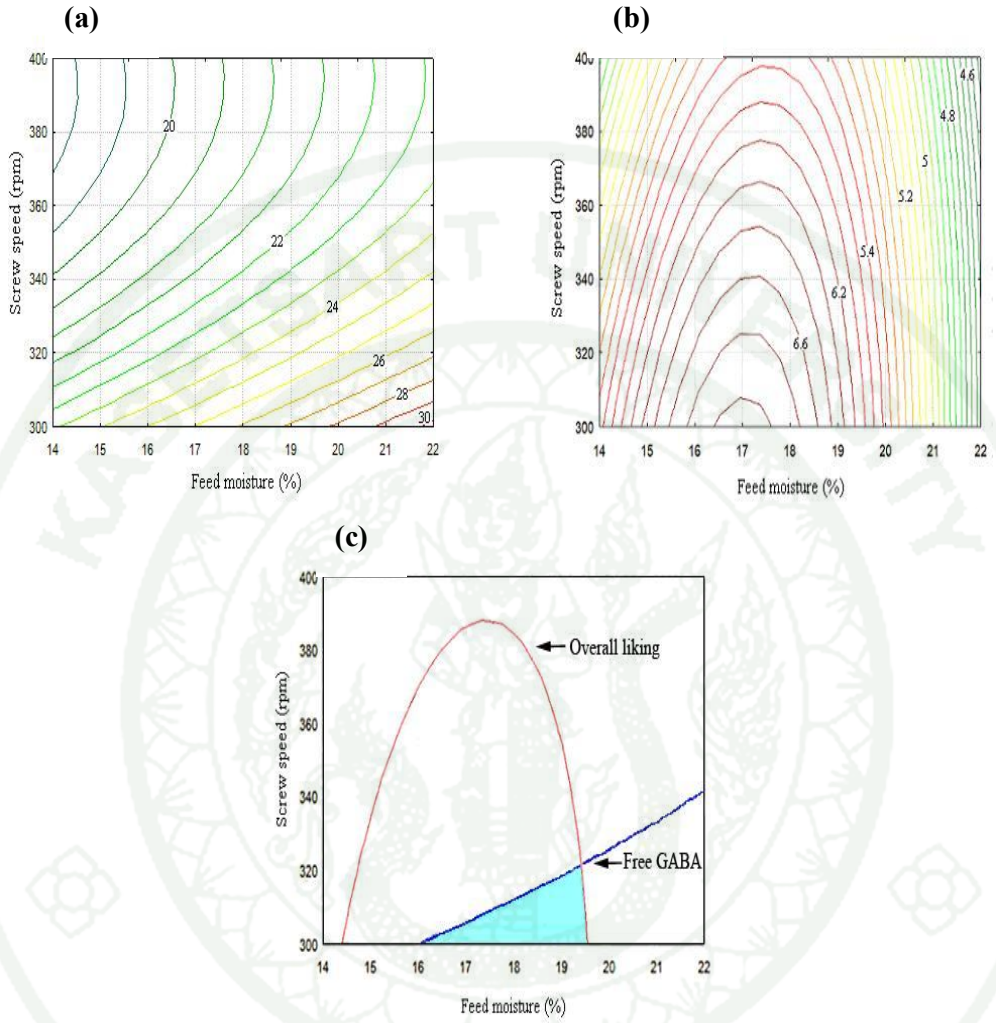

Figure 17 Contour plots of the free GABA (a), overall liking (b), and optimum condition area (c) from GKME.

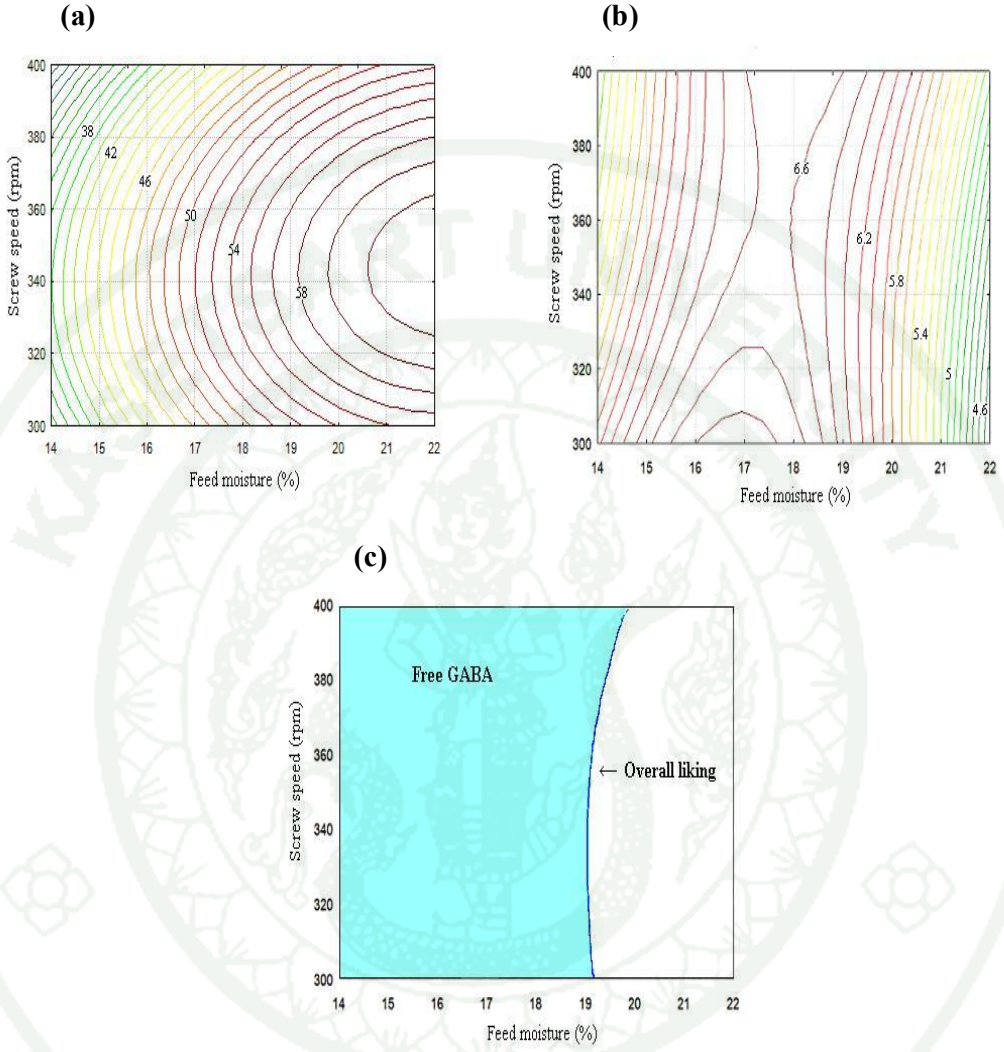

Figure 18 Contour plots of the free GABA (a), overall liking (b), and optimum condition area (c) from GHNE.

The optimum ranges of variables obtained from the contours plot of GHNF were 14-19% of feed moisture levels, and 300-400 rpm of screw speed (shown by the shaded area in Figure 18). Within this optimum range the characteristics for GBRF extrudates were free GABA content of 50.0-54.0 mg/100g and overall liking score was “slightly like” to “moderately like” (6.0-6.7). Extrusion condition was carried out for confirmation under the optimum process conditions (Table 14).
The model suitability was evaluated using the correlation coefficient (r) and the root

mean square error (RMSE). The observed and predicted values of extruded snack from selected conditions for verification of optimized region. The r value under all conditions from both rice varieties was more than 0.90. The RMSE values of free GABA and overall liking of GKME were 1.58 and 0.46, respectively) and those of GHNE were 24.822 and 0.478, respectively (Table 14). Therefore, the regression model could be used to predict reasonably well for the free GABA content and overall liking score of the extruded snack made from GKMF.

Table 14 Observed and predicted values of extruded snack from two rice varieties (GKME and GHNE) by selected conditions for verification of optimized region.

Properties	Predicted*		Observed		r**		RMSE***	
Feed moisture (%) Screw speed (rpm)	GKME	GHNE	GKME	GHNE	GKME	GHNE	GKME	GHNE
Free GABA								
15%, 350 rpm	23.56	43.88	23.90	21.69				
18%, 300 rpm	30.20	53.47	27.90	25.98	0.99	0.93	1.58	24.82
19%, 310 rpm	30.17	51.61	28.74	27.11				
Overall liking								
15%, 350 rpm	6.02	6.17	6.10	6.20				
18%, 300 rpm	6.81	6.66	6.20	6.10	0.99	0.90	0.46	0.48
19%, 310 rpm	6.70	6.64	6.20	6.03				

**r = The correlation coefficient.

***RMSE = Root mean squared error between observed and predicted values.

However, there are high validations in HN rice variety so the regression model could not be used to predict the GABA content of the extruded but the regression model could be used to predict reasonably well in the overall liking.This study demonstrated the feasibility of producing highly nutritious extruded snacks from GBRF.

3. Effect of barrel temperature on physicochemical properties of extruded snack from germinated brown rice flour fortified with crude xanthone powder

In this study, crude xanthone powder was fortified in GBRF with the objective of an improvement of antioxidant value. However, crude xanthone added in food product is not allowed by Food and Drug Administration of Thailand; therefore, the results of physicochemical properties from this part were evaluated except for sensory evaluation. Additionally, the resut of the physicochemical properties change in extrudates fortified with crude xanthone powder would be useful to the manufacturer or further study. From the previous result, the extrusion condition to produce extrudates made from GBRF (90.17 %) fortified with crude xanthone powder (0.36%), soy protein isolate (9.02 %) and $CaCO_3$ (0.45 %). The extrusion process was 18% of feed moisture (both rice varieties) and 300 rpm of screw speed for GKMF and 350 rpm of screw speed for GHNF. The physicochemical properties of extruded snacks from both GBRFs fortified with crude xanthone powder were analyzed as follow.

3.1 Physical properties of extrudates fortified with crude xanthone powder

3.1.1 a_w and color

The changes in the properties of extruded snack from both GBRFs fortified with crude xanthone powder were presented in Table 15. The a_w of extrudates ranged from 0.22 to 0.24 for GKME and from 0.19 to 0.21 for GHNE. a_w of extrudates from both rice varieties decreased with increased barrel temperature. This could be due to the fact that the water molecules in the starch are heated up by high temperature and change into a vapor. Moisture and a_w content of extruded product was found to be directly related to feed moisture and inversely related to extrusion temperature in previous work (Suknark *et al*., 1997). Decrease in a_w would be advantageous with regard to their shelf-life and stability of the extrudate against microbial growth. The color of extrudates fortified with crude xanthone powder had

more yellow color than extrudates from only GBRF, that caused by dark yellow pigment of crude xanthone powder (Figure 19). This observation confirmed by the previous result in section 2.1.1 shown yellowness (b* value) of extrudate from both rice varieties (Table 7 and 8). The b* value of extrudate from GBRF without crude xanthone powder was lower than that of extrudate fortified with crude xanthone powder. The results showed that barrel temperature had significant effect on the color of the products (Table 15).

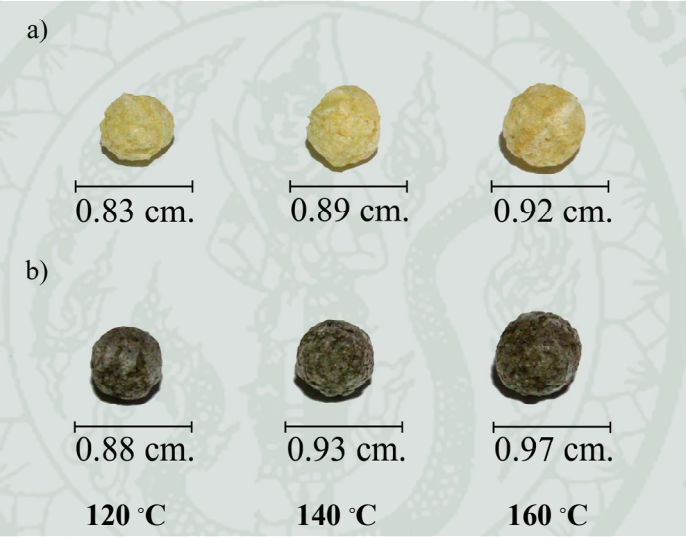

Figure 19 Color and dimension of GKME (a) and GHNE (b) fortified with crude xanthone powder at different barrel temperature levels (120°C, 140°C, and 160°C) at section 6.

Table 15 Physical properties of extruded snack made from GKMF and GHNF fortified with crude xanthone powder at different barrel temperature levels.

Germinated brown rice flour	Barrel temperature (°C)	a_w	Color			Bulk density (g/cm^3)	Expansion ratio	Hardness (N)
			L*	a*	b*			
GKMF	120	0.26±0.00[a]	76.97±0.40[a]	0.39±1.10[c]	37.92±0.20[a]	0.14±0.01[a]	3.31±0.03[c]	18.82±3.10[a]
	140	0.23±0.00[b]	76.51±0.10[b]	1.09±0.10[b]	36.81±3.50[b]	0.11±0.00[b]	3.55±0.74[b]	15.93±1.00[b]
	160	0.22±0.00[c]	75.52±0.20[c]	2.45±0.10[a]	36.67±0.20[c]	0.07±0.00[c]	3.69±0.77[a]	14.00±1.20[c]
GHNF	120	0.21±0.00[a]	53.60±0.20[a]	3.98±0.10[a]	14.43±0.20[a]	0.13±0.02[a]	3.54±0.06[c]	17.60±1.30[a]
	140	0.20±0.00[b]	53.07±0.40[b]	3.40±0.10[b]	14.40±0.20[a]	0.09±0.01[b]	3.71±0.06[b]	16.66±0.80[b]
	160	0.19±0.00[c]	52.97±0.20[b]	3.36±0.10[b]	14.00±0.10[b]	0.06±0.01[c]	3.88±0.13[a]	16.28±1.50[b]

Means ± SD of triplicate measurements for a_w and ten measurements for color, bulk density, expansion ratio and hardness.

For each variety of rice, means with different superscript letters in each column are significantly different ($P \leq 0.05$).

Increasing barrel temperature caused a significant decrease in lightness (L* value) and yellowness (b* value) of the extrudates from both rice varieties. The lowest L* value (75.52 and 52.97 from GKME and GHNE, respectively) and b* value (36.67 and 14.00 from GKME and GHNE, respectively) of the extrudates was found at the highest temperature of extrusion condition (160°C). This fact was probably due to Maillard reaction development according to extrusion temperature. In the present study, the redness (a* value) of the extrudates from GKME was enhanced by the high barrel temperature that promote browning reaction. In contrast, increasing barrel temperature tended to decrease a* value of GHNE. This might be due to degradation of anthocyanin at high temperature (Patrs *et al.*, 2010).

3.1.2 Bulk density and expansion ratio

An inverse relationship between bulk density and expansion ratio of extrudates has been earlier reported (Hagenimana *et al*., 2006; Ding *et al.*, 2006) as observed in this work (Table 15). The bulk density of the extrudate from three levels of barrel temperature ranged from 0.07-0.14 g/cm^3 for GKME and 0.06-0.13 g/cm^3 for GHNE. Increasing temperature gave low bulk density and high expansion ratio. Expansion ratio of extrudates (3.69 for GKME and 3.68 for GHNE) was greatest with the lowest a_w (0.22 for GKME and 0.19 for GHNE) under the highest barrel temperature (160°C). These relationships have been reported elsewhere for corn- and wheat-based snacks (Bhattacharya and Hanna, 1987; Ito *et al*., 1999). An increase in the barrel temperature decreased the melt viscosity, which was confirmed by the report of Mercier and Feillet (1975) that extrudate viscosity decreased with increased temperature. The reduced viscosity effect would favour the bubble growth during extrusion. Moreover, the degree of superheating of water in the extruder would increase at higher temperatures, also leading to greater expansion. The reduction of rice extrudate density caused by increased barrel temperature was also observed in this experiment. This likely caused by higher barrel temperature increases the extent of gelatinization and also the content of superheated steam that causes the rice extrudate to expand more, consequently leading to the production of a low bulk

density (Guha and Ali, 2006). The expansion ratio of extruded products ranged from 3.31-3.69 for GKME and 3.54-3.88 for GHNE.

3.1.3 Texture hardness

Barrel temperature also had a significant effect on the hardness of both rice extrudates. The hardness of extrudates from both rice varieties ranged from 14.00 N to 18.82 N (Table 15). Increasing temperature decreased the hardness of the extrudates. During the extrusion process, increasing temperature would decreased melt viscosity, which favoured the bubble growth and produced low density of extrudate with small and thin cells, thus decreasing the hardness of extrudate (Ding *et al*., 2005). The results showed that the lowest hardness (14.00 N for GKME and 16.28 N for GHNE) was found at the highest of barrel temperature (160 °C). Low hardness, which was also a favored property of extrudates, was observed at high barrel temperature. This research investigated the correlation coefficient (r) of barrel temperature and physical properties of extruded snack fortified with crude xanthone powder. The r value is a measure of the strength of the straight-line or linear relationship between two variables. The r values range between +1 (indicates a perfect positive linear relationship) and -1 (indicates a perfect negative linear relationship). This result showed that correlation of barrel temperature was positively related to the expansion ratio (r =0.99 for GKME and 1.00 for GHNE) and negatively related to a_w (r = -0.96 for GKME and -1.00 for GHNE), bulk density (r = -0.96 for GKME and -1.00 for GHNE) and hardness (r = -0.99 for GKME and -0.97 for GHNE) of extruded snack made from both rice varieties fortified with crude xanthone powder.

3.2 Chemical properties of extrudates

3.2.1 Free gamma-aminobutyric acid (GABA)

In this study, the free GABA content of both rice extrudates was significantly affected by barrel temperature. The free GABA content of GBRF fortified with crude xanthone powder before extrusion (non-extrudate) from both rice

varieties was significantly ($P \leq 0.05$) higher than those of extrudates fortified with crude xanthone powder. The reduction of free GABA content of GKME prepared at 120°C, 140°C and 160°C of barrel temperature was 22.52%, 26.50% and 28.49%, respectively compared to non-extrudates. For GHNE, the reduction of free GABA content was 39.97%, 40% and 41.72%, respectively compared to non-extrudates. The reduction of the free GABA content in extrudates is probably caused by the high temperature of extrusion conditions resulting in the destruction of the compounds. However, increasing of barrel temperature from 120 to 160°C had no significantly ($P > 0.05$) effect on free GABA content of GHNE. Free GABA content of GKME and GHNE was ranged from 24.10 to 26.11 mg/100g extrudate and 21.36 to 22.03 mg/100g extrudate, respectively (Table 16).

3.2.2 α-mangostin content

The chemical structure of xanthone forms the central core of a variety of naturally occurring organic compounds, such as mangostin, which are sometimes collectively referred to xanthones. Over 200 xanthones have been identified. Several studies have reported that xanthones particularly α- mangostin, a major xanthone, exhibits antioxidant (Jung *et al*., 2006; Chomnawang *et al*., 2007; Chen *et al*., 2008). The result showed that α-mangostin content of extrudates was significantly affected by extrusion process (Table 16). However, information on the effect of extrusion cooking on xanthones in snack product has been very limited. Although xanthone from mangosteen rind has also recently been used to produce

Table 16 Chemical and antioxidant properties of GKME and GHNE fortified with crude xanthone powder at different barrel temperature levels.

Rice varieties	Barrel temperature (°C)	Free GABA (mg/100g extrudate)	α-mangostin (mg/100g extrudate)	TPC (mg/100g extrudate)	DPPH (mg/100g extrudate)	FRAP (mg/100g extrudate)
KM	120	26.11±0.80^{a}	23.49±0.60^{a}	40.87±0.80^{b}	5.25±0.20^{c}	326.95±9.60^{a}
	140	24.77±0.60^{b}	21.89±1.30^{ab}	44.06±1.20^{ab}	5.26±0.01^{c}	331.91±7.50^{a}
	160	24.10±1.30^{b}	21.14±0.70^{ab}	45.04±0.10^{a}	6.07±0.02^{b}	382.12±4.20^{a}
	*Control	33.70±0.70	104.29±0.80	153.10±0.10	9.41±0.00	1388.43±34.40
HN	120	22.03±0.90^{a}	12.97±0.30^{a}	43.59±0.20^{c}	6.24±0.00^{c}	378.67±3.60^{c}
	140	21.99±0.80^{a}	12.80±0.50^{a}	50.93±1.30^{b}	6.41±0.00^{bc}	529.00±4.40^{b}
	160	21.36±0.90^{a}	12.59±0.70^{ab}	52.65±0.10^{a}	6.57±0.01^{b}	627.49±2.10^{a}
	*Control	36.65±0.80	43.18±0.10	184.16±1.10	9.23±0.00	1425.48±153.60

Means ± SD of triplicate measurements. For each variety of rice, means with different superscript letters in each column are significantly different ($P \leq 0.05$). *GBRF fortified with crude xanthone powder before extrusion served as the control.

various products such as dietary supplement products, beverages as well as antiseptic goods and herbal cosmetic, in many contries. Only one research studied by Suvarnakuta *et al.* (2011) is available on the study of drying methods on antioxidant activity of xanthones in mangosteen rind.

The results in this study showed that the contents of α-mangostin in extrudates significantly decreased during extrusion process due to thermal degradation. Increasing barrel temperature tended to decrease the α-mangostin content but it was not significantly different ($P>0.05$). Through this study, the reduction of α-mangostin content of GKME prepared at 120°C, 140°C and 160°C of barrel temperature was 77.48%, 76.89%, and 79.73% respectively compared to non-extrudates. In case of GHNE, the reduction of α-mangostin content was 69.96%, 70.36% and 70.84% %, respectively. The α-mangostin content of GKME and GHNE ranged from 21.14 to 23.49 mg/100g extrudate and 12.59 to 12.97 mg/100g extrudate, respectively. A similar result was observed for degradation of xanthones (i.e., α-mangostin and γ-desoxygartanin) and their antioxidant activity in mangosteen rind by drying methods (hot-air drying, vacuum drying and low-pressure superheated steam drying) at 60°C, 75°C and 90°C (Suvankuta *et al.*, 2011).

3.2.3 Total phenolic compound (TPC)

Mangosteen has been shown to contain a variety of phenolic compounds such as condensed tannins, anthocyanins, xanthones and their derivatives (Chin *et al.*, 2008; Maisuthisakul *et al.*, 2007). This confirmed by the previous result in section 2.2.2 shown that extrudates from both GBRFs fortified with crude xanthone powder had higer TPC than those of extrudates without crude xanthone powder. The TPC of GKME fortified with crude xanthone powder (44.06 mg GAE/100 g extrudate) and without crude xanthone powder (13.80 mg GAE/100 g extrudate in study 2) in the same extrusion condition at 18% of feed moisture and 140°C of barrel temperature and 300 rpm. The TPC of GHNE fortified with crude xanthone powder (50.93 mg GAE/100 g extrudate) higher than GHNE without crude xanthone powder

(31.61 mg GAE/100 g extrudate in study 2) produced at the same extrusion condition (18% of feed moisture, 140°C of barrel temperature and 350 rpm). The TPC of extrudates from both rice varieties fortified with crude xanthone powder was significantly ($P \leq 0.05$) lower than those of non-extrudates. This could be due to thermal degradation. In addition, this decrease may be due to the decarboxylation of phenolic acids during extrusion which may promote polymerisation of phenols leading to reduce extractability and antioxidant activity (Repo-Carrasco-Valencia *et al*., 2009; Dlamini *et al*., 2007). Yagci and Gogus (2010) also observed that barrel temperature caused significant decrease in total phenolic content of rice-based extruded foods.

The extrudates from GHNF had higher TPC than that of GKMF. The difference in the concentration of phenolic compounds between rice varieties related to the pericarp color. Many studies have reported that black rice (i.e., HNR) is more abundant in anthocyanin and other phenolic compounds compared to that of white rice (i.e., KMR) (Zhang *et al*., 2006; Sutharut and Sudarat, 2012). In the present study, higher barrel temperature levels increased retention of TPC in both GKME and GHNE. The retention of TPC in GKME was 26.69%, 28.78% and 29.42% at 120, 140 and 160 °C, respectively. In case of GHNE, the retention of TPC was 23.67%, 27.66% and 28.59%, respectively. Furthermore, an increase in temperature increases the rate of development of Maillard reaction products (i.e., melanoidins) acts as antioxidant. This result was the same trend as described about TPC in the previous result section 2.2.2. A similar increase in TPC was reported after extrusion cooking of sweet potato (Shih *et al*., 2009) and cereal based ready-to-eat expanded snacks (Stojceska *et al*., 2008).

3.2.4 Antioxidant activity

Xanthones and their derivatives have been reported to have high antioxidant activity (Jung *et al*., 2006; Okonogi *et al*., 2007). This study confirmed by the previous result of antioxidant activity in section 2.2.2 shown that antioxidant activity from both extrudates (Table 9 and 10) was lower than that of extrudates

fortified with crude xanthone powder (Table 16) compared to the same extrusion condition (barrel temperature at section 6 =140°C). The antioxidant activity was also significantly affected by extrusion process, which reduced DPPH radical scavenging activity and FRAP of extrudates made from both GBRFs. Significant differences ($P \leq 0.05$) of DPPH radical scavenging activity and FRAP values were found between GBRF before extrusion and extrudates (Table 16). It is indeed well recognized that thermal processing affects natural antioxidants in plant materials.

Antioxidant activity of extruded prodcts is dependent not only on the level of bioactive compounds but also on the composition of bioactive compounds. Most of the bioactive compounds are temperature sensitive and barrel temperature plays a significant role in the stability of these bioactives. Furthermore, the increase in antioxidant activity in extrudates under severe extrusion (high level of barrel temperature) may be explained by Maillard reaction. An increase in temperature increases the rate of development of Maillard reaction product (Davis and Labuza, 2003). Maillard reaction products have been reported by several authors to inhibit lipid oxidation in food products and acts like antioxidant (Wang *et al*., 2011). The mechanism of antioxidative action of Maillard reaction products postulate that the products are also effective in reducing peroxides and inactivating radicals as well as in complexing of heavy metals (Yilmaz and Toldeo, 2005).

4. Develop formulation of syrup coating for extruded snack from germinated brown rice flour

Based on the results of the optimization of extrusion condition in study 3, 18% of feed moisture at 300 and 350 rpm of screw speed were selected for produce extruded snack from GKMF and GHNF, respectively. Syrup coating formulations were developing from basic formulations from Institute of Food Reseach and Product Development as shown in the method of section 4. Syrup coating formulations for extruded snack from GKMF and GHNF consisted of glucose syrup, sucrose, coconut sugar, salt, margarine, water and butter milk cream flavor as shown in Table 17.

Table 17 Syrup coating formulations of GKME and GHNE.

Formulation composition (%)	Extruded snack	
	GKME	GHNE
Glucose syrup	6.25	5.65
Sucrose	37.50	33.90
Coconut sugar	15.00	13.56
Water	31.25	33.90
Salt	0.62	0.56
Margarine	6.88	6.21
Butter milk cream flavor	2.50	3.40
Cocoa powder	-	2.82
Total	100.00	100.00

In case of GHNE cocoa powder were added because the color of product is dark. Cocoa powder could improve products with good color and flavor.

This study with reasons of the improvement of taste and acceptance in term of all sensory attributes include two sections: the first part was serve extruded snack without milk (color, aroma, flavor, crispness and sweetness) and the second part was serve extruded snack with milk (aroma, flavor, crispness, sweetness and overall liking). To improved the product attributes, the just about right (JAR) for the attributes of extruded snack from GBRF was explored in term of percentage. The product attributes with more than 70% of JAR means accepted by consumers were not needed to be improved.

For the product attributes with less than 70% of JAR, they were analyzed further using binomial test in order to improve the products. The extruded snacks from both GBRFs coating with syrup was conducted to the consumer acceptance test by 9-point hedonic scale as demonstrated in Table 18 and 19.

Table 18 Hedonic score and frequency of Just about right (JAR) of GKME.

Attributes	Hedonic score	Just About Right (%)		
		Not enough	About Right	Too much
Serve without milk				
Color	7.5±0.7	-	-	-
Aroma	7.5±0.9	6.7	86.7	6.7
Flavor	7.7±0.9	3.3	93.3	3.3
Crispness	7.7±0.7	3.3	96.7	-
Sweetness	7.6±0.8	6.7	83.3	10.0
Serve with milk				
Aroma	7.5±0.9	10.0	90.0	-
Flavor	7.6±0.7	13.3	86.7	-
Crispness	7.6±1.1	3.3	83.3	13.3
Sweetness	7.7±1.0	20.0	80.0	-
Overall liking	7.7±0.8			

Means ± SD based on 30 consumer responses and on a 9-point hedonic scale, wherein 1 = dislike extremely, 5 = neither dislike nor like and 9 = like extremely.

Table 19 Hedonic score and frequency of Just about right (JAR) from GHNE.

Attributes	Hedonic score	Just About Right (%)		
		Not enough	About Right	Too much
Serve without milk				
Color	7.6±0.8	-	-	-
Aroma	7.3±0.9	16.7	76.7	6.7
Flavor	7.6±0.8	10.0	83.3	6.7
Crispness	7.9±0.8	-	100.0	-
Sweetness	7.7±0.8	6.7	90.0	3.3
Serve with milk				
Aroma	7.5±1.0	23.3	76.7	-
Flavor	7.5±0.9	26.7	73.3	-
Crispness	7.9±0.8	6.7	86.7	6.7
Sweetness	7.7±0.8	20.0	76.7	3.3
Overall liking	7.7±0.8			

Means ± SD based on 30 consumer responses and on a 9-point hedonic scale, wherein 1 = dislike extremely, 5 = neither dislike nor like and 9 = like extremely.

The result of sensory evaluation indicated that liking scores of all sensory attributes and overall liking from both rice varieties ranged from to 7.3-7.9 (like moderately). Each sensory attribute was “just right” with JAR scores of more than 70%. Therefore, the product did not have to be improved and then this formula was brought to consumer test.

5. Quality evaluation of developed snack from germinated brown rice flour

5.1 Determination of the qualities of developed snack from germinated brown rice flour

The developed snack from both GBRFs containing 90.5% of GBRF (obtained from steeped at pH 3 for 48 h), 9.05% of soy protein isolate and 0.45% of $CaCO_3$. The extrusion conditions to produce extruded snack obtained high GABA content and acceptable for consumer were 18% of feed moisture (for both rice varities), 300 rpm of screw speed for GKMF and 350 rpm of screw speed for GHNF. After that coating extrudates with syrup as described in the previous result section 4 to obtained the developed snack. The quality of developed snack (Figure 20) was determined and described as follows (Table 20).

Based on the estimation, the cost of the extruded snack from GBRF including cost of raw materials plus other variable costs such as man power and the electricity expenses (exclude the packaging cost) would be approximately 5.44 bath/30 g and 6.82 bath/30 g for GKME and GHNE, respectively (Appendix E Appendix Tables E1-E5).

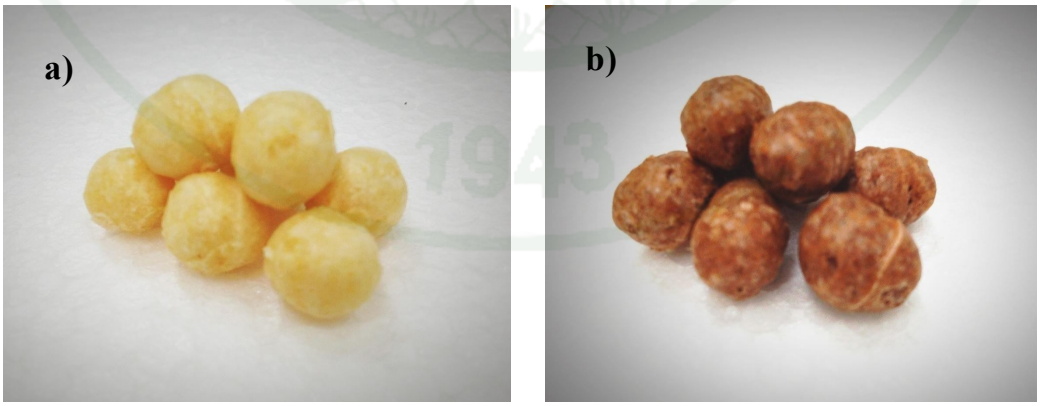

Figure 20 Developed snack made from GKMF (a) and GHNF (b).

Table 20 Physical, chemical, microbiology qualities and proximate composition of developed snack made from GKMF and GHNF.

Qualities	GKME	GHNE
Physical qualities		
a_w	0.25±0.00	0.25±0.00
L* (lightness)	78.30±0.28	45.14±0.26
a* (redness)	6.42±0.20	7.32±0.09
b* (yellowness)	24.46±0.52	6.69±0.10
Bulk density (g/cm^3)	0.10±0.01	0.10±0.03
Expansion ratio	3.84±0.13	3.83±0.14
Hardness (N)	21.50±1.62	16.16±1.26
Water absorption index (g swollen gel/g)	2.89±0.15	2.60±10.10
Water soluble index (%)	30.50±0.10	34.28±1.16
Chemical qualities		
Free GABA (mg/100g extrudate)	27.90±2.31	48.07±1.64
TPC (mg GAE/100g extrudate)	13.80±2.08	31.61±0.13
DPPH (mg GAE/100g extrudate)	2.24±0.07	4.89±0.07
FRAP (mg $FeSO_4$/100g extrudate)	500.76±6.29	575.10±2.08
Microbiological qualities		
Total plate count (cfu/g)	$< 2.5 \times 10^2$	5.5×10^2
Yeast & mold (cfu/g)	< 10 (est.)	< 10 (est.)
Proximate composition		
Moisture (%, wb)	2.41	2.46
Crude protein (%,db)	11.00	11.00
Crude fat (%,db)	2.27	2.80
Ash (%,db)	1.00	1.00
Crude fiber (%,db)	0.50	0.50
*Carbohydrate (%,db)	85.23	84.70

Means ± SD of triplicate measurements. wb= wet basis, db = dry basis.

*Carbohydrate content obtained from the calculation of

100%-(% crude protein+ % crude fat+ % crude fiber+ % ash, db).

The result of physical qualities showed both developed snack has the same value of a_w, bulk density and expansion ratio. The color lightness (L* value) and yellowness (b* value) of developed snack from GKMF were higher than those of GHNF while the redness (a* value) was lower. The different color value of the developed snack depened on the pigment from rice. In addition, extruded snack from GHNF added cocoa powder (dark brown color) gave the product lower lightness and yellowness value but higher redness value.

The commercial brands of brand A and brand B were selected to compare with this developed product because they have the shape, texture quality similar to that of this developed product. Both commercial brands of brand A and brand B had texture hardness value of 17.00 and 20.00 N, respectively, water absorption index (WAI) 2.54 and 2.96 g swollen gel/g, respectively and water soluble index (WSI) 36.50 and 32.64 %, respectively. In comparison with these two commercial brands, it was found that developed snacks made from GKMF and GHNF had texture hardness (16.00 and 21.00 N, respectively), water absorption index (WAI) (2.60 and 2.89 g swollen gel/g, respectively) and water soluble index (WSI) (30.50 and 34.28 %, respectively) were in the same rage of those two commercial brands. The WAI measures the volume occupied by the extrudate starch after swelling in excess water, which maintains the integrity of starch in aqueous dispersion (Ding *et al*., 2005). The WAI of extruded products is related to the level of gelatinization of starch and to the length of the starch chains (Kandan *et al*., 2003). The WAI increase with an increase in gelatinization. The WSI is a measure of the extent the extrudate is solubilized during hydration and often used as an indicator of degradation of molecular components. The degradation of starch increase caused WSI increased. Sing *et al*. (2007) reported that WAI and WSI of rice grits increased by extrusion cooking. Similar results were reported by Mercier and Feillet (1975) who found that soluble starch increased after extrusion.

5.3 Chemical qualities of the developed snack

5.3.1 Free GABA content

Germinated brown rice flours used as the main ingredient in this part were obtained from rice harvested in 2010 and kept paddy rice for 4 month (free GABA content of 36.84 and 58.60 mg/100g flour for GKMF and GHNF).
The developed snack had higher free GABA content than the one from 2009 (average free GABA content from all the year round of 23.70 and 36.40 mg/100g extrudate for GKME and GHNE, respectively). The different amounts of GABA content found among the rice varieties are mainly caused by their genetic constitution (Karladee and Suriyong, 2012). In addition, various factors including harvest time of rice, rice quality, environment factors during storage rice and cooking process are affected the free GABA content.

The developed snack contained free GABA of 27.90 and 48.07 mg/100 g extrudate prepared from GKMF and GHNF. Thus, the free GABA content in developed snack had been lost about 24.27 % and 17.97% for GKME and GHNE, respectively. That might be mainly due to the application of high temperature during extrusion process and drying process after coated syrup. Moreover, if we consume one serving of developed snack (30 g) we would acquire 8.36 and 14.42 mg/serving of free GABA from GKME and GHNE, respectively. As free GABA is a compound of bio-functionality, this could become a promising method of developing a GABA-rich snack product, while also enhancing GBR consumption in Thailand as well as in all others rice consuming countries.

5.3.2 Total phenolic content and antioxidant activity

The TPC and antioxidant activity of the developed snack are given in Table 20. The result showed that developed snack from GHNF had higher TPC, DPPH radical scavenging actiactivity and FRAP than those qualities from GKMF. The TPC of GKME and GHNE was 31.61 and 13.80 mg GAE/100g

extrudate, respectively. DPPH radical scavenging actiactivity of GKME and GHNE was 2.24 and 4.89 mg GAE/100g extrudate, respectively. FRAP of GKME and GHNE was 500.76 and 13.8575.10 mg GAE/100g extrudate, respectively. In fact, the kernel colour (black purple) which is formed by deposits of anthocyanins as a potent source of TPC and antioxidants (Yawadio *et al*., 2007). This indicated the interesting of the local rice varieties (KM and HN) for adding nutritional value to functional snack products.

5.3.3 Proximate composition

The chemical qualities showed that developed snack from both rice varieties contained moisture content less than 4% according to the specification of the crispy cereal-based snacks (Thai Industrial Standard, 2008). The result showed that both developed snacks had protein content (11%) that came from GBRF and soy protein isolate. Developed snack from GHNE has a little bit higher of fat content than that of GKME this was probably due to the diffent composition of rice varieties. Rujirapisit *et al.* (2012) reported that Hom nin rice had a little bit higher fat content (3.0 mg/100 g) than Khao Dawk Mali 105 (2.4 mg/100g). Moreover, the coating syrup formulation of GHNE had cocoa powder that contain about 20-22% fat. This might be the reson of higher fat content in GHNE while GKME without cocoa powder in the syrup formulation.

5.4 Microbiological quality

Total plate count of developed snacks was less than $2.5x10^2$ cfu/g. Yeast & mold count were less than 10 cfu/g (est.) which was considered safe for consumption (Thai Industrial Standard, 2008).

5.5 Sensory evaluation of the developed snack

Sensory evaluation of the developed snack was presented in Table 21. The result of sensory evaluation indicated that liking scores of all sensory attributes

and overall liking from both rice varieties ranged from 7.5-7.8 (like moderately to like very much).

Table 21 Sensory evaluation of the developed snack made from GKMF and GHNF.

Attributes	GKME	GHNE
Serve without milk		
Color	7.8±0.6	7.5±0.8
Aroma	7.5±0.9	7.5±0.9
Flavor	7.6±0.5	7.5±0.7
Crispness	7.8±0.8	7.7±1.0
Sweetness	7.8±0.9	7.8±0.8
Serve with milk		
Aroma	7.5±1.0	7.5±0.9
Flavor	7.6±0.9	7.6±0.7
Crispness	7.8±0.8	7.6±1.0
Sweetness	7.7±0.6	7.7±0.9
Overall liking	7.6±0.6	7.4±0.8

Means ± SD based on 30 consumer responses and on a 9-point hedonic scale, wherein 1 = dislike extremely, 5 = neither dislike nor like and 9 = like extremely.

6. The consumer acceptance of extruded snack prepared from germinated brown rice flour

Participating consumers in Thailand (n=200) were 32.50% male and 67.50% female; between 18 and 60 years of age whereas in the US. (n=100) 59.00% were male and 41.00% were female; between 18 and 60 years of age. Consumer acceptability scores from Thai and US consumers and purchase intent of GKME and GHNE are shown in Table 22. The results demonstrated that the developed extruded snack from both GBRFs was acceptable by both Thai and US. consumers.

Table 22 Consumer acceptability scores from Thai and US consumer and purchase intent of GKME and GHNE.

Attributes	Thai		US	
	GKME	GHNE	GKME	GHNE
Acceptability*				
Serve without milk				
Shape	6.4±1.5^{c}	6.5±1.3^{c}	6.9±1.3^{c}	6.9±1.5^{ab}
Color	7.0±1.1^{b}	6.9±1.4^{b}	6.7±1.4^{c}	6.8±1.5^{b}
Overall aroma	7.1±1.5^{b}	6.6±1.2^{c}	7.4±1.2^{a}	6.9±1.2^{ab}
Overall flavor	7.1±1.3^{b}	6.6±1.3^{c}	7.2±1.2^{b}	6.7±1.4^{b}
Crispiness	7.2±1.2^{a}	7.1±1.1^{a}	7.6±1.0^{a}	7.4±1.1^{a}
Sweetness	7.0±1.4^{b}	6.7±1.2^{b}	7.1±1.3^{b}	6.8±1.3^{b}
Serve with milk				
Overall aroma	7.2±1.0^{a}	6.8±1.2^{b}	7.2±1.2^{b}	6.9±1.3^{b}
Overall flavor	7.2±1.0^{a}	6.8±1.2^{b}	7.5±1.1^{a}	7.0±1.3^{ab}
Crispiness	7.0±1.4^{b}	6.8±1.4^{b}	7.4±1.0^{a}	7.2±1.0^{a}
Sweetness	7.2±1.1^{a}	6.9±1.1^{b}	7.2±1.2^{b}	6.9±1.3^{ab}
Overall liking	7.4±1.04^{a}	7.0±1.2^{b}	7.4±1.0^{a}	7.0±1.2^{ab}
Accept	96.0%	95.5%	99.0%	97.0%
Purchase	83.0%	83.5%	74.0%	61.0%

*Mean±standard deviation from 200 responses of Thai and 100 responses of US consumer based on a 9-point hedonic scale (1= dislike extremely, 5= neither like nor dislike, 9= like extremely). Means with different superscript letters in each row are significantly different ($P \leq 0.05$).

The acceptability scores for 10 sensory attributes from both Thai and the US. consumers ranged from 6.4-7.4 and from 6.5-7.4 for GKME and GHNE, respectively. The overall liking score of extruded snack from two rice varieties was “like moderately” and the mean overall liking scores of developed snack from GKMF was slightly higher (7.4 for both Thai and US. consumers) than from GHNE (7.0 for both

Thai and US. consumers, respectively). At least 83% and 61% of both Thai and US. consumers would purchase developed snack from GKMF and GHNF, respectively when commercially available. This study demonstrated the feasibility of producing highly nutritious extruded snacks from GBRF that were acceptable to both Thai and US. consumers.

To identify attributes that influenced overall acceptance and purchase intent, the logistic regression analysis (LRA) was performed using all parameters of the sensory attributes. LRA specifies the probabilities of the particular outcomes (i.e., overall acceptance and purchase intent) for each unit or case (in this case, product) involved. Table 23 showed parameter estimates and probability for predicting overall acceptance and purchase intent of extruded snack from GBRF. Among 2 samples of developed snack from GKMF and GHNF, results indicated that overall liking was the most critical attribute influencing overall acceptance of consumers. Similarly result was observed for purchase intent. It indicated that overall liking was the most critical attribute followed by crispness, color and sweetness for samples served without milk. While overall liking and crispness were critical for samples served with milk.

In this study, there were some significant differences in the level of overall acceptance and purchase intent of each developed snack sample as affected by nationality and gender. Table 24 and 25 showed parameter estimates and probability for predicting purchase intent of extruded snack from GBRF as affected by nationality and gender. The result showed that sweetness and overall liking attributes influenced purchase intent of Thai consumers. This could be due to the fact that much of the Thai foods are sweet and one of the tastes that Thai people preferred is sweetness (Owen, 2000). Moreover, Thailand's Public Health Ministry (2010) reported that the consumption of sugar in Thai people were 3 times higher than recommended daily requirement levels. The average quantity of sugar intake per Thai person was 29.6 kg per year or an average of 20 teaspoons per day, while the recommended daily showed that consumers should recived sugar no more than 10 kg per year or an average daily 6-8 teaspoons (Anonymous, 2010). US consumers indicated that crispness and overall liking attributes influenced purchase intent. This supported by the research of Ondo

and Ryu (2013) who observed that consumer acceptance of extruded foods was mainly due to the convenience, attractive appearance and texture. For gender, the result indicated that crispness and overall liking attributes influenced purchase intent in female while for male, indicated that color and overall liking attributes influenced purchase intent.

Table 23 Parameter estimates and probability for predicting overall acceptance and purchase intent of extruded snack from GBRF[A].

Parameter	Overall acceptance		Purchase intent	
	Estimate	$Pr > \chi^2$	Estimate	$Pr > \chi^2$
Served without milk				
Shape	0.0810	0.7245	0.1191	0.2490
Color	-0.2668	0.2534	0.3023	0.0057*
Aroma	0.1015	0.6817	-0.0002	0.9986
Flavor	0.2364	0.3785	0.2750	0.0509
Crispness	-0.2299	0.4275	-0.4271	0.0041*
Sweetness	0.4350	0.0682	0.3467	0.0075
Served with milk				
Flavor	-0.1947	0.5228	-0.3025	0.0831
Crispness	0.1695	0.4381	-0.3064	0.0287*
Sweetness	-0.0853	0.7680	0.1287	0.4292
Overall liking	1.3557	0.0005*	1.3676	<.0001*

[A]Based on the logistic regression analysis (LRA), using a full model with 10 sensory attributes. The analysis of maximum likelihood estimates was used to obtain parameter estimates.*Parameter estimates were considered significant when probability of the Wald χ^2 value was less than 0.05.

Table 24 Parameter estimates and probability for predicting purchase intent of extruded snack from GBRF as affected by nationality [A].

Parameter	Nationality			
	Thai		US	
	Estimate	Pr > χ^2	Estimate	Pr > χ^2
Served without milk				
Shape	0.1296	0.3536	0.3426	0.0695
Color	0.2595	0.0859	0.1460	0.4359
Aroma	0.0159	0.9262	0.2918	0.1398
Flavor	0.2356	0.1890	0.1195	0.6485
Crispness	-0.3487	0.0682	-0.3755	0.1769
Sweetness	0.3933	0.0152*	0.3457	0.1695
Served with milk				
Flavor	-0.0462	0.8442	-0.5329	0.0706
Crispness	-0.0375	0.8300	-0.6595	0.0236*
Sweetness	-0.1292	0.5937	0.3019	0.2502
Overall liking	1.0679	0.0005*	1.7143	<.0001*

[A]Based on the logistic regression analysis (LRA), using a full model with 10 sensory attributes. The analysis of maximum likelihood estimates was used to obtain parameter estimates.*Parameter estimates were considered significant when probability of the Wald χ^2 value was less than 0.05.

Table 25 Parameter estimates and probability for predicting purchase intent of extruded snack from GBRF as affected by gender[A].

Parameter	Gender			
	Female		Male	
	Estimate	Pr > χ^2	Estimate	Pr > χ^2
Served without milk				
Shape	0.0513	0.7278	0.1788	0.2637
Color	0.1431	0.3430	0.4861	0.0055*
Aroma	0.1557	0.3513	-0.1743	0.3511
Flavor	0.3330	0.0623	0.1426	0.5756
Crispness	-0.4669	0.0167*	-0.3911	0.1100
Sweetness	0.3090	0.0677	0.4033	0.0789
Served with milk				
Flavor	-0.4830	0.0607	-0.2289	0.3710
Crispness	-0.2488	0.1555	-0.4345	0.0707
Sweetness	0.0293	0.8909	0.2934	0.2671
Overall liking	1.5792	<.0001*	1.2924	0.0001*

[A]Based on the logistic regression analysis (LRA), using a full model with 10 sensory attributes. The analysis of maximum likelihood estimates was used to obtain parameter estimates.*Parameter estimates were considered significant when probability of the Wald χ^2 value was less than 0.05.

Odds is defined as a ratio of the probability that an event will occur divided by the probability that an event will not occur. The probability will always have a value ranging from 0 to 1.00, but the odds can be greater than 1.00. An odds ratio of 1.0 indicates total independence. The effect of a predictor variable on a dichotomous outcome can be represented by an odds ratio or Exp (β) or e^{β} (Allison, 1999).
The odds ratio of a product for being classified as acceptable or purchased for a given one unit increase in the value of the hedonic score on the combination of some significant predictors (shape, color, aroma, flavor, crispness, sweetness and overall

liking rated on a 9-point hedonic scale). Odds ratio estimates for predicting overall acceptance and purchase intent of extruded snack from GBRF based on overall liking scores were presented in Table 26.

Table 26 Odds ratio estimates for predicting overall acceptance and purchase intent of extruded snack from GBRF based on overall liking scores[A].

Parameter	Odds ratio	
	Overall acceptance	Purchase intent
Overall consumer	3.880	3.926
Thai	3.985	2.909
US	4.951	5.553
Female	3.052	4.851
Male	7.116	3.642
Thai-Female	3.042	4.060
US-Female	N/A	7.640
Thai-Male	N/A	1.529
US-Male	N/A	8.545

[A]Based on the logistic regression analysis (LRA), using a full model with 10 sensory attributes. The analysis of maximum likelihood estimates was used to obtain parameter estimates. Parameter estimates were considered significant when probability of the Wald χ^2 value was less than 0.05. N/A refers to "not applicable".

In this experiment, the odds ratio of overall consumer (both Thai and US. consumers) was 3.880 for overall acceptance, indicating the probability of the product being accepted was 3.880 times higher (than not being accepted, $P<0.0001$) with every 1-unit increase of the overall liking score (based on a 9-point hedonic scale). For purchase intent, overall liking was an influencing attribute with the odds ratio estimate of 3.926. The odds ratio estimate of overall consumer (3.926) for purchase intent was higher than that for overall acceptance (3.880), indicating that consumers perceived purchase intent as more critical than overall acceptance. Similarly results

were observed for parameters of US consumers, gender of female from both Thai and US and Thai-female (Table 26). In contrast, the odds ratio estimate of Thai (3.985) and male (7.116) for overall acceptance was higher than that for purchase intent (2.909 and 3.642, respectively), indicating that Thai consumers and male consumers from both Thai and US perceived overall acceptance as more critical than purchase intent.

In addition, parameter of male had the highest scores of odds ratio estimates on overall acceptance (7.116). The highest score of purchase intent was shown in the parameter of US-male (8.545) followed by US-female (7.640) whearas these parameters (i.e., US-female, US-male and Thai-male) were not significant on the overall acceptance (Table 26). From the consumer studies, understanding how each consumer segment differently perceives about extruded snack from GBRF. This will give manufacturers a better direction for developing extruded snack products with expected sensory qulity.

CONCLUSION AND RECOMMENDATIONS

Conclusion

The significant physicochemical property changes of GBRF prepared from KMR and HNR were observed under different storage times of paddy rice and germination conditions. Increasing storage time of paddy rice up to 8 months caused an increase in free GABA content in both GBRFs. GBRF obtained at pH 3 had free GABA content and reducing sugar content higher than those of GBRF obtained at pH 6.8 during storage. The PCA results also confirmed that pH of steeping water and storage time of paddy rice had significant effects on the physicochemical properties of GBRF obtained from both brown rice varieties. GBRFs produced from paddy rice stored for 6 to 10 months and prepared under a specific condition (i.e., buffer at pH 3 and 48 h steeping time) possessed the desirable physicochemical properties and high free GABA content.

The properties of extruded snack from GBRF by a twin-screw extruder were mainly dependent on the feed moisture content. Screw speed (300-400 rpm) had no significant effect on the physicochemical properties of extrudates. The increasing feed moisture from 14% to 22% resulted in increased retention of free GABA content but decreased retention of TPC, DPPH radical scavenging activity and FRAP of extrudates from both rice varieties. Optimum conditions to produce high GABA snack and acceptable to consumer predicted by regression model using GKMF and GHNF were 16 to 19% of feed moisture and 300 to 310 rpm of screw speed and 14 to 19 % of feed moisture and 300 to 400 rpm of screw speed, respectively. Within this optimum region, the extruded snacks contain free GABA content of 25-30 mg/100g extrudate and 50-54 mg/100g extrudate for GKMF and GHNF, respectively and acceptable for consumer (slightly like). This study demonstrated that taste and acceptance of extrudates could improve by coating extruded snack with syrup and flavor. The result of sensory evaluation on coated extruded snack indicated that liking scores of all sensory attributes and overall liking from both rice varieties were higher (moderately like) than those of uncoated (slightly like).

This research investigated the correlation coefficient of barrel temperature was positively related to the expansion ratio and negatively related to a_w, bulk density and hardness of extruded snack fortified with crude xanthone powder. Increasing barrel temperature from 120 °C to 160°C caused a decrease in free GABA and α-mangostin content of extrudates fortified with crude xanthone powder. The TPC, DPPH radical scavenging activity and FRAP of extrudates fortified with crude xanthone powder made from both GBRFs were significantly reduced by extrusion process.

The formulation of developed snack contained 90.5% of GBRF obtained from steeped brown rice grains at pH 3 for 48 h, 9.05% of soy protein isolate and 0.45% of $CaCO_3$. The optimum extrusion conditions to produce extruded snack obtain high free GABA content and acceptable for consume prepared from GKMF and GHNF were 18% of feed moisture and 300 and 350 rpm of screw speed, respectively. The developed snack contained free GABA of 8.36 and 14.42 mg/serving from GKMF and GHNF, respectively. Regarding consumer acceptability (n=200 for Thai and 100 for US. consumers), the mean overall liking scores of developed snack made from GKMF was slightly higher (7.4 for both Thai and US. consumers) than the GHNF (7.0 for both Thai and US. consumers). The logistic regression analysis identified overall liking as the critical attributes influencing overall acceptance and purchase intent of the developed snack. This study demonstrated the feasibility of producing highly nutritious extruded snacks from GBRF that were acceptable to both Thai and US. consumers.

Recommendation

This developed snacks which would have a healthy but they still contain high sucrose from coating syrup. Thus, further studies on investigation of the sweeteners or low sugar extruded snack are necessary.

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APPENDICES

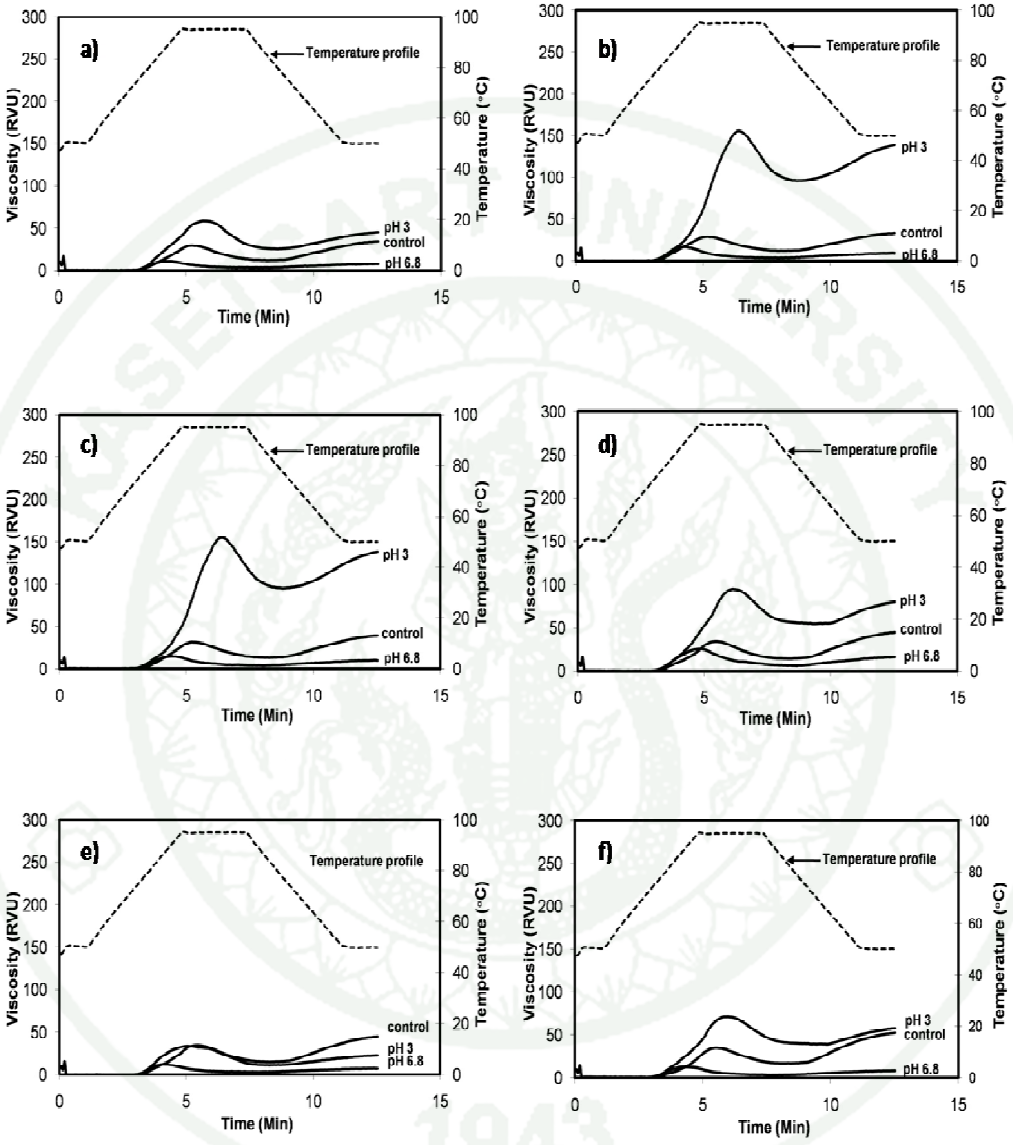
Appendix A
GABA snack

a) b) c)

Appendix Figure A1 New GABA snack products in Thailand: Instant embryo rice with multi grain cereal; Brand Richie Healthy Buddy (a), Organic sprouted muesli; Brand Xongdur Fruity (b) and Kim Chi flavoured rice snack; Brand CAL Nutri Crisp (c).

Source : Mintel's Global New Products Database (GNPD, 2013)

Appendix B
Pasting properties and stirring number of GHNF



Appendix Figure B1 Pasting profile of GHNF during storage periods: 2 months (a), 4 months (b), months (c), 8 months (d), 10 months (e), and 12 months (f) obtained from steeping at 35°C for 48 h in buffer solution (pH 3) and reverse osmosis water (RO, pH 6.8) compared to that of non-GBRF (control) measure by RVA.

Appendix Table B1 Pasting characteristics of GHNF as affected by various steeping condition and storage time.

Germination condition		Viscosity (RVU)					
Steeping condition	Storage time (months)	Pasting Temperature (°C)	Peak Viscosity	Trough	Breakdown	Final viscosity	Set back from through
pH 6.8	2	72.33 ±5.70^{d}	11.59±0.50^{i}	3.12 ±0.20^{e}	8.47 ±0.50^{g}	8.00±0.50^{j}	4.88±0.90^{g}
pH 3	2	72.55 ±5.20^{cd}	61.35 ±5.10^{d}	26.52 ±3.80^{d}	34.83 ±1.50^{c}	47.45±5.30^{e}	20.93±3.40^{d}
Control	2	75.70 ±1.20^{abcd}	32.04 ±4.30^{efg}	12.89 ±2.10^{gh}	19.15 ±2.20^{e}	37.60±5.70^{fg}	24.71±1.90^{c}
pH 6.8	4	73.07 ±3.20^{bcd}	17.61 ±0.30^{h}	3.51 ±0.20^{i}	14.10±0.10^{f}	9.64 ±0.30^{j}	6.13±1.20^{f}
pH 3	4	76.76 ±1.60^{abc}	163.07 ±2.80^{a}	100.22 ±5.70^{a}	64.52 ±4.70^{a}	145.06±4.10^{a}	44.84±0.30^{a}
Control	4	76.18 ±0.90^{abcd}	30.31 ±1.40^{fg}	12.48 ±0.70^{gh}	17.83 ±0.60^{e}	34.86 ±1.90^{g}	22.38±4.30^{d}
pH 6.8	6	76.27 ±2.50^{abcd}	16.10 ±0.50^{hi}	3.93 ±0.20^{i}	12.18 ±0.50^{fg}	10.53±0.40^{j}	6.60±3.00^{f}
pH 3	6	77.52 ±0.00^{ab}	162.07 ±2.80^{a}	99.53±2.40^{a}	62.74 ±5.20^{a}	144.94 ±3.40^{a}	45.41±3.00^{a}
Control	6	76.70 ±0.80^{abc}	32.99±3.00^{ef}	13.65 ±1.60^{efg}	19.34 ±1.40^{e}	40.84 ±3.60^{f}	27.19±3.00^{c}
pH 6.8	8	75.65 ±1.20^{abcd}	26.94±1.52^{g}	5.96 ±0.10^{i}	20.98 ±1.50^{de}	17.13 ±0.30^{i}	11.17±0.80^{e}
pH 3	8	78.55 ±0.40^{a}	99.40 ±3.00^{b}	57.09±2.80^{b}	42.32 ±0.70^{b}	83.25±4.30^{b}	26.16±0.50^{c}
Control	8	77.55 ±2.00^{ab}	36.03±2.10^{e}	15.04±0.60^{ef}	20.98 ±1.50^{de}	46.98±1.70^{e}	31.94±3.10^{b}
pH 6.8	10	75.18 ±0.80^{abcd}	12.50±0.70^{hi}	3.03 ±0.50^{i}	9.40 ±0.30^{g}	7.40±0.50^{j}	4.30±0.30^{f}
pH 3	10	75.50 ±0.90^{abcd}	35.50±3.00^{ef}	11.30±0.90^{h}	24.30±2.20^{d}	23.70±1.80^{h}	12.50±1.50^{e}
Control	10	77.00 ±0.40^{ab}	36.20±0.90^{e}	15.90±0.20^{ef}	20.30±1.00^{e}	45.70±0.50^{e}	29.80±1.00^{bc}
pH 6.8	12	75.70±0.50^{abcd}	13.20±0.70^{hi}	2.90±0.10^{i}	10.20±0.60^{g}	8.60±0.50^{j}	5.60±0.40^{f}
pH 3	12	77.20±0.40^{ab}	74.80±4.50^{c}	40.70±1.80^{c}	34.00±2.80^{c}	60.40±2.80^{c}	19.70±1.80^{d}
Control	12	77.20±0.40^{ab}	36.50±1.10^{e}	17.30±0.90^{e}	19.20±0.90^{e}	55.00±1.60^{d}	37.80±1.30^{b}

Appendix Table B2 Strring number (SN) of GHNF as affected by various steeping condition and storage time[A].

Germination condition		SN
Steeping condition	Storage time (months)	(RVU)
pH 6.8	2	8.90±0.60^{m}
pH 3	2	95.00±1.70^{d}
Control	2	38.70±0.20^{l}
pH 6.8	4	12.00±0.60^{kl}
pH 3	4	118.80±1.60^{b}
Control	4	39.90±1.20^{f}
pH 6.8	6	12.50±0.50^{k}
pH 3	6	124.70±1.90^{a}
Control	6	38.00±0.80^{g}
pH 6.8	8	21.00±0.90^{j}
pH 3	8	125.80±2.60^{a}
Control	8	37.40±0.30^{i}
pH 6.8	10	9.70±0.50^{lm}
pH 3	10	103.80±3.80^{e}
Control	10	39.30±2.10^{g}
pH 6.8	12	9.80±0.10^{lm}
pH 3	12	59.57±0.10^{c}
Control	12	32.70±1.10^{h}

[A]Means ± SD of triplicate measurements. Means with different superscript letters in each column are significantly different ($P \leq 0.05$).

Nongerminated brown rice flour served as the control

Appendix C

Physicochemical measurements

1. Measurement of α-amylase activity with the Rapid Visco Analyser (RVA) AACC method 22-08 (AACC, 2000)

Procedure

1.1 Instrument preparation

Switch on instrument and allow at least 30 min to warm up. Switch on computer, if required, and run control software. Configureure the various models to run SN test as described in manual or as follows;

Series 4: Select SN profile from instrument keypad, or connect to computer and run control software.

Idle tolerance and speed settings are normally preconFigureured. Idle temperature is 95°C and the idle tolerance is 1°C. Where control software is used, inter a file name and select run option. The instrument disperses sample by rotating paddle at 960 rpm for first 10 sec of the test, after which viscosity is sensed using a constant paddle rotation speed of 160 rpm.

1.2 Determination

1.2.1 Weigh 4.00 g whole meal or 3.50 g flour (14% moisture basis) into a weighing vessel before into test canister.

1.2.2 Dispense 25.0 mL water (14% moisture basis) into a canister.

1.2.3 Transfer sample onto water surface in canister. Place paddle into canister and vigorously jog blade through sample up and down 10 times. If any lumps remain on water surface or adhere to paddle, repeat jogging action.

1.2.4 Place paddle into canister and insert paddle and canister assembly firmly into paddle coupling so that paddle is properly centered. Initiate measurement cycle by depressing motor tower of instrument. Do not allow sample to stand in the water for more than 1 min before commencing test. Test will proceed and terminate automatically. Discard canister after use.

1.2.5 Record SN shown on display of RVA at end of test (total of 3 min), or displayed as final viscosity on computer.

2. Measurement of reducing sugar (Somogyi, 1951)

2.1 Apparatus

2.1.1 Analytical balance
2.1.2 Thermosatted water baht set at 80-85°C
2.1.3 Vortex mixer
2.1.4 Spectrophotometer set at 520 nm
2.1.5 Bench centrifuge, required speed 3000 rpm
2.1.6 Filter paper, Whatman no.1, or equivalent
2.1.7 Stop clock

2.2 Reagents

2.2.1 Alkaline copper reagent

a) di-sodium hydrogen orthophosphate dodecahydrate ($Na_2HPO_4.12H_2O$)		71 g
b) Potassium sodium (+) tartrate (Rochelle Salt)		40 g
c) Sodium hydroxide (NaOH) 1N	100 ml	(4 g)
d) Copper (II) sulphate ($CuSO_4.5H_2O$) 10%	80 ml	(8 g)
e) Sodium sulphate (Na_2SO_4)		180 g

The $Na_2HPO_4.12H_2O$ and Rochelle Salt are dissolved in about 250 mLof water, then the dissolved NaOH pallet is introduced with stirring, and this is followed by the addition of the $CuSO_4.5H_2O$. The sodium sulfate is dissolved in about 500 mL of hot water and boiled to expel air. After cooling, the two solutions are united and diluted to volume in a 1000 mLvolumetric flask. The solvent should be prepared few days or a week before using according to solvent stability. The filtration is required before use.

2.2.2 Nelson reagent

a) Ammonium molydate [$(NH_4)_6Mo_7O_{24}.4H_2O$)]	53.2 g
b) 96% Sulfuric acid (H_2SO_4)	21.0 mL
c) di-sodium hydrogen arsenate heptahydrate 12% ($NaHAsO_4.7H_2O$)	50.0 mL (6 g)

All reagents are dissolved in distilled water one by one, respectively and adjusted to volume in 1000 mL volumetric flask. The solvent should be prepared few days or a week before using according to solvent stability. The filtration is required before use.

2.3 Sample preparation

Sugar was extracted from100 mg of samples by 5 mL of 80% ethyl alchol, and incubate tube at 80-85°C for 5 min. Mix contents on vortex stirrer and add another 5 mL of 80% aqueous ethanol. Centrifuge the tube for 10 min at 1000 x g (about 3000 rpm) on bench centrifuge (AACC, 2000). Made up to volume (10 mL).

2.4 Procedure

2.4.1 Pipette 1 mL extracted solution in a test tube.

2.4.2 Add 1 mLalkaline copper reagent, mix, and immerse test tube in

vigorously boiling water bath. Surface of liquid in test tube should be 3-4 cm below surface of boiling water. Delay between filtering of extract and treatment in boiling water bath hold not exceed 10-15 min. Further delay may cause error due to sucrose hydrolysis in acid solution. Let test tube remain in boiling water bath exactky 15 min.

2.4.3 Cool test tube in ice bath, add 1 ml of Nelson reagent, mix thoroughly, let it stand for 30 min at room temperature.

2.4.4 Add 5 mL of distilled water, mix and read color absorbance at 520 nm immediately.

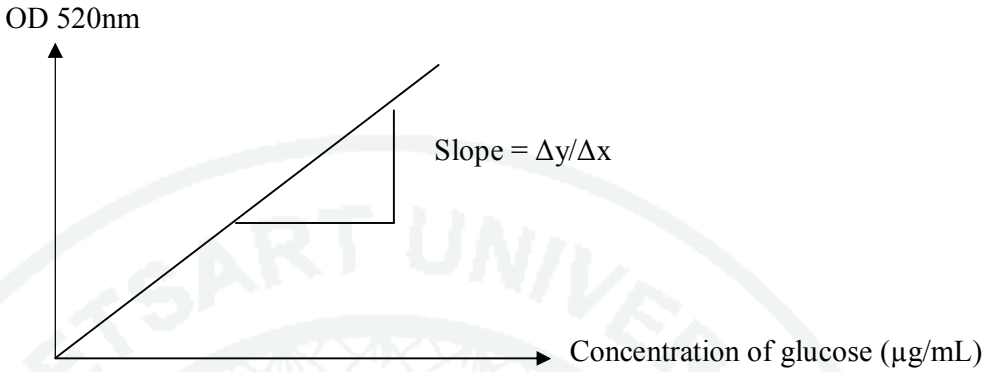
2.5 Standard curve

2.5.1 Prepare standard solutions of glucose 200 µg/mL (0.02 g of dry glucose/ 100 ml).

2.5.2 Determine qualities needed and prepare working solutions of standard glucose solution for the standard curve as the following table by the same procedure as in steps 1-4 of procedure above.

Appendix Table C1 Preparation of standard glucose solution with different concentration

Concentration of glucose (µg/mL)	Distilled water (mL)	Standard solution of glucose 200 µg/mL (mL)
0	10	0
40	8	2
80	6	4
120	4	6
160	2	8
200	0	10



Appendix Figure C1 Standard curve of standard glucose solution with different concentration.

3. Measurement of total phenolic content by Folin-Ciocalteu method

3.1 Apparatus

3.1.1 Vortex mixer
3.1.2 Spectrophotometer set at 760 nm
3.1.3 Bench centrifuge, required speed 3300 rpm
3.1.4 Filter paper, Whatman no.1
3.1.5 Stop clock

3.2 Reagents

3.2.1	0.01 Folin- Ciocalteu's reagent	10 mL/100 mL
3.2.2	7.5% Sodium carbonate (Na_2CO_3)	75g/L
3.2.3	Absolute Methanol	

3.3 Sample preparation

Extract was prepared from ground extrudate weighed (2.0 g) accurately and extracted at a room temperature with methanol under agitation using a magnetic

stirrer for 30 min. The mixtures were centrifuged at 2500 x *g* for 10 min and the supernatants were collected. The residues were reextracted twice under the same conditions, resulting finally in 50 mL extracted in methanol. All extracts were used as they were after centrifugation to determine total phenolic content and antioxidant activity.

3.4 Procedure

3.4.1 Pipette 0.5 mL extracted solution in a test tube.

3.4.2 Add 2.5 mL Folin-Ciocalteu's reagent, mix thoroughly, and let it stand for 3 min at room temperature.

3.4.3 Add 2 ml of 7.5% Sodium carbonate. The mixture was allowed to stand in the dark.

3.4.4 Read color absorbance at 760 nm immediately. The total phenolic content was expressed as mg gallic equivalents per 100 g extrudate.

3.5 Standard curve

TPC values were determined from a calibration curve prepared with a series of gallic acid standards (0.5g/L) 0.00, 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 mg/mL.

4. Measurement of DPPH radical scavenging activity

4.1 Apparatus

4.1.1 Vortex mixer

4.1.2 Spectrophotometer set at 517 nm

4.1.3 Bench centrifuge, required speed 3300 rpm

4.1.4 micropipate 100 μL

4.1.5 Filter paper, Whatman no.1

4.1.6 Stop clock

4.2 Reagents

4.2.1 0.2 mM DPPH

4.2.2 Absolute Methanol

4.3 Sample preparation

Extract was prepared the same procedure as described in the previous Appendix section 3.3.

4.4 Procedure

4.4.1 Pipette 3 mL extracted solution in a test tube.

4.4.2 Add 2 mL 0.2 mM DPPH. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min.

4.4.3 Read color absorbance at 517 nm immediately.

4.5 Calculation

The percentage of free radical scavenging activity was calculated as followed.

DPPH radical scavenging activity (%) $= (1- (A_{sample}-A_{control})) \times 100$

where $A_{control}$ is the absorbance of the control (DPPH solution without test sample), A_{sample} is the absorbance of the test sample (DPPH solution with test sample). Then, DPPH radical scavenging activity was converted to mg gallic equivalents per 100 g extrudate.

4.6 Standard curve

DPPH radical scavenging activity were determined from a calibration curve prepared with a series of gallic acid standards (0.5g/L) 0.00, 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 mg/mL.

5. Measurement of Ferric reducing antioxidant power (FRAP)

5.1 Apparatus

5.1.1 Vortex mixer
5.1.2 Spectrophotometer set at 595 nm
5.1.3 Bench centrifuge, required speed 3300 rpm
5.1.4 micropipate 100 µL
5.1.4 Filter paper, Whatman no.1
5.1.5 Stop clock
5.1.6 Water Bath

5.2 Reagents

5.2.1 20 mM $FeCl_3 \cdot 6H_2O$
5.2.2 10 mM 2,4,6-tripyridyls-triazine (TPTZ)
5.2.3 40 mM Hydrochloric Acid (HCl)
5.2.4 300 mM Acetate buffer pH 3.6
5.2.5 Absolute Acetic acid

The FRAP reagent was freshly prepared by mixing 100 mL of acetate buffer (300 mM, pH 3.6), 10 mL TPTZ solution (10 mM TPTZ in 40 mM/HCl), 10 mL $FeCl_3 \cdot 6H_2O$ (20 nM) in a ratio of 10:1:1 and 12 mL distilled water, at 37°C.

5.3 Sample preparation

Extract was prepared the same procedure as described in the previous Appendix section 3.3.

5.4 Procedure

5.4.1 A solution of 10 mM TPTZ and 20 mM ferric chloride was diluted in 300 mM sodium acetate buffer (pH 3.6) at a ratio of 1:1:10.

5.4.2 Pipette 3 mL of mix solution as described above from 5.4.1 in a test tube.

5.4.3 The test tube was incubated at 37 °C for 30 min in a water bath.

5.4.4 Pipette 100 µL extracted solution in a test tube.

5.4.5 Add 300 µL of DI. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min.

5.4.6 Read color absorbance at 595 nm immediately.

5.5 Standard curve

Methanolic solutions of known Fe (II) concentration, ranging from 100 to 2000 µmol/l, ($FeSO_4 \cdot 7H_2O$) were used for the preparation of the calibration curve. Prepare standard solutions of $FeSO_4$ 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 mM.

6. Water absorption index and water solubility index measurement

Water absorption index (WAI) and water solubility index (WSI) were determined according to the method of Anderson *et al.* (1969). A 2.5 g sample of ground extruded snack was suspended in 30 ml of water at 30° C, in a 50 ml pre-weighed centrifuge tube, stirred intermittently over a 30 min period, and centrifugal at 3000 rpm for 1 min. The supernatant liquid was carefully poured into a pre-weighed evaporating dish. The remaining gel was weighed and the WAI was recorded as the gel weight (g)/dry sample weight (g). The amount of dried solids recovered by evaporating the supernatant from the water absorption test was expressed as a percentage of dry solids in the 2.5 g sample and defined as WSI. Assays were performed in triplicate.

Appendix D
Questionnaire and consumer acceptance test

Research Consent Form

I, _____, agree to participate in the research entitled "Consumer Acceptance of Breakfast Cereal Made from Brown Rice Flour" which is being conducted by Dr. Witoon Prinyawiwatkul, Professor of the Department of Food Science at Louisiana State University, Agricultural Center, phone number (225) 578-5188.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. Up to 120 consumers will participate in this research. For this particular research, about 10 minutes participation will be required for each consumer.

The following points have been explained to me:
1. In any case, it is my responsibility to report prior to participation to the investigator any food allergies I may have.
2. The reason for the research is to gather information on sensory acceptability of breakfast cereal made from brown rice flour. The benefit that I may expect from it is a satisfaction that I have contributed to quality improvement of these products.
3. The procedures are as follows: 2 coded samples will be placed in front of me, and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.
4. Participation entails minimal risk: The only risk which can be envisioned is that of an allergic reaction to common food ingredients [rice and rice products, milk and dairy products, table sugar, and salt (sodium chloride), and plain unsalted crackers]. However, because it is known to me beforehand that the food to be tested contains common food ingredients, the situation can normally be avoided.
5. The results of this study will not be released in any individual identifiable form without my prior consent unless required by law.
6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me, and all of my questions have been answered. I understand that additional questions regarding the study should be directed to the investigator listed above. In addition, I understand the research at Louisiana State University, Agricultural Center, which involves human participation, is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Dr. Michael Keenan, Chair of LSU AgCenter IRB, (225) 578-1708. I agree with the terms above and acknowledge.
I have been given a copy of the consent form.

_____ _____
Signature of Investigator Signature of Participant
Witness: _____ Date: _____

Demographic information:

Gender ( ) Female ( ) Male

Age (years): ( ) 18-20 ( ) 21-30 ( ) 31-40

( ) 41-50 ( ) 51-60 ( ) >60

Product information:

Have you ever eaten breakfast cereal? ( ) Yes ( ) No

Do you like breakfast cereal? ( ) Like ( ) Neither like Nor Dislike ( ) Dislike

Sample code ……..

1. Please evaluate the product and mark a score **[**√**]** that best reflects your feeling about the product.
2. Between the samples, you are required to drink water and eat unsalted cracker to clean your palate.

SERVED WITHOUT MILK:

1. How would you rate the **SHAPE** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

2. How would you rate the **COLOR** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

3. How would you rate the **OVERALL AROMA** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

4. How would you rate the **OVERALL FLAVOR (aroma and taste)** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

5. How would you rate the **CRISPINESS** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

6. How would you rate the **SWEETNESS** of this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

SERVED WITH MILK :

7. How would you rate the **OVERALL AROMA** of this product served with milk?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

8. How would you rate the **OVERALL FLAVOR (aroma and taste)** of this product served with milk?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

9. How would you rate the **CRISPINESS** of this product served with milk?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

10. How would you rate the **SWEETNESS** of this product served with milk?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

11. **OVERALL,** how do you **"LIKE"** this product?

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
1	2	3	4	5	6	7	8	9

12. Do you **accept** this product? ( ) Yes ( ) No

13. Will you **buy** this product? ( ) Yes ( ) No

.....

HEALTH BENEFIT:

"Breakfast cereal made from germinated brown rice flour is a healthier product containing high gamma-aminobutyric acid (GABA) and fiber to promote health. These GABA have been proved to have reduced anxiety, prevents Alzheimer's disease, prevents chronic alcohol-related diseases, regulates blood pressure and maintenance of brain function."

14. After knowing its health benefit, will you purchase this product?

( ) Yes ( ) No

15. Will you purchase this product if the price is increased by 20%?

( ) Yes ( ) No

Appendix E

Cost estimation

Appendix Table E1 Cost estimation of germinated brown rice flour.

Raw material[A]	Process	Price (baht/kg)	Yield (%)	Cost (baht/kg)	Grand total cost (baht)
KM	Paddy rice → Brown rice	24.00	70.00	34.29	
	Brown rice → GBRF	34.29	95.00	36.09	
GKMF (Cost of rice+buffer cost = 36.09 +6.0*)					42.09
HN	Paddy rice → Brown rice	45.00	70.00	64.29	
	Brown rice → GBRF	64.29	95.00	67.67	
GHNF (Cost of rice+buffer cost = 67.67 +6.0*)					73.67

[A]Raw material price as of April, 2013

*Buffer as citrate buffer was used at rice;water of 1:5, thus using 1 kg of brown rice needed about 15L (including the water used in changing twice during steeping time) of water (assumed as 15 kg). Citrate buffer (0.4 baht/kg) were prepared from citric acid and sodium citrate which cost of 65 baht/kg. Thus, total cost of buffer per 1 kg of rice was 6.0 baht (obtained from 0.4 baht/kgx15 kg).

Appendix Table E2 Cost estimation of syrup coating for extruded snack made from GKMF based on 100g syrup.

Ingredient	Price (baht/unit)[A]	Formulation (g)	Cost (baht)
Glucose syrup	37.50/kg	6.25	0.25
Sucrose	25.00/kg	37.50	1.13
Paste sugar	55.00/kg	15.00	0.90
Drinking water[B]	1.00/L	31.25	0.03
Salt	57.58/kg	0.62	0.04
Margarine	213.33/kg	6.88	1.47
Butter milk flavor[B]	192.66/L	2.50	0.48
Cocoa powder	-	-	
		Grand total (baht/100g syrup)	4.30

[A]Price of raw materials as of April, 2013

[B]Estimated that 1 mL of drinking water and butter milk flavor equivalent to 1 g

Appendix Table E3 Cost estimation of syrup coating for extruded snack made from GHNF based on 100g syrup.

Ingredient	Price (baht/unit)[A]	Formulation (g)	Cost (baht)
Glucose syrup	37.50/kg	5.65	0.21
Sucrose	25.00/kg	33.90	0.85
Paste sugar	55.00/kg	13.56	0.75
Drinking water[B]	1.0/L	33.90	0.03
Salt	57.58/kg	0.56	0.03
Margarine	213.33/kg	6.21	1.32
Butter milk flavor[B]	192.66/L	3.40	0.65
Cocoa powder	600.00/kg	2.82	1.69
		Grand total (baht/100g syrup)	5.53

[A]Price of raw materials as of April, 2013

[B]Estimated that 1 mL of drinking water and butter milk flavor equivalent to 1 g

Appendix Table E4 Total cost estimation of extruded snack made from GKMF and GHNF based on 30 g/ serving.

Cost of raw materials	KM	HN	KM	HN
	(price/kg)		(price/unit)	
GBRF (90.5%)	42.09	73.67	1.43	2.50
Soy protein isolate (9.05%)	256.80	256.80	0.88	0.88
$CaCO_3$ (0.45%)	35.00	35.00	0.01	0.01
Syrup coating	43.00	55.30	2.06	2.65
Total			4.38	6.04

Appendix Table E5 Cost of another variable costs of extruded snack per day.

Expenditure[A]	Price baht/day/100kg	Price baht/day/30 g
Electricity expense	5.0	0.14
Tap water	0.8	0.02
Maintenance	5.5	0.17
Depreciation assets	11.0	0.32
Man power	50.0	1.50
Total	72.3	2.15

[A]Extrusion process 100 kg/batch/ day yield 80% (37.50 g flour = 30 g extrudates)

Therefore, the total cost of extruded snack made from GKMF and GHNF exclude the packaging cost would be about baht based on 30g/ serving.

Total cost (baht/box) = Cost of raw materials + Cost of another variable costs

GKME = 4.38 + 2.15 = 6.53

GHNE = 6.04 + 2.15 = 8.19

CIRRICULUM VITAE

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SCHOLARASHIP : Graduate School Scholarship, Kasetsart University 2007. Royal Golden Jubilee Ph.D. program joint funding KasetsartUniversity (2009-2013) Grant number PHD/0261/2550.