MICROWAVE ASSISTED EXTRACTION, ENCAPSULATION AND STABILITY OF HOMNIN RICE BRAN EXTRACTS

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ABSTRACT

Thai colored rice has been reported as a potential source of antioxidants. Hom Nin rice is a kind of black rice and it contains higher nutritional value amongst other varities, especially antioxidant agents including phenolic compounds, flavonoids and anthocyanins. The aims of this thesis was investigate the effects of defatting process on yields, phytochemicals and antioxidant activities of HomNin rice bran extract. The results indicated that defatting process did not significantly affected extraction yields. The results of proximate analysis of the defatted Homnin rice bran and the control (non-defatted) found that the moisture, ash, fiber, protein and fat contents were in the range of 10.55-11.63, 8.39-9.37, 7.51-8.15, 1.03-1.07, and 1.33-14.89%. The effects of defatting process on the total phenolic content (TP), total flavonoids (TF) and anthocyanin (AT) resulted in higher TP, TF and AT than those of the control. The defatted Homnin rice bran extracts had higher antioxidant activity values of 31.42, 25.53 and 162.89 μmol TE/g DM for DPPH, ABTS and FRAP assays, respectively. Therefore, defatting process was used for study the suitable of solvent type and citric acid concentration for the rice bran extraction.

In second part, the effects of solvents (water, methanol and ethanol) combined with citric acid at concentrations of 0, 0.05 and 0.1 mol/dm^3 for the extraction on antioxidants of Homnin rice bran were investigated, then the antioxidant

extraction from Homnin rice bran was optimized by using microwave-assisted extraction and ~~its~~ microencapsulation by freeze drying was examined. The results showed that the methanolic extract with 0.1 mol/dm^3 of citric acid gave the highest yield (43.72%) whereas TP, TF and AT were 86.63 mg of GAE/g DM, 14.67 mg CE/g DM and 6.18 mg C3G/g DM, respectively. While, the antioxidant activities as DPPH, ABTS and FRAP were 29.02, 25.76 and 140.57 µmol TE/g DM, respectively. Even though, the aqueous extracts had slightly lower ($P>0.05$) TP, TF, AT and antioxidants (52.54 mg of GAE/g DM, 13.38 mg CE/g DM and 2.76 mg C3G/g DM 29.47 µmol TE/g DM, 24.18 µmol TE/g DM and 153.40 µmol TE/g DM, respectively) therefore, the acidified water was selected for the next study since the extraction cost would be less and also safer.

The Response Surface Methodology of antioxidant extraction of Homnin rice bran by using microwave found that the predicted values were close to the predicted results of total phenolic content, total flavonoids, anthocyanin were 89.89 mg GAE/g DM, 76.98 mg CE/g DM and 18.08 mg/g DM, respectively and antioxidant activities (DPPH, ABTS and FRAP) were 107.72 µmol TE/g DM, 56.60 µmol TE/g DM and 165.63 µmol TE/g DM, respectively. Comparing to the optimum extraction condition which the three factors obtained from the model were followings: 648 Watt of power energy, 0.076 mol/dm^3 of citric acid concentration and extraction time of 83 second and the experimental values of total phenolic content, total flavonoids, anthocyanin were 90.59 mg GAE/g DM, 77.53 mg CE/g DM and 18.82 mg C3G/g DM, respectively and antioxidant activities (DPPH, ABTS and FRAP) were 109.35 µmol TE/g DM, 58.09 µmol TE/g DM and 168.26 µmol TE/g DM, respectively. Hence, the obtained rice bran extract from this condition was used for the encapsulation study.

The encapsulated extracts by using maltodextrin (MD) (20%) (w/v), maltodextrin + soy protein isolate (MD:SPI) (15:5), maltodextrin + whey protein isolate (MD:WPI) (15:5) and maltodextrin+ maltodextrin+whey protein isolate+ soy protein isolate (MD:WPI:SPI)(10:5:5) were investigated and its stability regarding to pH, temperature and sugar concentration was studied. It was found that encapsulating agents affected the encapsulation efficiency of the powdered extract and the retentions of TP, TF and AT after being encapsulated were in the range of 43.90-62.42%, 33.06-39.20% and 53.24-86.66% and DPPH, ABTS and FRAP were in the range of 71.51-

81.62%, 63.38-100.86% and 56.87-70.87%, respectively. The stability of encapsulated DHRBs in terms of TP, TF, AT and antioxidant activities was also decreased during storage at 25 C for 7 weeks. The DHRB encapsulated with MD:SPI:WPI had the highest stability which the retentions of TP, TF and AT were 67.74%, 70.22% and 70.36%, respectively and antioxidant activities (DPPH, ABTS and FRAP) were 75.18%, 69.72 and 80.63%, respectively after 7 weeks at 25°C. The pH and heating also affected the stability of encapsulated DHRB extracts regarding phytochemicals and antioxidant activity ($P<0.05$) while sucrose concentration had slightly affected. It was found that DHRB extract encapsulated with MD:SPI:WPI in acidic pH (4.5) exhibited the highest of TP, TF, AT, DPPH, ABTS and FRAP ($P<0.05$).

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CHAPTER I

INTRODUCTION

Background

Rice (*Oryza sativa* L.) is the most important cereal crop cultivated in the world, which fodder more than half the world's population (Danielski et al., 2005; Xia et al., 2006; Lu et al., 2008). It is believed to provide more health benefits than other carbohydrate based foods, since it contains several nutrients and antioxidant compounds. Furthermore, rice is a good source of calories provided by its high content of starch, fat, fiber, vitamins, minerals and high nutritional quality proteins since it is hypoallergenic, easily digested and antioxidant properties are also exceptional (Mazza, 1998; Kim, 2005). In particular, the grains with pigments include black glutinous rice, black rice, red rice and purple rice. The purple black rice pigments of the flavonoids are synthesized in crops and most of them were in the groups of anthocyanin (Yawadio, Tanimori, & Morita., 2007). Thai rice, especially, has gained popularity worldwide for its nutrients and fragrance (Vijittra et al., 2011). Homnin rice is of interesting because it contains higher nutritional value than that of other rice species including protein, vitamins and minerals (Suzuki et al., 2004). It contains more vitamin B, niacin, vitamin E, calcium, magnesium, iron and zinc compared to white rice and it could possibly be one of a significant source of anthocyanins. Anthocyanins are characterized as the molecules having an electron deficiency due to their particular chemical structure, which makes them very reactive toward free radicals present in the body, consequently enabling them to be powerful natural antioxidants (Galvano, 2005). Anthocyanins in foods also provide advantages in anti-cancer, liver protection, prevent heart disease, decrease dyslipid, reduction of coronary heart disease and improved visual acuity applications (Mazza, & Miniati, 1993; Chen et al., 2006; Lee et al., 2010).

Rice bran is a by-product from rice milling. It comprises about 10% of the total rice grain, which is normally used as animal feed. It also contains the same nutrients and anti-oxidative compounds as rice, including vitamin E complex (tocopherols and tocotrienols), phytic acid, phenols and tricin. (Vijittra et al., 2011). Besides, rice bran contains many valuable substances such as γ-oryzanol and phytosterols. The major component of vitamin E in rice bran is α-tocopherol which is an antioxidant and can lower the risk of cancer formation and coronary heart diseases (Renuka, & Arumughan, 2007). It is also reported to prevent Alzheimer's disease and allergies. γ-oryzanol can be used to reduce blood cholesterol levels, treat nerve imbalance, as well as an antioxidant and preservative (Imsanguan et al., 2008).

Anthocyanins are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts (Mazza, & Miniati, 1993). Besides giving color to plants, anthocyanins have various healthy functions such as protecting from DNA cleavage, inhibiting inflammation, and preventing cardiovascular and neurodegenerative diseases (Matsumoto et al., 2002). The previous studies indicated that black rice bran contains a high amount of anthocyanins (Xia et al., 2003), and extraction of anthocyanins has become one of the alternative ways to use the black rice waste. Usually, conventional solvent extraction of anthocyanins is time and solvent consuming and the efficiency is low. Moreover, thermal extraction with a long time could cause the degradation of anthocyanins and decrease the antioxidant activity of the extracts (Lapornik, Prošek, & Wondra, 2005). Microwave-assisted extraction (MAE) utilizes the energy of microwaves to cause molecular movement and rotation of liquids with a permanent dipole leading to a very fast heating of the sample and the solvent, offering advantages such as improved efficiency, reduced extraction time, low solvent consumption, and high level of automation compared to conventional extraction techniques (Sun et al., 2007). For those antioxidants that are unstable in alkaline and neutral solutions, acidic aqueous solvents have been used for extracting, so as to disrupt the cell membranes and at the same time dissolve the water-soluble antioxidative compounds. Usually, citric acid, hydrochloric acid, and acetic acid are chosen for acidulating the extraction solvent but using citric acid is safer for human consumption (Li et al., 2012; Mateus, & Freitas, 2009).

Therefore, this research is aimed to investigate the extraction of antioxidants from Homnin rice bran which is a by-product from milling in order to obtain higher antioxidant activity and anthocyanin content by using microwave-assist extraction compare to the conventional solvent extraction. Thus, the development of safer natural antioxidant and color from the extracts of by-product materials could be possible to compensate the synthetic antioxidants and colors.

Objectives

1. To study on the effects of defatting process on yields, phytochemicals and antioxidant activities
2. To investigate the effects of solvent type and citric acid concentration on the extraction of antioxidants from Homnin rice bran
3. To improve the extraction efficiency by using microwave-assisted extraction from Homnin rice bran by Response Surface Methodology (RSM)
4. To study the stability of antioxidants from Homnin rice bran in order to be applied in food products

CHAPTER II

LITERATURE REVIEW

Rice

Rice (*Oryza sativa* L.) is the most common crop, and very important in the world. In Asia, rice is so central to the culture that the word is almost synonymous with food (Nakornriab, 2009). In Asia alone, more than 2,000 million people obtain 60-70 percent of their calories from rice and its products. (Sriseadka, 2011). It is also the main exported product of Thailand. About 20 million tons per year of rice are produced in Thailand, which produces about 1.6 million tons of rice bran (USDA Foreign Agricultural Services, 2010) In Thailand, rice exhibits many colors, such as white, black, red and purple depending on the pigments accumulated in the pericarp and bran layer of the rice kernels (Deng et al., 2013).

Rice is a short-lived plant related to the grass family, with a life cycle of 3-7 months. Rice can grow to 1–1.8 m. tall, occasionally more depending on soil fertility and the variety. The grass has long, slender leaves 50–100 cm long and 2–2.5 cm broad. The small wind-pollinated flowers are produced in a branched arching to pendulous inflorescence 30–50 cm long. The edible seed is a grain (caryopsis) of 5–12 mm long and 2–3 mm thick. The span of one cycle varies depending on its type and the growing environment (Nakornriab, 2009)

Rice is roughly divided into two types, *Japonica* and *Indica*. There are three main races of domesticated rice: *Indica*, or South Asian rice (Basmati type, center above); *Japonica*, or East Asian rice (short-grain, right above); and *Javanica*, or Southeast Asian rice. *Indica* and *Javanica* varieties are generally adapted to the tropics, while *Japonica* rices are adapted to more temperate growing regions. Both *Japonica* and *Indica* types of rice include non-glutinous and glutinous rice. Each type of rice has its own special characteristics and each has its own place in rice cooking. Non-glutinous rice is popularly used in general rice cooking. This rice is somewhat transparent and when cooked it is less sticky than glutinous rice. It is usually cooked in water and served plain. Glutinous rice tends to be white and opaque and is very sticky

when cooked. It is commonly used to make rice cakes and various kinds of desserts, and processed to make rice snacks. There are different types of rice presently grown and used in Thailand; white, black, and red rice (Nakornriab, 2009).

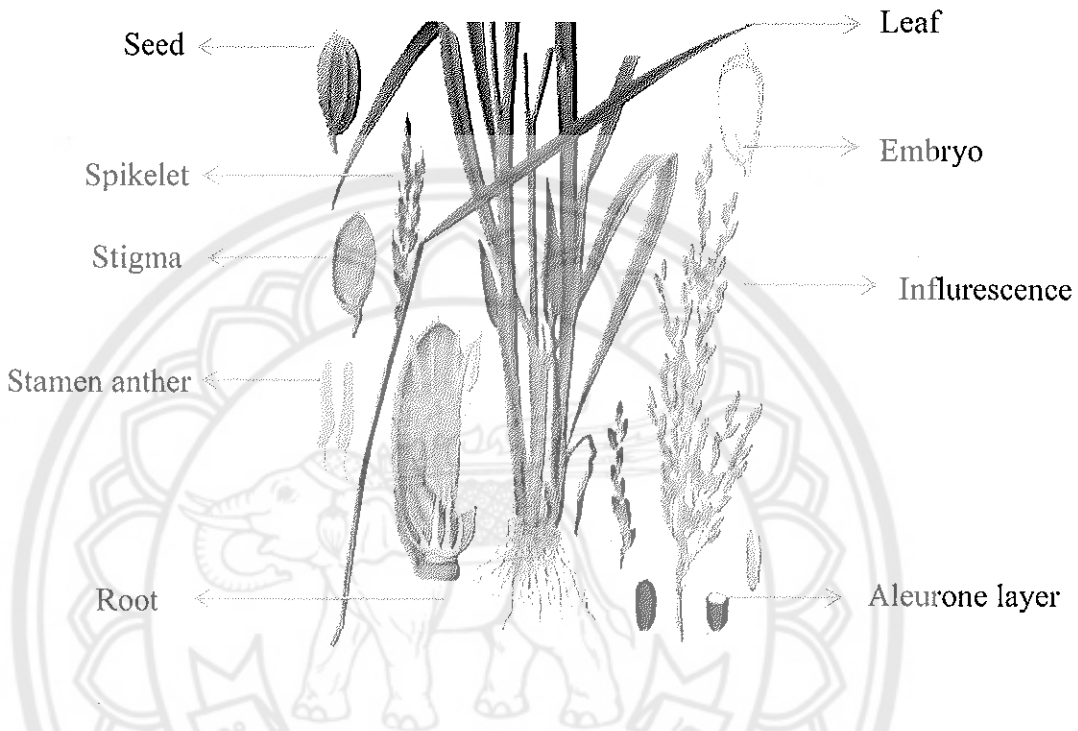

Figure 1 Schematic of rice

Source: Sriseadka, 2011

Rice composition

Rice paddy contains 20% of rice husk, 1–2% of rice germ, 8–9% of rice bran and 70% of rice starchy endosperm (Figure 2). Rice is a staple food due to nutrient content such as carbohydrate (64–73%), protein (5–7%), ash (3–5%), dietary fiber (16–19%) and fat (1.5–2.3%) of a whole grain (Nivikul et al., 2008; Orthoefer, 2005).

Orthoefer, & Eastman (2004) Rice grain structure consist of hull, pericarp, seed coat, aleuronic layer, embryo and endosperm (Figure 2).

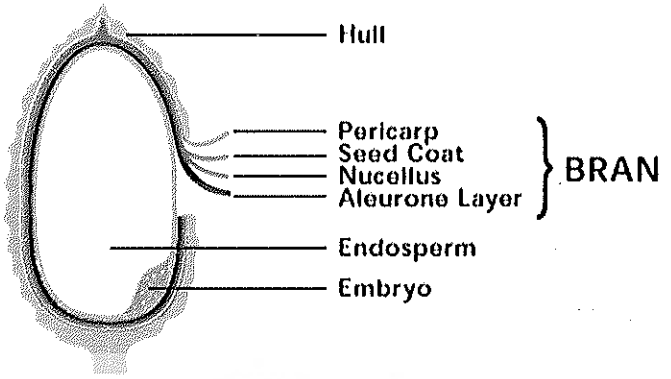

Figure 2 Component of rice grain

Source: Orthoefer, & Eastman, 2004

1. Hull

Rice hulls, the hard outer covering brown rice which protect rice grain during growing season from insect and changing of external environment.

2. Pericarp

The most outer fibrous layer which thick about 10 μm and locates the pigment in colored rice. Main function of pericarp is to serve as protective layer against molds and quality deterioration from oxidation and enzymes due to the movement of oxygen, carbon dioxide and vapor of water.

3. Seed coat

Seed coat is only a few cells in thickness 0.5 μm. This layer is rich in oil and protein contents but low in starch content.

4. Aleuronic layer

Aleuronic layer is part of endosperm which is the most outer. Therefore, bran portion of rice grain comprise of pericarp, seed coat, nucellus and aleuronic layer and typically includes embryo. Rice bran contains 18–22% oil. This part is removed by milling. Bran is the most nutritious part of rice and rich in fiber, protein, oil, vitamins and minerals although it has very low starch.

5. Embryo or germ

Embryo is very small because weight only 1–3% of total rice grain. It locates at central bottom portion of the grain. Embryo can grow to be a new plant

because it is the living organism in the grain. Embryo is removed by milling result in indented shape at the end of the milled rice grain.

6. Endosperm

After removing the hull, the bran and the embryo, endosperm remains. Endosperm is the largest portion of the rice grain because weight 80% of total rice grain. In addition, endosperm is rich in carbohydrate but low in vitamin and mineral.

Pigmented rice

Pigmented rice (e.g., black red and purple rice) are some varieties of rice that have a color on the palea, lemma and another inside part such as pericarp tegmen and aleurone layer (Chiang et al., 2006). Pigmented rice varieties have potential to promote human health because they contain antioxidant compounds that have the ability to inhibit the formation or to reduce the concentration of reactive cell-damaging free radicals. These antioxidant compounds include flavonoids, anthocyanins, γ-oryzanols, vitamin E and phenolic compounds. Besides, it is well documented that consumption of pigmented rice bran can produce a hypocholesterolemic effect as well as antioxidant activity, attributed in major part to the presence of tocotrienols and γ-oryzanol (Zullaikah et al., 2009). A recent report showed that the supplementation of atherogenic diets with black rice pigment markedly reduced oxidative stress and inflammation in addition to modulating atherosclerotic lesions in the apolipoprotein E deficient mice. These findings indicated that compounds present in pigmented rice, which absent in white rice, provided cardiovascular protection in addition to the lipid-soluble components present in rice bran. Moreover, the recent study showed that pigmented rice varieties had greater antioxidant and free radical scavenging activities than the non pigmented ones (Sriseadka, 2011).

Generally, these colored compounds or pigments fall into a number of large groups such as chlorophylls, riboflavin, carotenoids, flavonoids and quinones. The structure of the pigmented rice kernel is illustrated in Figure 3. Most of these pigments are reported to form in plant for vital functions, which could benefit human health in a meaningful way. Their impotant bioactivities include free-radical scavenging (Chiang et al., 2006), enhancement of the immune system (Choi et al., 2007) and reduction risk of cancer (Chen et al., 2006) and heart disease (Wang et al., 2007). Pigmented rice is,

thus, anticipated the greater functional dietary potential than that of the white rice (Toyokuni et al., 2002).

Black rice and by-product

Black rice (*Oryza sativa* L.) is a special cultivar of rice and widely consumed since ancient times in Eastern Asia countries. (Guo et al., 2007) There are many varieties of black rice from China, Thailand and Indonesia. In Thailand, black rice is the second most common rice and grown in the Northern and Northeastern parts of country. It could be either medium or long grain. It contains high amounts of protein, minerals, vitamins, fats, cellulose, and niacin. The functional properties of black rice including mutagenic, carcinogenic, and antioxidative activities (Chen et al., 2006; Nam et al., 2005; Kaneda et al., 2006). Black rice is rich in anthocyanins such as cyanidin-3-*O*-β-D-glucoside, pelagonidin-3-*O*-β-D-glucosideand delephinidin-3-*O*-β-D-glucoside (Hu et al., 2003), which are important to suppress oxidation in the body, and these benefits are not found in white rice. Their name refers to the kernel color (black, red or purple) which is formed by deposits of anthocyanins in different layers of the pericarp, seed coat and aleurone (Chaudhary, 2003). The structure of the pigmented rice kernel is illustrated in Figure 3. It contains phytochemicals that are responsible for their color. Most of these pigments are reported to form in plant for vital functions, which could benefit human health in a meaningful way. Their important bioactivities include free-radical scavenging, enhancement of the immune system and reduction risk of cancer and heart disease. Pigmented rice is anticipated greater functional dietary potential than that of the white rice (Toyokuni et al., 2002; Wang et al., 2007; Chen et al., 2006; Choi et al., 2007).

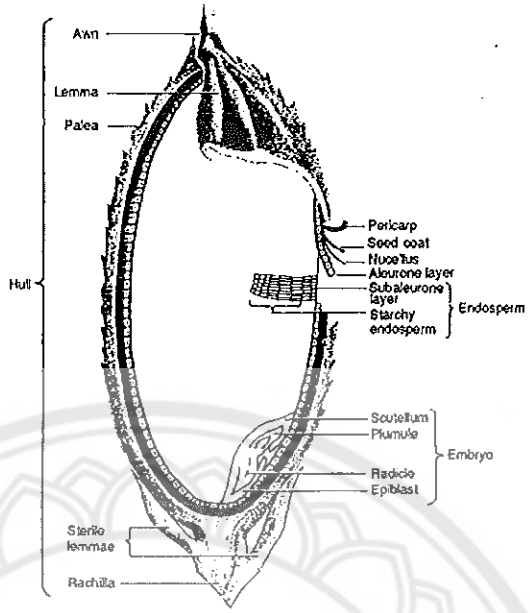

Figure 3 Longitudinal section of rice grain

Source: Rice in human nutrition, 2013

Pigmented rice bran is the hard outer layer of grain and consists of combined aleurone and pericarp. Along with germ, it is an integral part of whole grains, and is often produced as a by-product of milling in the production of refined grains. While comprising just 8% of total weight, rice bran accounts for 60% of the nutrients found in each rice kernel. When bran is removed from grains, they lose a portion of their nutritional value. Bran is particularly rich in dietary fiber, and omegas and contains significant quantitative of starch, protein, vitamins, and dietary minerals (Nakornriab, 2009). Besides, It is an important source of rice oil and other phytochemicals which possess antioxidative and disease fighting properties. Antioxidative polyphenols in rice bran include ferulic acids, its esterified derivatives (oryzanols), tocopherols and other phenolic compounds (Carlos et al., 2007).

Rice by-products generally contain higher amount of nutrients when compared to the polished rice. Table 1 shows the proximate composition and major minerals of several rice by-products. Rice bran, which is derived from the outer layer of the rice grain, is composed of an aleurone layer of the rice kernel, with some proportion of the endosperm and germ (Justo et al., 2013) accounting for

approximately 10% of the weight of the rice grain (Rondanelli et al., 2011). This part is composed of both lipophilic antioxidants (tocopherols, tocotrienols and γ-oryzanol) and phenolics (Min et al., 2011). These substances protect against chronic diseases of the cardiovascular system and help to quench the free radicals and anticancer effects (Abdul et al., 2007; Devi et al., 2007).

In addition, the consumption of whole grains has been reported to protect against colorectal cancer in human interventions (Slavin et al., 1997). The chemopreventive properties of whole grain consumption have been attributed both to fibers and to other phytochemicals (Steinmetz, & Potter, 1991) that are mostly present in the bran layer. Table 2 summarizes several previous findings on the effect of rice by-products on cancer.

Table 1 Proximate composition and minerals content of rice by-products

Nutrients	Content (% of dry matter)				
	Broken	Husk	Bran	Polishing	Straw
Dry matter	87.0-89.0	87.0-92.5	89-94	90	90.9
Protein	6.7-9.8	2.1-4.3	10.6-16.9	11.2-13.4	1.2-7.5
Crude fat	0.5-1.9	0.30-0.93	5.1-19.7	10.1-13.9	0.8-2.1
Crude fiber	0.6	30.0-53.4	7.0-18.9	2.3-3.6	33.5-68.9
Ash	5.0	13.2-24.4	8.8-28.8	5.2-8.3	12.2-21.4
Carbohydrate	No data	22.4-35.3	90	51.1-55.0	39.1-47.3
Calcium	0.09-0.19	0.04-0.21	0.08-1.4	0.05	0.30-0.71
Phosphorus	0.03-0.04	0.07-0.08	1.3-2.9	1.48	0.06-0.16

Source: Norhaizan et al., 2013

Table 2 Summary of anti-cancer effect of rice by-products

Authors	Components	Findings
Boateng et al. (2009)	Rice bran	5% and 10% rice bran significantly ($p < 0.05$) reduce the incidence of azoxymethane (AOM) induced colon tumors in male Fisher 344 rats after 44 weeks feeding
Jeon et al. (2006)	Methanol extracts (rice husk)	Possess significant reactive oxygen activity scavenging and metal chelating activities and protective against oxidative DNA damage using human lymphocytes
Kawabata et al. (1999)	Rice germ	Inhibit ACF formation and reduce incidence of colonic adenocarcinoma
Kannan et al. (2010)	Peptides (rice bran)	Inhibit 84% of colon cancer cells (Caco-2 and HCT-116) growth, 80% for breast cancer cells (MCF-7, MDA-MB-231) growth and 84% for liver cancer cells (HepG-2) growth
Kim et al. (2007)	Methanol extracts (rice husk)	Highly cytotoxic, with IC_{50} values of 0.5 μg/mL *in vitro* reduced colonic pre-neoplastic ACF formation by 35%

Homnin rice

Among the black rice, the most popular is probably Hom-nin rice or fragrant purple rice developed by Kasetsart University (Thailand's agricultural university). (Apichat, 2001). Characteristics of grain of Homnin rice is a slender, dark purple when cooked to a light purple, soft and fragrant. More importantly, it contains a substance called proanthocyanidin which gives the rice its dark color, and is a more potent antioxidant. Many studies have been reported that black rice contains rich of anthocyanin and other polyphenolic compounds much more abundantly than white

rice (Ryu et al., 1998; Zhang et al., 2006). Table 3 shows the nutritional information of Hom-nin rice.

Table 3 Nutritonal infornation of un-milled Hom-nin (Black) rice

Phytonutrients	Content
Protein	10-12.5%
Complex carbohydrate	70-86%
Amylose	7-16%
Amylopectin	80-57%
Omega-3 (Fat)	1-2%
Digestible fiber	2-10%
Sodium	0.001%
Iron	2.25-3.26 mg/100 g
Zinc	2.9 mg/100 g
Copper	0.1 mg/100 g
Calcium	4.2 mg/100 g
Potassium	20 mg/100 g
Vitamin B1	0.34 mg/100 g
Vitamin B2	0.05 mg/100 g
Vitamin B3	4.7 mg/100 g
Vitamin B6	0.62 mg/100 g
Folic acid	339.4 µg/100 g
Antioxidants-Bioflavonoids: anthocyanin (cyanidin), proanthocyanidin (procyanidin), and vitamin E	293 µmol/100 g

Source: Chrispeels, & David, 1994; Apichat, 2001

Classification, chemical composition and distribution of bioactive compounds in Homnin rice bran

Phenolic

Plant phenolics, derived from a wide range of plant secondary metabolites, have attracted increasing attention for their antioxidant properties and marked effects in the prevention of various oxidative stress associated diseases such as cancer (Dai, & Mumper, 2010). Phytochemicals, especially phenolics in fruits and vegetables are the major bioactive compounds known for health benefits. Plant phenolics are commonly found in both edible and non-edible parts of the plants and have been reported to have multiple biological effects, including antioxidant activity. The scavenging activity of phenolics is mainly due to their redox properties, which allows them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Babbar et al., 2011). Therefore, in the last few years, the extraction and identification of phenolic compounds from different plants has become a major area of health and medical-related research (Cvjetko et al., 2016).

Structurally, phenolic compounds comprise an aromatic ring, bearing one or more hydroxyl substituents and range from simple phenolic molecules to highly polymerised compounds (Bravo, 1998). Despite this structural diversity, the group of compounds are often referred to as polyphenols. Most naturally occurring phenolic compounds are present as conjugates with mono- and polysaccharides, linked to one or more of the phenolic groups, and may also occur as functional derivatives such as esters and methyl esters (Harborne, 1980; Harborne, Baxter, & Moss, 1999; Shahidi, & Naczk, 1995). Though such structural diversity results in the wide range of phenolic compounds that occur in nature, phenolic compounds can basically be categorized into several classes as shown in Table 4 and Fig 4.

Table 4 Classes of phenolic and phenolic compounds in plants

Number of carbon atoms	Class	Basic skeleton	Examples
6	Simple phenolics, benzoquinones	C_6	Catechol, Hydroquinone 2,6-Dimethoxybenzoquinone
7	Phenolic acids	$C_6 - C_1$	Gallic acid, Salicylic acid
8	Acethophenones, Tyrosine derivatives, phenylacetic acids	$C_6 - C_2$	3-Acetyl-6-methoxybenzaldehyde Tyrosol p-Hydroxyphenylacetic acid
9	Hydroxycinnamic acids, phenylpropanoids (coumarins, isocoumarins, chromones, chromenes	$C_6 - C_3$	Caffeic acid, Ferulic acid Myristicin, Eugenol Umbelliferone, Aesculitin Bergenon Eugenin
10	Napthoquinones	$C_6 - C_4$	Juglone, Plumbagin
13	Xanthones	$C_6 - C_1 - C_6$	Mangiferin
14	Stilbenes, anthraquinones	$C_6 - C_2 - C_6$	Resveratrol, Emodin
15	Flavonoids, isoflavonoids	$C_6 - C_3 - C_6$	Quercetin, Cyanidin Genistein
18	Lignans, neolignans	$(C_6 - C_3)_2$	Pinoresinol, Eusiderin
30	Biflavonoids	$(C_6 - C_3 - C_6)_2$	Amentoflavone
n	Lignins Condensed tannins (proanthocyanidins or flavolans)	$(C_6 - C_3)_n$ $(C_6)_n$ $(C_6 - C_3 - C_6)_n$	Lignins Catechol melanins Flavolans (Condensed Tannins)

Source: Harborne, 1980; Balasundram et al., 2006

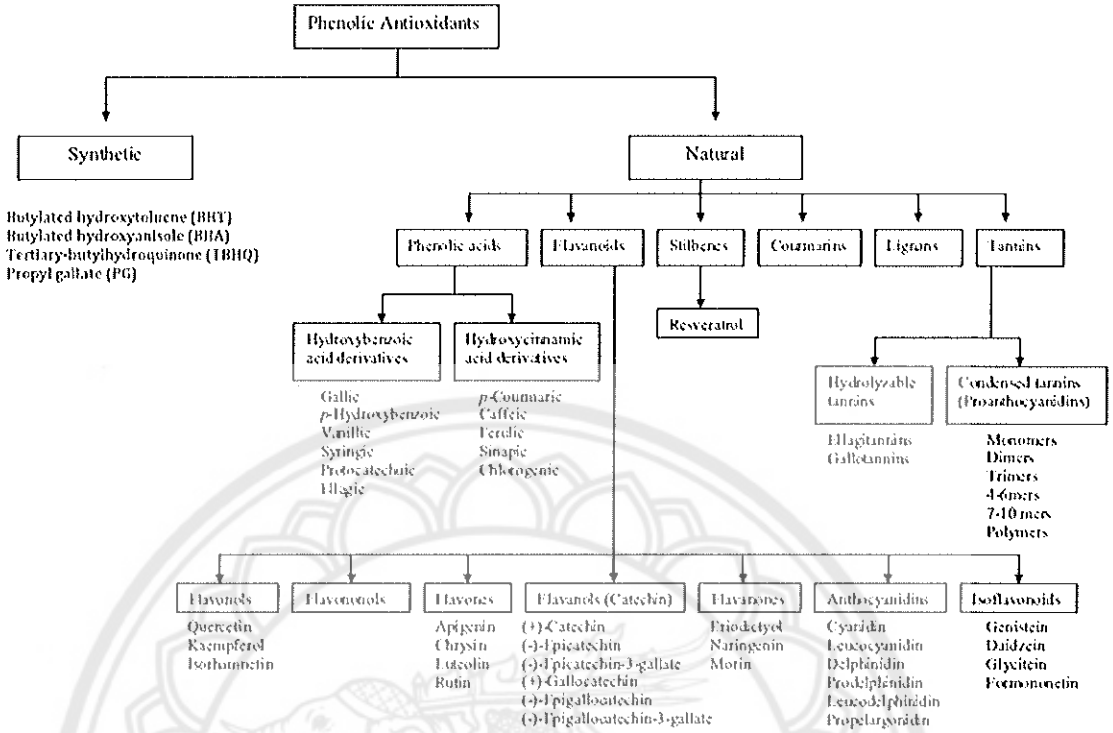

Figure 4 Classification of phenolic antioxidants

Source: Shahidi, & Ambigaipalan, 2015

Phenolic compounds

Phenolic compounds, or polyphenols, constitute one of the most numerous and widely-distributed groups of substances in the plant kingdom, with more than 8,000 phenolic structures currently known (Harborne, 1980). Phenolic compounds are among the most widely distributed plant secondary metabolites that play a key role in the sensory and nutritional quality of fruits, vegetables and other plants (Moreno-Montoro et al., 2015; García-Marino et al., 2006). Evidence indicates that phenolic compounds have potent antioxidant properties and free radical scavenging capabilities (Shashidi, & Wanasundara, 1992). Phenolic compounds are known to exert various physiological effects in humans, such as preventing oxidative damage of lipid and low density lipoproteins (Morton et al., 2000) and reducing the risk of coronary heart disease and cancer (Martinez et al., 2000). Cereal grains contain unique free phenolic compounds and their glycosides, which exist in solution, and a significant amount of

insoluble phenolic compounds, most of which are bound to polysaccharides in the cell wall (Miller et al., 2000). These compounds are concentrated in the bran layers and are lost with the separation of seed coat during processing. Similarly, most phenolic compounds in rice, which is a major staple cereal all over the world, particularly in Asia, are also lost with rice bran. Figure 5 illustrates the chemical structures of phenolic compounds in rice.

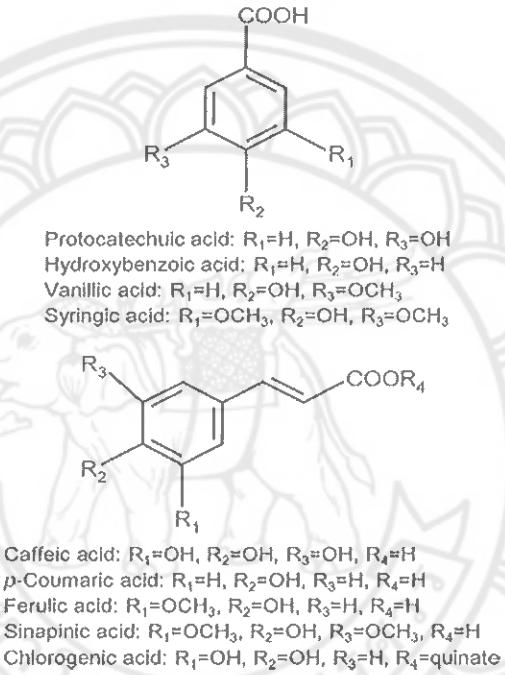

Figure 5 Chemical structures of phenolic compounds in rice

Source: Ayumi et al., 1999

Phenolic compounds are important antioxidants, because of their high redox potentials. They act as reducing agents, hydrogen donors, singlet oxygen quenchers and as metal chelating agents (Tsao, & Yang, 2003). Health-related effects of phenolic compounds such as antibacterial, antimutagenic, anticarcinogenic, antithrombotic and vasodilatory activities have been reported (Ezoubeiri et al., 2005; Pedreschi, & Cisneros, 2007; Kähkönen, & Heinonen, 2003). The cited beneficial effects have been related to their antioxidant properties. The number, type and concentration of phenolics in plants

exhibit extreme diversity. Phenolic compounds vary in structure. Hydroxybenzoic and hydroxycinnamic acids have a single-ring structure. However flavonoids comprise three ring structures and can be further classified into anthocyanins, flavan 3-ols, flavones, flavanones and flavonols. Some flavonoids such as flavan 3-ols can be found in the form of dimers, trimers and polymers (Tsao, & Deng, 2004). In plants, phenolics mainly occur as glycosylated forms through O-glycosidic bonds with a number of different sugars such as glucose, galactose, rhamnose, arabinose, xylose and rutinose (Justesen et al., 1998). In addition, phenolic compounds are also present acylations with phenolic or aliphatic acids, which complicates the identification task. Distinctions are thus made between the phenolic acids, flavonoids, stilbenes, and lignans. Among these phenolic substances, flavonoids, and in particular, anthocyanins are of interest because of their high occurrence in foods, especially in fruits, vegetables, and green leafy vegetables including green tea (Naczk, & Shahidi, 2006).

Flavonoid

Flavonoids constitute the largest group of plant phenolics, accounting for over half of the eight thousand naturally occurring phenolic compounds (Harborne et al., 1999). Flavonoids are the important class of phenolic compounds that found in food. They are C15 compound (C6-C3-C6) present as aglycone or in a glycoside form bound to various sugars, such as arabinose, glucose, galactose, rhamnose, and xylose. These polyphenols form a diverse range of compouds can be classified into many classes (flavone, flavonol, flavonone, flavonol, anthocyanin, chalcone, isoflavanone and isoflavone) (Sriseadka, 2011). Essentially the structure consists of two aromatic rings A and B, joined by a 3-carbon bridge, usually in the form of a heterocyclic ring, C (Fig. 6).

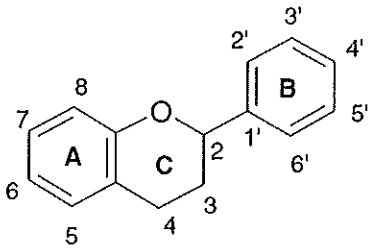

Figure 6 Generic structure of a flavonoid molecule

Source: Balasundram et al., 2006

The aromatic ring A is derived from the acetate/malonate pathway, while ring B is derived from phenylalanine through the shikimate pathway. Variations in substitution patterns to ring C result in the major flavonoid classes, i.e., flavonols, flavones, flavanones, flavanols (or catechins), isoflavones, flavanonols, and anthocyanidins (Fig. 7), of which flavones and flavonols are the most widely occurring and structurally diverse (Harborne et al., 1999). Substitutions to rings A and B give rise to the different compounds within each class of flavonoids. These substitutions may include oxygenation, alkylation, glycosylation, acylation and sulfation.

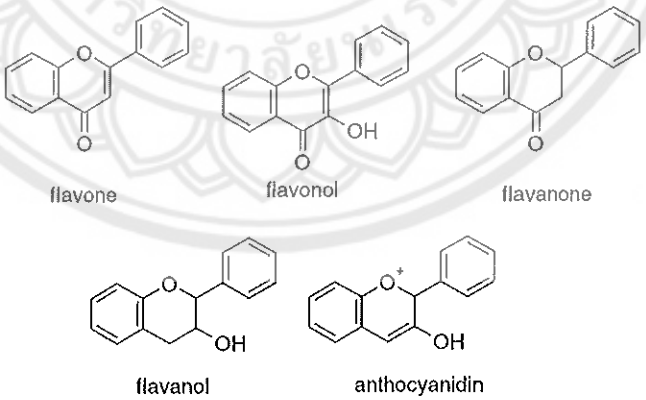

Figure 7 Generic structure of major classes flavonoids

Source: Balasundram et al., 2006

The antioxidant activity of flavonoids depend substantially on the number and position of hydroxyl groups in the molecule (Farkas et al., 2004). Antioxidant compounds include vitamins, phenols, carotenoids, and flavonoids. Among the last group, flavones, isoflavones, flavonones, flavonols, anthocyanins, and catechins are the most important and exhibit substantial antioxidant activity (Wang et al., 1997). Generally, antioxidants can be categorized into two major groups, i.e., synthetic and natural species (Fig 4). Whereas, the synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) are commonly used as additive in food products to increase shelf life (Baydar et al., 2007), natural ones have protective roles for human body against certain diseases. Recently, natural antioxidants have come to the attention as nutritionists for maintaining optimum health and well-being (Orak, 2007). Nowadays, with the current upsurge of interest in the efficacy and use of naturally derived antioxidants, foods of plant origin have received much attention.

Anthocyanins

Anthocyanins are the largest group of water-soluble pigments widespread in the plant kingdom. They are responsible for cyanic colors ranging from salmon pink through violet to dark red and blue of most flowers, fruits and leaves of angiosperms. These natural pigments are usually associated with red fruits but also occur in vegetables, roots, legumes and cereals (Mazza, & Miniati, 1993; Francis, 1989; Andersen, & Markham, 2006). Anthocyanins are interesting pigments regarding their chromatic features. The oldest anthocyanin extract used in the food industry is enocyanin obtained from red grape pomace and marketed in Italy since 1879. Nowadays, grape extracts are often used as colorants for sugar confectionary, dairy products, ice creams, etc. (Mateus, & Freitas, 2009).

Besides their color features, anthocyanins have recently attracted even more interest due to their possible health attributes, such as a reduced risk of coronary diseases, reduced risk of stroke, anticarcinogen activity, anti-inflammatory effects, cardiovascular diseases, virus inhibition, Alzheimer's disease. Anthocyanins and other flavonoids are regarded as important nutraceuticals mainly due to their antioxidant

effects, which give them a potential role in prevention of the various diseases associated with oxidative stress (Gould et al., 2004; Andersen, & Markham, 2006).

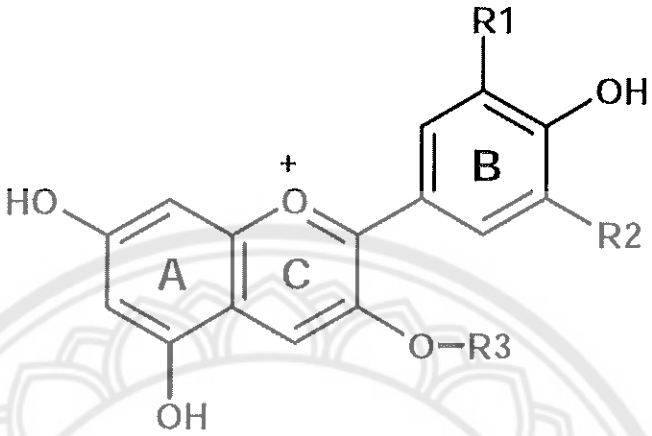

Figure 8 Structures of the major anthocyanin-3-O-glucoside and respective wavelength at the maximum of absorption in the visible region

Source: Zhang et al., 2005

Table 5 Structures of the major anthocyanin-3-O-glucoside and respective wavelength at the maximum of absorption in the visible region

Anthocyanin	R_1	R_2	λmax (nm)*	
			R_3=H	R_3=gluc
Delphinidin	OH	OH	546	541
Petunidin	OH	OCH_3	543	540
Malvidin	OCH_3	OCH_3	542	538
Cyanidin	OH	H	535	530
Peonidin	OCH_3	H	532	528
Pelargonidin	H	H	520	516

Source: Giusti et al., 1999

Rice bran obtained after removal of hulls contains proanthocyanins and anthocyanins, leading to black or red colors (Min et al., 2011). In black rice, the anthocyanins are mainly in free form which accounts for 99.5–99.9% of total anthocyanin (Zhang et al., 2010). The main anthocyanins in black rice are cyanidin-3-glucoside (C3G) and peonidin-3-glucoside (P3G) with the content of the former being significantly higher than the latter (Lee, 2010; Ryu et al., 1998; Yawadio et al., 2007). Anthocyanins are located in certain layers of the kernel, which could be separated into anthocyanin rich fractions for use as functional food ingredients or colorants. In black rice, the pigments are concentrated in the pericarp layers (Abdel-Aal, & Hucl, 1999; Zeven, 1991). The highest concentration of anthocyanin pigments in corn was found in the pericarp, whereas the aleurone layer contained small concentrations (Moreno et al., 2005).

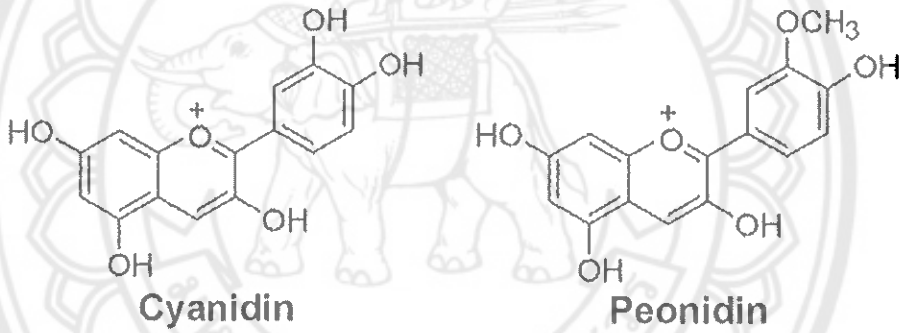

Figure 9 The main of anthocyanidin in black rice

Source: Zhang et al., 2005

Antioxidants

The term of antioxidant referred to any substance that hindered the reaction of substance with dioxygen. Since such reactions frequently involve radicals, now antioxidant generally refers to any substance which inhibits a free radical reaction. In living systems this often involves oxygen in one manner or another (Baskin, & Salem, 1997). Antioxidants are classified into two broad divisions, depending on whether they are soluble in water or lipids. Generally, water-soluble antioxidants react with oxidants in the cell cytoplasm and the blood plasma, while lipid-soluble antioxidants protect

cell membranes from lipid peroxidation (Sies, 1997). The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells, while others such as uric acid are more evenly distributed throughout the body. Plants, which are sources of phytochemicals with strong antioxidant activity, have attracted a great deal of attention in recent years.

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. It is well-known that plant produces these chemicals to protect itself, however, current researches have demonstrated that many phytochemicals can protect humans against diseases. Some of the notorious phytochemicals are lycopene in tomatoes, isoflavones in soy and flavanoids in fruits. Many phytochemicals are polyphenol antioxidants that impart bright colors to fruits and vegetables. Lycopene makes tomatoes red, carotene makes carrots orange and anthocyanin makes blueberries blue. Both the bright colors and the antioxidant activities are due to alternating single-bonded and double-bonded carbons. There is abundant evidence from epidemiological studies showing that the phytochemicals in fruits and vegetables can significantly reduce the risk of cancer, probably due to polyphenol antioxidant and anti-inflammatory effects (Nakornriab, 2009).

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. There is an increasing interest in natural antioxidants, e.g., polyphenols, present in medicinal and dietary plants, which might help prevent oxidative damage (Fu et al., 1995). Besides, natural antioxidant from plant extracts have attracted increasing interest due to consumer concern about the safety of the synthetic antioxidants in food. Extract of fruits, vegetables, cereals and their by-products, such as the cereal hulls, apple peel, rice bran, and citrus peel and seed all showed effective antioxidant activity in a model system (Sun, & Ho, 2005).

Black rice contains rich of lipophilic and hydrophilic antioxidant. The main lipophilic antioxidant are α-tocopherol and γ-tocopherol, the major components of vitamin E, rice bran contains higher levels of α -tocotrienol and γ-tocotrienol, which are not found or are present in much lower amounts in most grains and cereals

(Godber, & Juliano, 2004; Xu, & Godber, 1999). And the main hydrophilic antioxidant are anthocyanin, L-ascorbic acid, phenolic acid etc (Sungjoon, & Zhimin, 2009).

According to the study of Sungjoon, & Zhimin (2009) show that on table 6. It was found that total phenolic content and DPPH Free radical scavenging capability were significantly. By hydrophilic antioxidants and their antioxidant activity in color rice bran was significantly higher than that of lipophilic antioxidants. Because anthocyanins in the color rice bran contributed to the higher antioxidant activity of the hydrophilic extract than that of the lipophilic extract.

Table 6 Yields, Total phenolic content, and DPPH Free radical scavenging capability (TEAC) of the OBF and IBF

	lipophilic extract		hydrophilic extract	
	OBF	**IBF**	**OBF**	**IBF**
yield (%)	13.5±0.4a	14.1±0.2a	10.8±0.1b	11.0±0.3b
total phenolic content (μg of catechin equiv/g)	6.0±0.1a	2.7±0.1b	113.9±1.9c	489.1±3.8d
TEAC (μmol of trolox eq./g)	5.6±0.2a	4.6±0.3b	78.2±2.5c	433.6±9.4d

Source: Sungjoon, & Zhimin, 2009

Rice bran is a rich source of natural antioxidants which can be used as free radical scavengers. It is widely recognized that many of the today's diseases are due to the oxidative stress that resulted from an imbalance between formation and neutralization of free radicals. The antioxidants are very efficient in reducing low density lipoprotein and total serum cholesterol. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so

antioxidants are often reducing agents such as γ-oryzanol, anthocyanins, phenolic acids and vitamin E (Helmut, 1997).

Measurement of antioxidant activity

The need to measure antioxidant activity is well documented; these are carried out for meaningful comparison of foods or commercial products and for provision of quality standards for regulatory issues and health claim (Shahidi, & Zhong, 2005).

There are numerous methods for measuring antioxidant activity; these may be classified into two categories. The first category measures the ability of antioxidants in inhibiting oxidation in a model system by monitoring the associated changes using physical, chemical or instrumental means. Radical scavenging assays include methods based on hydrogen atom transfer (HAT) or single electron transfer (SET) mechanisms. Oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP) and crocin bleaching assays are the major methods that measure HAT whilst trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays represent SET-based methods (Shahidi and Ambigaipalan, 2015). HAT-based methods measure the classical ability of an antioxidant to quench free radicals by hydrogen donation whilst SET-based methods detect the ability of a potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyls and radicals (Shahidi, & Zhong, 2005). The most frequently used analytical tests are DPPH, ABTS, FRAP, ORAC, TEAC, TRAP, CUPRAC (cupric reducing antioxidant capacity) and others (Table 7).

Table 7 Comparison of methods for assessing antioxidant activity

Antioxidant assay	Simplicity	Instrumentation required	Biological relevance	Mechanism	End point	Quantitation	Lipophilic and hydrophilic AOC
CUPRAC	+++	+++		SET	Time	ΔOD fixed time	---
DPPH	+	+	-	SET	IC_{50}	ΔOD fixed time	-
FRAP	+++	+++	--	SET	Time, varies	ΔOD fixed time	---
ORAC	++	+	+++	HAT	Fixed time	AUC	+++
TRAP	-	-specialized	+++	HAT	Lag phase	IC_{50} lag time	--
	-						
TEAC	+	+	-	SET	Time	ΔOD fixed time	+++
TOSC	-	-	++	HAT	IC_{50}	AUC	---
LDL oxidation	-	+++	+++	HAT	Lag phase	Lag time	---
PHOTOCHEM	+	-specialized	++	?	Fixed time	Lag time or AUC	+++

Note: +, ++, +++ = desirable to highly desired characteristic.

-, –, — = less desirable to highly undesirable based upon this characteristic.

SET = single electron transfer

HAT = hydrogen atom abstraction

Source: Embuscado, 2015

Extraction of Antioxidant from black rice bran

Rice bran is a natural source carefully to contain high amounts of nutrients and a large amount of biologically active phytochemicals, such as tocopherols, tocotrienols, oryzanols, vitamin B complex, phenolic compounds and anthocyanins (Pitija et al., 2013; Min et al., 2011). In black rice, the anthocyanins are mainly in free form which counting for 99.5–99.9% of total anthocyanin (Zhang et al., 2010).

Anthocyanins have become to be one the most studied polyphenol group of compounds in research and development projects connected with the food industry. Therefore, methods for their extraction, purification and analysis have been refined. For anthocyanins are not stable in alkaline and neutral solutions, acidic aqueous solvents have been used for extraction solvents in order to disrupt cell membranes and at the same time dissolve the water-soluble pigments. Usually, citric acid, hydrochloric acid, acetic acid are chosen for acidulating the extraction solvent but using citric acid is safe for human consumption (Mateus, & Freitas, 2009; Li et al., 2012). The most commonly used solvents are ethanol or methanol, but it may also compose of n-butanol, acetone, methanol/water mixtures or boiling water (Garcia et al., 1998). The addition of water will be depending on the nature of the sample as it may be useful to complete anthocyanin extraction. For food applications, although having a lower extraction capacity and being difficult to eliminate afterwards, ethanol is usually preferred due to its low toxicity. Usually, conventional solvent extraction of anthocyanin is time and solvent consuming and the efficiency is low. Moreover, thermal extraction with a long time could cause the degradation of anthocyanin and reduce the antioxidant activity of the extracts (Lapornik et al., 2005). Thus, researchers have developed a new method for extraction of antioxidant and anthocyanin for increase yield of them.

Pressurized-liquid extraction (PLE), which is also known as accelerated solvent extraction (ASE), applies pressure and elevated temperature with liquid solvents to achieve fast and efficient extraction. The use of PLE can improve sample through put by reducing extraction time and minimizing or eliminating the use of toxic solvents. PLE has been used as an innovative technique in the extraction of anthocyanins from other fruits and vegetables including spinach, berries and grape pomace (Truong et al., 2012).

Solvent extraction

Solvent extraction is usually used to recover a component from either a solid or liquid. The sample is contacted with a solvent that will dissolve the solutes of interest. Solvent extraction is of major commercial importance to the chemical and biochemical industries, as it is often the most efficient method of separation of valuable products from complex feedstocks or reaction products (Birch, 2000). Solvent extraction is using organic solvents such as hexane, ethanol, petroleum ether, or methanol. Amongst others, organic solvent has been used as the solvent for rice bran extraction by many researchers and industrialists due to the availability, high yield extractability and easy operation (Amarasinghe, & Gangodavilage, 2004). But the disadvantages of the conventional solvent extraction include long extraction times, large solvent consumption and not environment-friendly (Valeèrie, 2000).

However, extraction yield and antioxidant activity not only depend on the extraction method but also on the solvent used for extraction. The presence of various antioxidant compounds with different chemical characteristics and polarities may or may not be soluble in a particular solvent (Nihal et al., 2006). Polar solvents are frequently used for polyphenols extraction from plant sample. The most suitable solvents are aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate. Ethanol is a good solvent for polyphenol extraction and is safe for human consumption. Methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols, whereas aqueous acetone is good for extraction of higher molecular weight flavanols (Jin, & Russell, 2010). For extracting flavonoids from tea, aqueous ethanol performed better than aqueous methanol and aqueous acetone (Wang, & Helliwell, 2001). Extracts with the greatest antioxidant activity were obtained in mate tea and black tea by using 50% aqueous ethanol and 50% aqueous acetone, respectively (Nihal et al., 2006). Therefore, in this research, it was selected for water, methanol and ethanol used in the extraction of antioxidants from Homnin rice bran.

Microwave-assisted extraction

Microwave heating was first introduced commercially in 1947. It took some time to catch on but it is now a standard item in most kitchens for cooking uses. Its use in the chemistry laboratory has significantly lagged behind domestic applications. Microwaves were first used in 1975 as a heating source for acid digestion under atmospheric conditions. The sample preparation step was reduced from 1-2 hour to under 15 min, producing an overall reduction in analysis time. This work initiated the use of microwave energy as a heating source for the chemistry laboratory. Other applications include distillation, organic and inorganic synthesis, evaporation and solvent extraction (LeBlanc, 2000).

Table 8 Advantage and disadvantage of each extraction technique

Technique	Conventional	SC-CO_2	UAE	MAE
High efficiency		✓	✓	✓
Low cost	✓		✓	✓
Extraction time		✓	✓	✓
Used low solvent		✓	✓	✓
environmentally friendly		✓	✓	✓

Source: Spigno, & Faveri, 2009; Deng et al., 2006; Huie, 2002; Grigonis et al., 2005; Pan et al., 2002

Microwave-assisted extraction (MAE) is a process that uses microwave energy and solvents to extract target compounds from various matrices. The highly localized temperature and pressure can cause selective migration of target compounds from the material to the surroundings at more rapid rate and with similar or better recoveries compared with conventional extraction, with the main advantages of reducing both extraction time and solvent consumption (Spigno, & Faveri, 2009).

There has recently been widespread interest in the application of microwave heating to the analysis of active compounds in plant herbs. MAE was used for the isolation of volatile and active compounds from plant materials. The main virtue of MAE is the reduction of extraction time and organic solvent. Prior to analysis, the analytes in the extracts obtained by MAE needed further extraction and concentration. So, a simple, rapid, and low-cost technique with extraction and concentration capacity is desirable (Deng et al., 2006). However, some methods of extraction are time consuming (Soxhlet, ultrasonic), require large amounts of solvent, are expensive (Supercritical fluid extraction) and/or energy intensive (pressurized liquid extraction) (Huie, 2002; Grigonis et al., 2005). Recently, microwaves have been used to assist in extraction, as microwave extraction can be a more environmentally and economically friendly process (Pan et al., 2002).

Encapsulation

Encapsulation is a process to entrap one substance within another substance, thereby producing particles with diameters of a few nm to a few mm. The substance that is encapsulated may be called the core material, the active agent, fill, internal phase, or payload phase. The substance that is encapsulating may be called the coating, membrane, shell, carrier material, wall material, external phase, or matrix. The carrier material of encapsulates used in food products or processes should be food grade and able to form a barrier for the active agent and its surroundings (Nicolaas, & Viktor, 2010). Encapsulation has been used in the pharmaceutical industry for many years, for controlled release and delivery of drugs (Jeroen, & Berger, 2006). The main types of encapsulates might be distinguished reservoir type, matrix type and coated matrix type (Figure 10).

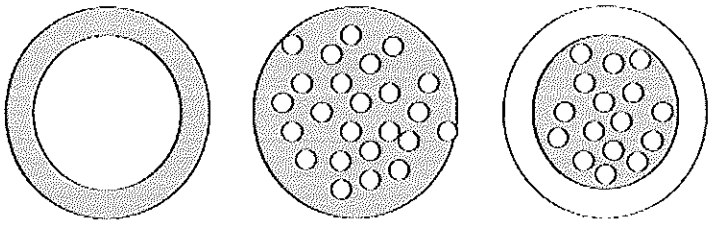

Figure 10 Reservoir type (left), matrix type (middle), and coated matrix type (right) encapsulates

Source: Nicolaas, & Eyal, 2010

The reservoir type has a shell around the active agent. This type is also called capsule, single-core, mono-core or core-shell type. Application of pressure can lead to breakage of the reservoir type of encapsulates and thus to the release of its contents. Matrix type and coated matrix type these type are called poly- or multiple-core type. The active agent in the matrix type is much more dispersed over the carrier material; it can be in the form of relatively small droplets or more homogenously distributed over the encapsulate (Nicolaas, & Eyal, 2010).

Many techniques have been used for encapsulation such as freeze drying, spray drying, spray cooling, extrusion, co–acervation, liposome, fluidized bed coating, entrapment, centrifugal suspension separation, inclusion complexation, lyophilization, and cocrystallization. There are three steps to encapsulate bioactive substances. The first step is the formation of the wall material around substance to be encapsulated. The second step is making sure that the undesired leakage does not occur and the final step is ensuring that the undesired materials are kept out (Champagne, & Fustier, 2007; Madene et al., 2006; Mozafari, 2006).

The type of coating material selected can vary depending on the objective of microencapsulation process (enteric protection, time–release, taste masking) and the desired barrier properties. By the selection of the appropriate coating material, a reservoir system can be designed to protect the bioactive ingredient within the core, until it reaches a favorable biological environment, based on the temperature, the pH, the enzyme activity, the ionic strength or other environmental factors. Figure 11 shows

the different categories of coating materials and some common examples. These materials are normally applied to the core by spraying a solution or suspension of the coating material (Felton et al., 1997; Anal et al., 2007; Kuang et al., 2010).

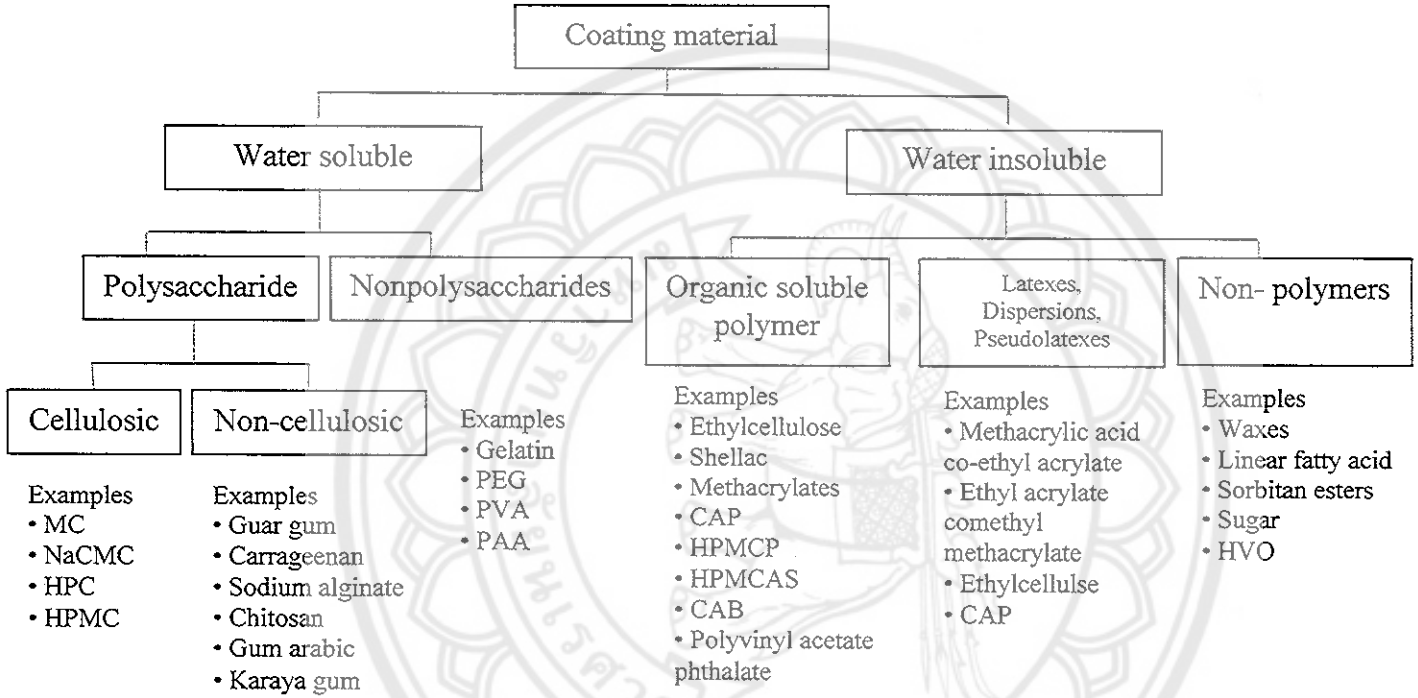

Figure 11 Different categories of coating materials and common examples

Note: methylcellulose (MC); sodium carboxylmethy cellulose (CMC); hydroxypropyl cellulose (HPC); polyethylene glycol (PEG); polyvinyl alcohol (PVA); polyacrylic acid (PAA); cellulose acetate phthalate (CAP); hydroxypropyl methylcellulose phthalate (HPMCP); hydroxypropylmethyl cellulose acetate succinate (HPMCAS); cellulose acetate butyrate (CAB); hydrogenated vegetable oil (HVO)

Source: Kuang et al., 2010

Materials used in film coating or matrix formation include several categories:

1. Waxes and lipids: beeswax, candelilla and carnauba waxes, wax micro- and wax macroemulsions, glycerol distearate, natural and modified fats.

2. Proteins: gelatins, whey proteins, zein, soy proteins, gluten, and so on. All these proteins are available both in native and modified forms.

3. Carbohydrates: starches, maltodextrins, chitosan, sucrose, glucose, ethylcellulose, cellulose acetate, alginates, carrageenans, chitosan, and so on.

4. Food grade polymers: polypropylene, polyvinylacetate, polystyrene, polybutadiene, and so on (Jamileh, 2007).

The possible benefits of microencapsulated ingredients in the food industry. Jeroen et al. (2006).

1. Reduce the reactivity of the fragrance with the outside environment, for example oxygen, pH and water

2. Decrease the evaporation rate of the fragrance, control the release rate and provide sustained release

3. Promote the ease of handling of the fragrance

4. Prevent lumping

5. Improve the compatibility with other constituents

6. Convert a gas or liquid to a solid form

7. Promote easy mixing

8. Dilute the core material to achieve uniform dispersion in the product

9. Stabilise and protect the fragrance during storage

10. Reduce the losses (of top notes) during repeated opening of the packages

11. Increase use levels without affecting solubility and dispersing behaviour

12. Reduce loss levels in washing water and sewers

13. Extend shelf life

14. Increase deposition and adhesion on textiles

Theory of response surface methodology

Response surface methodology

Response surface methodology (RSM) is a collection and multivariate statistical techniques useful for developing, improving and optimizing processes, i.e.,

to discover the conditions in which to apply a procedure in order to obtain the best possible response in the experimental region studied. This methodology involves the design of experiments and multiple regression analysis as tools to assess the effects of two or more independent variables on dependent variables (Myers et al., 2009). This performance measure or quality characteristic is called the response (output variable). It is typically measured on a continuous scale, although attribute responses, ranks and sensory response are not unusual. The most applications of RSM will involve more than one response. The input variables are called independent variables and they are subject for purposes of a test or an experiment. The RSM is equipped with statistical tools to determine the significance of a factor over a response. The evaluation of factors using the RSM uses experimental design in order to distribute the selected variables within the boundaries of the design. The response of experiment can be presented graphically, either in the contour plot or three dimension that help visualize the shape of the response (Myers et al., 2009; Khuri, & cornell, 1996).

One additional advantage is the possibility of evaluating the interaction effect between the independent variables on the response. This technique is based on the fit of a polynomial equation to the experimental data to describe the behavior of a set of data. In this way, a mathematical model which describes the studied process is generated. The objective is to simultaneously optimize the levels of the studied variables to attain the best possible performance of the process (Myers et al., 2009). Recently, the researchers have been published on the application of RSM to optimize extraction bioactive compounds and antioxidant activities. Bachir Bey et al. (2014) studied the optimum conditions for extracting total phenolic compounds (TPC) and antioxidant activity from fresh dark fig (*Ficus carica* L.) whereas Yuan et al. (2015) investigated the methanol concentration, extraction time, and liquid/solid ratio as factors and eight bioactive compounds contents as responses. Şahin et al. (2013) was determined HCl concentration between 0.41 and 0.44 mol/L, methanol volume between 55% and 59% (v/v), extraction temperature between 64 and 70°C, extraction time between 101 and 107 min for extraction of phenolic content and antioxidant capacity from Artemisia absinthium. Yuan et al. (2012) was determined energy density between 20 and 40 (Watt/ml), citric acid concentration between 0.2 and 0.6 mol/dm^3, liquid:solid ratio between 1:15 and 1:20, extraction time between 20 and 60

second for extraction of anthocyanins yield from grape peel._Ranic et al., (2014) was studied extraction process from espresso spent coffee grounds that is a waste material abundantly produced by restaurants and cafeterias using RSM with microwave-assisted extraction. They found that the reduced time of extraction, low power and medium liquid to solid ratio while using minimal concentration of ethanol, the polyphenols extract with high antioxidant activity can be achieved.

The Box–Behnken Design

Another class of response surface designs are called Box-Behnken designs (BBD) that provides three levels for each factor (-1, 0, 1) and consists of a particular subset of the factorial combinations from the 3k factorial design. Some Box–Behnken designs are rotatable, but, in general, this design is not always rotatable. They are very useful in the same setting as the central composite designs. Their primary advantage is in addressing the issue of where the experimental boundaries should be, and in particular to avoid treatment combinations that are extreme. By extreme, the corner points and the star points are thinking, which are extreme points in terms of region in the experiment. The BBD avoids all the corner points, and the star points. Therefore, the BBD is popular in industrial research because it is an economical design and requires only three levels for each factor (Myers et al., 2009;_Khuri, & cornell, 1996). However, both the CCD and the BBD can work but they have different structures, so if the experimental region is such that extreme points are a problem then there are some advantages to the BBD. Two important models are commonly used in RSM. These are special cases of model (1) and include the first-degree model (d = 1),

$$Y = b_0 + \sum_{i=1}^{k} b_i x_i + \varepsilon \quad (1)$$

and the second-degree model (d = 2)

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=1}^{3} b_{ij} X_i X_j + \varepsilon \quad (2)$$

Where Y represent the response variables, b0 is a constant, bi , bii and bij are the linear, quadratic and interactive coefficients, respectively. Xi and Xj are the levels of the independent variables (Myers et al., 2009).

The Central Composite Design

The central composite design (CCD) is one of the most commonly used response surface designs for fitting second-order models. The factorial portion is used to fit all linear and interaction terms. The axial points provide additional levels of the factor for purposes of estimation of the quadratic terms. This is perhaps the most popular of all second-order designs. This design consists of the following three portions:

1. A complete (or a fraction of) 2k factorial design whose factors' levels are coded as −1, 1. This is called the factorial portion.

2. An axial portion consisting of 2k points arranged so that two points are chosen on the axis of each control variable at a distance of α from the design center (chosen as the point at the origin of the coordinates system). The values of α (or the axial parameter) and is chosen so that the CCD can acquire certain desirable properties.

3. n0 center points.

Thus, the total number of design points in a CCD is $n = 2k + 2k + n0$. For example, a CCD for $k = 2$, $\alpha = \sqrt{2}$, $n0 = 2$ has the form (Myers et al., 2009; Khuri, 2005).

CHAPTER III

RESEARCH METHODOLOGY

Materials

Freshly milled Homnin rice bran (*Oryza sativa* L.) (after milling less than 24 hours) was collected from Nongpingkai rice mill group Ampher Muaeng, KamphaengPhet province follow by sieving to separate grain from rice bran. Rice bran was ground, then passed through 100 mesh (150 µm) sieves and heated by hot air oven at 105 °C for 15 minute to inactivate endogenous lipase and then cooled to room temperature and stored in laminated bag at -20°C until use (Juliano, 1985).

Chemicals

1. Acetonitrile (RCI Labscan, Thailand)
2. Aluminum chloride (Ajax, Australia)
3. Catechin (Sigma, China)
4. Chloroform (RCI Labscan, Thailand)
5. Ethanol (Merck, Germany)
6. Ferric chloride (Sigma-Aldrich, st. Louise, U.S.A)
7. Folin-Ciocalteu reagent (Sigma-Aldrich, st. Louise, U.S.A)
8. Gallic acid (J.T. Baker,U.S.A.)
9. Glacial acetic acid (RCI Labscan, Thailand)
10. Hexane (RCI Labscan, Thailand)
11. Hydrochloric acid (RCI Labscan, Thailand)
12. Methanol (Merck, Germany)
13. Petroleum ether (RCI Labscan, Thailand)
14. Potassium persulfate ((Ajax Finechem, Auckland, New Zealand))
15. Sodium carbonate (Ajax Finechem, Auckland, New Zealand)
16. Sodium hydroxide (RCI Labscan, Thailand)
17. Sodium nitrite (Loba Chemie, Mumbai, India)

18. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) (Sigma-Aldrich, st. Louise, U.S.A)

19. 2,2-diphenyl-l-picrylhydrazyl (DPPH) (Sigma-Aldrich, st. Louise, U.S.A)

20. 2,4,6-tripyridyl-s-triazine (TPTZ) (Sigma-Aldrich, st. Louise, U.S.A)

21. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma-Aldrich, st. Louise, U.S.A)

Apparatuses

1. Analytical balance 2 decimals (Metteler Toledo, PB3002-S, Switzerland)

2. Analytical balance with 4 decimal points (Mettler, model AB 204-5, Switzerland)

3. Centrifuge (Hettich Zentrifugen, model Universal 32 R, Germany)

4. High performance liquid chromatography (HPLC) (Perkin Elmer series 200, USA)

5. Hot air oven (Memmert, UNE 500, Germany)

6. Magnetic stirrer (Nuova, model Thermolyne, USA)

7. Micro pipet (Rainin, U.S.A)

8. Microwave extraction

9. pH meter (Mettler toledo, China)

10. Orbital shaker (Gemmy, model VRN-480, USA)

11. Rotary evaporator (Buchi, model V-500, Switzerland)

12. UV-Vis spectrophotometer (Thermo, Genessys 10 Series, U.S.A.)

13. Vacuum pump (Buchi, model V-500, Switzerland)

14. Vortex mixer (Vortex, model Genie 2, USA)

15. Water activity (Aqualab, model Series 3TE, USA)

16. Water bath (Memmert, model Wb22, Germany)

Scope of the study

This study, the research methodology was divided into 5 sections as follows.

Section 1, Effects of defatting process on yields, total phenolic content, total flavonoids, anthocyanin and antioxidant activities of Homnin rice bran extract were investigated and selected to be used in the next section.

Section 2, Effect of type of solvent (water, methanol and ethanol) and citric acid concentration (0, 0.05 and 0.1 mol/dm^3) were investigated on yields, total phenolic content, total flavonoids, anthocyanin and antioxidant activities of Homnin rice bran extract and the best extracting condition was selected to be used in the next section.

Section 3, Application of RSM for optimization of Homnin rice bran extraction using microwave-assist extraction (MAE with parameters i.e. power energy, citric acid concentration (mol/dm^3) and extraction time and the total phenolic content (TP), total flavonoids (TF), anthocyanin (AT) and antioxidant activities (DPPH, FRAP and ABTS) of the extract were investigated.

Section 4, Encapsulation of the DHRB extract using maltodextrin, whey protein and soy protein and also the combined encapsulating agents was investigated and selected for the next study.

Section 5, Investigation of the stability of encapsulated DHRB for its application in food products.

Section 1, The effects of defatting process on phytochemical and antioxidant activities of Homnin rice bran extract

1. Sample preparation

Homnin rice bran was devided into 2 groups. The first group (control) and the second group was defatted with hexane using the ratio of bran and solvent at 1:10 (w/v) for 2 hours at room temperature in a shaker at 100 rpm. The homogenized mixture was filtered through Whatman No. 1 filter paper and the residue was re-extracted twice following the same procedure and dried in a hood for 12 h in order to remove any residual solvent left then stored at -20°C in laminated bag until use (Kim et al., 2013).

2. Extraction of Homnin rice bran

2 groups of Homnin rice bran were extracted by shaking method with an orbital shaker at 120 rpm for 5 h at room temperature. 2.0 g of Homnin rice bran was extracted with methanol (1:20, w/v) and the extract was centrifuged for 10 min at 5,000 rpm, then the supernatant was collected. Both supernatants were then combined and the methanol and ethanol were evaporated by using rotary evaporator at 40°C and 100 mbar before freeze drying and the dried extracts were then used for phytochemical analyses (Graciele et al., 2011; Nontasan et al., 2012; Tananuwong, & Tewaruth, 2010). For freeze drying, the samples were previously frozen and then put into a chamber at -55 °C under pressure of 0.05 bar, being maintained under these conditions for 48 h.

3. Determination of total phenolic content

The total phenolic content of Homnin rice bran extracts (HNBE) was determined using the method of Folin-Ciocalteu reagent as described by Singleton et al. (1999). Briefly, 150 µl of the extract was mixed with 800 µl Folin-Ciocalteu reagent (diluted with water 1:10 v/v and freshly prepared) and 2 mL of 7.5% sodium carbonate. The final mixture was diluted to 7 mL with deionized water. The mixture was allowed to keep in the dark at room temperature for 2 h to complete the reaction. The absorbance was measured at 750 nm using UV-Vis spectrophotometer. Gallic acid was used as a standard and total phenolic content was expressed as mg of gallic acid equivalents per gram of Homnin rice bran dry matter (mg GAE/g DM).

4. Determination of total flavonoid content

The total flavonoid content was determined by Jia et al. (1999) with some modifications. 250 µl of sample was mixed with 1.25 ml deionized water and 75 µl of 5% sodium nitrite and the mixture was incubated in the dark at room temperature for 5 min. Then, 150 µl of 10% aluminum chloride solution was added and allowed to stand in the dark at room temperature for 5 min before addition of 500 µl 1.0 M sodium hydroxide. The absorbance was measured at 510 nm using UV-Vis spectrophotometer compared to catechin standard. Total flavonoid content of the sample was expressed as mg of catechin equivalents per gram of Homnin rice bran dry matter (mg CE/g DM).

5. Determination of anthocyanin

Total anthocyanin content of HNBE was measured using the pH differential method described by Fuleki, & Francis (1968; Elisia et al., 2007) with minor modifications. The extracts were dissolved in potassium chloride buffer (KCl, 0.025 M, pH 1.0) and sodium acetate ($CH_3COONa.3H_2O$, 0.4 M, pH 4.5) with a pre-determined dilution factor. The absorbances of measured samples were read at 510 and 700 nm against a blank cell containing deionized water (H_2O). The absorbance (A) of the diluted sample was then calculated as follows:

$$A = (A_{510} - A_{700}\text{nm})\ \text{pH 1.0} - (A_{510} - A_{700}\text{nm})\ \text{pH4.5}.$$

The monomeric anthocyanin pigment concentration in the original sample was calculated according to the following formula:

$$\text{Anthocyanin content (mg/dm}^3) = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1}$$

in which cyanidin-3-glucoside molecular weight (MW = 449.2), the dilution factor or dilution multiple (DF = 1) and the molar absorptivity constant (ε = 29,600) were used.

6. DPPH radical scavenging assay

The DPPH method was determined as described by Nuengchamnong et al. (2009) with some modofications. Briefly, 100 µl of samples were added to 3 ml of 0.2 mM DPPH methanolic solution. The reaction mixture was agitated and allowed to stand at room temperature in the dark for 30 min. The absorbance at 517 nm was used to measure the concentration of the remaining DPPH using a spectrophotometer. The calibration curve was performed with Trolox solution (a water soluble vitamin E analog). Total antioxidant activity was expressed as micromoles of Trolox equivalents per gram of Homnin rice bran dry matter (µmol TE/g DM).

7. ABTS radical scavenging assay

The ABTS assay was determined according to Arnao et al. (2001) with some modifications. The ABTS radical cation was generated from 7.4 mM ABTS solution with 2.45 mM potassium persulfate. The mixture was allowed to stand in the

dark at room temperature for 12-16 h before use. The $ABTS^{\bullet+}$ solution was diluted with methanol to obtain an absorbance of 0.70±0.02 at 734 nm. Sample solutions were mixed with ABTS•+ solution and allowed to stand in a dark for 6 min at room temperature. The absorbance was then measured at 734 nm and the results were expressed as micromoles of Trolox equivalents per gram of Homnin rice bran dry matter (μmol TE/g DM).

8. Ferric reducing antioxidant power (FRAP)

The FRAP assay was determined using a modified method of Maier et al. (2009). The FRAP reagent was performed by 300 mM acetate and glacial acetic acid buffer (pH 3.6), 20 mM ferric chloride and 10 mM of TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl. The working FRAP reagent was freshly prepared by mixing three solutions together in the ratio of 10:1:1 and the reagent was incubated at 37°C in water bath. 3 ml of FRAP reagent was mixed with 100 μl of the sample and incubated at 37°C for 30 min. The absorbance was read at 593 nm and expressed as micromoles of Trolox equivalents per gram of Homnin rice bran dry matter (μmol TE/g DM).

9. Statistical analysis

The measurements were carried out in three replicates and the results were reported as mean±standard deviation (S.D.).

Section 2, Effects of solvent type and citric acid concentration on bioactive compounds and antioxidant activities of Homnin rice bran extract

For this part, the effects of 2 factors, including type of solvent and citric acid concentration on yields, total phenolic content, total flavonoids, anthocyanin and antioxidant activities were determined. The best condition was selected for the next section.

1. Sample preparation

Defatted Homnin rice bran (DHRB) with best condition (selected from section 1) 2 g was extracted by 0, 0.05, 0.1 mol/dm^3 of citric acid in water, methanol and ethanol (1:20 w/v) with rotary shaker, 120 rpm for 2 hours at room temperature. After pouring out the supernatant, the precipitate was re-extracted again using the same procedure. Both supernatant were combined and adjusted to 100 ml then used for phytochemical analyses (modified from Tananuwong, & Tewaruth, 2010; Graciele

et al., 2011; Nontasan et al., 2012). The sample of methanol and ethanol were evaporated by using rotary evaporator at 40°C and 100 mbar before freeze drying and the dried extracts were then used for phytochemical analyses. For freeze drying, the samples were previously frozen and then put into a chamber at -55 °C under pressure of 0.05 bar, being maintained under these conditions for 48 h. A Christ alpha 1–4 LD equipment (SciQuip, UK) was used.

2. Total phenolic content, total flavonoids, anthocyanin and antioxidant activities (DPPH, FRAP and ABTS)

The measurement was performed following a method as described in section 1.3 - 1.8, respectively. Each analyzed was assayed in triplicate.

3. Statistical analysis

All the experiments were carried out in triplicate using factorial in completely randomized design and the results were reported as mean±standard deviation (S.D.). Analysis of variance ($P<0.05$) and significant differences among means were tested by Duncan's new multiple range test (DMRT). SPSS was used for statistical analysis and Excel software was applied to depict plots.

Section 3, Application of RSM for optimization of DHRB extraction using MAE with parameters i.e. power energy, citric acid concentration and extraction time on phytochemicals and antioxidant activities of the extract

In this part, the effects of 3 parameters, including power energy, citric acid concentration and extraction time on total phenolic content, total flavonoids, anthocyanin and antioxidant activities of DHRB were determined using MAE. The optimization condition of DHRB extraction was applied by RSM.

1. Sample preparation

2 g DHRB from the best condition (selected from section 2) was placed into Erlenmeyer flasks and extracted with different proportion of power energy from 298-800 watt, citric acid concentration from 0.033-0.117 mol/dm^3, and extraction time from 13-147 second (Table 9). The mixtures were carried in microwave reactor. Then, the extracts were centrifuged at 10°C and 5,000 rpm for 10 min and the supernatants were collected, and stored at -20°C until use.

Table 9 Variables and their levels employed in a central composite design for optimization of DHRB extracts

Variable	Range and levels (coded)				
	-α(-1.68)	**-1**	**0**	**+1**	**+α(+1.68)**
Power energy (Watt)	298	400	550	700	800
Citric acid concentration (mol/dm^3)	0.033	0.05	0.75	0.1	0.117
Extraction time (second)	13	40	80	120	147

2. Analytical methodology

Determination of total phenolic content, total flavonoid, anthocyanin and antioxidant activities (DPPH, FRAP and ABTS) were the same methods previously designed in section 1.

3. Experimental design and data analysis

The optimum of extraction parameters from DHRB by using RSM was employed in the optimum study. A central composite design (CCD) was used to investigate the effect of the independent variable as power energy (X_1), citric acid concentration (X_2), extraction time (X_3) to be optimized for the extraction (Table 9). The complete design consisted of 20 experimental points including eight factorial points, six axial points and six center points. The data was fitted with a second order polynomial equation, which expressed the total phenolic content (Y_1), total flavonoid (Y_2), anthocyanin (Y_3), DPPH (Y_4), ABTS (Y_5) and FRAP (Y_6). The equation was as follows:

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i=1}^{3}\sum_{j=1}^{3} a_{ij} X_i X_j \qquad (1)$$

Where Y represents the response variables, b_0 is a constant, b_i , b_{ii} *and* b_{ij} are the linear, quadratic and interactive coefficients, respectively. X_i and X_j are the levels of the independent variables. Three-dimensional surface response plots were generated by varying two variables within the experimental range and holding the

other constant at the central point. The analytical steps as analysis of variance (ANOVA), regression analysis and coefficients of the response surface equation were estimated by using Design expert v 6.10. Fisher's F-test determined the second-order polynomial model equation at a probability (P) of 0.001, 0.01 or 0.05. The test of statistical significance was based on the total error criteria with a confidence level of 95% ($P < 0.05$).

4. Verification of model

The predictive performance of the models depending on the combined effect of power energy (X_1), citric acid concentration (X_2), extraction time (X_3) was validated with an optimum extraction conditions as predictive models of RSM. The criterion of fitting efficiency of data to the model was the average mean deviation Eq. (2).

$$E(\%) = \frac{1}{n_e}\sum_{i=1}^{n}\left\|\frac{V_E - V_P}{V_E}\right\| \times 100 \qquad (2)$$

Where n_e is the number of experiment data, V_E is the experimental value and V_P is the predicted value (Hossain et al., 2012).

5. Total phenolic content, total flavonoid, anthocyanin and antioxidant activity (DPPH, FRAP and ABTS)

The analysis methods for total phenolic content, total flavonoid, anthocyanin and antioxidant activity (DPPH, FRAP and ABTS) analyses were performed following the methods as described in section 1.3 - 1.8. Each analyzed was assayed in triplicate.

Section 4, Effect of encapsulation on phytochemicals and antioxidants activity of defatted Homnin rice bran extract

For this part, the effect of encapsulating agent, including MD WPI and SPI on moisture content, water activity, color (L*, a*, b*), solubility, TP, TF, AT and antioxidant activities were determined. The ratio of encapsulating agents is shown in Table 10.

Table 10 The coated matrix for encapsulation of DHRB extract

sample	coated matrix		
	Maltodextrin (MD, %)	Whey protein isolate (WPI, %)	Soy protein isolate (SPI, %)
1	20	-	-
2	15	-	5
3	15	5	-
4	10	5	5

1. Encapsulation of defatted Homnin rice bran extract

The optimum condition for extraction from section 3 was selected and the obtained antioxidant extract was encapsulated using 20% (w/v) coating material concentration of four different wall materials including maltodextrin (MD), combination of maltodextrin and whey protein isolate (WPI) (MD +WPI; the ratio of 15:5), maltodextrin and soy protein isolate (SPI) (MD+ SPI; the ratio of 15:5), and maltodextrin, whey protein isolate and soy protein isolate (MD+ WPI+ SPI; the ratio of 10:5:5). All the samples were stirred gently with magnetic stirrer for 30 min to dissolve solid particle prior to freeze drying. The microencapsulated powders were collected, packed in laminate bag and stored at -20 °C until use (modified from Deladino et al., 2008; Ezhilarasi et al., 2013).

2. Total phenolic content, total flavonoids, anthocyanin and antioxidant activities (DPPH, FRAP and ABTS)

The defatted Homnin rice bran extract powders were analyzed for total phenolic content, total flavonoid anthocyanin and antioxidant activity (DPPH, ABTS and FRAP) analyses. The measurements were performed following the methods as described in section 1.3- 1.8, respectively. Each analyzed was assayed in triplicate.

3. Physical properties of freeze–dried powder

3.1 Moisture content of defatted Homnin rice bran extract powder was determined by AOAC (2005) method. Moisture (g water/100 g sample) was determined by drying a 5 g sample at 105°C to constant weight.

3.2 Water activity (aw) was measured at 25°C by using Aqualab, model Series 3TE, USA.

3.3 The color values of defatted Homnin rice bran extract powder were measured using colorimeter and recorded using CIE system (L*, a*, b*). The L*, a*, b* color system consists mean L* values (lightness-darkness), (a* values) redness-greenness and (b* values) yellowness-blueness coordinates to determine the color. Ten measurements were taken on each sample and values for L*, a* and b* were averaged.

3.4 Solubility was determined according to the method proposed by Cano-Chauca et al. (2005), with some modifications. 1 g of sample was mixed with 100 mL of distilled water and the mixture was stirred in a magnetic stirrer for 30 min. Then, the solution was centrifuged at 5000×g for 5 min. Aliquot of 25 mL of the supernatant was transferred to preweighed petridish and oven-dried at 105 °C for 5 h. The solubility was calculated by weight difference and expressed in percentage (%).

4. Encapsulation efficiency

The encapsulation efficiency was calculated as the ratio between the mass of phytochemicals and antioxidant activity in the final product and its initial mass to be encapsulated. About 0.5 g exactly weighed microcapsulated sample was dissolved in distilled water to form homogeneous solution (Ezhilarasi et al., 2013). The measurement was performed following the methods described in section 1.3-1.8, respectively. Each analyzed was assayed in triplicate.

The sample of each materials was selected investigated the stability of antioxidant from defatted Homnin rice bran extract.

5. Statistical analysis

All the experiments were carried out in triplicate at a completely randomized design and the results were reported as mean±standard deviation (S.D.). Analysis of variance ($P<0.05$) and significant differences among means were tested by Least Significant Difference (LSD). SPSS was used for statistical analysis and Excel software was applied to depict plots.

Section 5, Stability of defatted Homnin rice bran extract powder on phytochemicals and antioxidant activity

The stability of antioxidant from the extract was studied with various pH, temperature levels and sugar concentrations in order to investigate the feasibility to apply in food products.

1. Effect of storage on phytochemicals and antioxidant stability of Homnin rice bran extract powder

Homnin rice bran extract powder was kept in plastic bag at 25°C for 7 weeks. Aliquots were taken from the same plastic bag weekly for 7 weeks to analyse for phytochemicals and antioxidant activity.

2. Effect of pH on phytochemicals and antioxidant stability of Homnin rice bran extract powder

1 g/100ml of extract powder solution with water was adjusted to pH 2.5 (to mimic orange juice or soft drinks), 4.5 (to mimic yogurt) and 6.5 (to mimic milk) using 1 M HCl and 1 M NaOH (modified from Ho et al.(2017). The solutions was stored at room temperature (RT) and 25 °C for 24 hours.

3. Effect of temperature and time on phytochemicals and antioxidant stability of Homnin rice bran extract powder

1 g/100ml of powder solutions at pH 5.0 heated at 80 °C for 0, 10, 20, 30, 40, 60 and 90 min. After cooling down to 21 °C in a water bath (modified from Guan and Zhong, 2015), the measurements were performed following the methods described in section 1.3, 1.4, 1.5, 1.7, and 1.8, respectively. Each analyzed was assayed in triplicate.

4. Effect of sucrose concentration on phytochemicals and antioxidant stability of Homnin rice bran extract powder

1 g/100ml solutions were adjusted to 0, 10, 20 % of sucrose and then heated at 90 °C for 2 hours (modified from Tsai et al., 2005). After cooling down to 21 °C in a water bath, the measurements were performed following the methods described in section 1.3, 1.4, 1.5, 1.7, and 1.8, respectively. Each analyzed was assayed in triplicate.

5. Statistical analysis

All the experiments were carried out in triplicate with a completely randomized design and the results were reported as mean±standard deviation (S.D.). Analysis of variance ($P<0.05$) and significant differences among means were tested by Duncan's New Multiple's Range test (DMRT). SPSS was used for statistical analysis and Excel software was applied to depict plots.

CHAPTER IV

RESULT AND DISCUSSION

This chapter provides the results and discussions of the study which were divided into 5 sections as following.

Section 1 Effects of defatting process on yields, phytochemical and antioxidant activities.

Section 2 Effects of solvent type and citric acid concentration on phytochemical and antioxidant activities.

Section 3 Application of RSM for optimization of Homnin rice bran extraction using microwave-assist extraction (MAE with parameters i.e. power energy, citric acid concentration (mol/dm^3) and extraction time and the total phenolic content (TP), total flavonoids (TF), anthocyanin (AT) and antioxidant activities (DPPH, FRAP and ABTS) of the extract were investigated.

Section 4 Encapsulation of the DHRB extract using maltodextrin, whey protein and soy protein and also the combined encapsulating agents was investigated and selected for the next study.

Section 5 Investigation of the stability of encapsulated DHRB for its application in food products.

Section 1 The effects of defatting process on phytochemical and antioxidant activities of Homnin rice bran extract

1. Effects of defatting process on yields, total phenolic content, total flavonoids, anthocyanin and antioxidant activities of Homnin rice bran

To obtain acceptable yields and antioxidant activity with minimal changes of functional properties of the extract required, the extraction technique is one of the most important stage (Zhu et al., 2010). In this experiment, the effects of defatting process on yields, moisture, ash, crude fiber, crude protein, crude fat, TP, TF, AT and antioxidant activity were summarized in table 10. The results indicated that defatting process did not significantly affected extraction yields. The yields of the control and

the defatted Homnin rice bran were between 8.41 to 8.52%. The results of proximate analysis of the defatted Homnin rice bran and the control (non-defatted) found that the moisture, ash, fiber, protein and fat contents of defatted Homnin rice bran and the control were in the range of 10.55-11.63, 8.39-9.37, 7.51-8.15, 1.03-1.07, and 1.33-14.89 %, respectively. The proximate analysis found in this study was similar to Huang and Lai (2016), who studied the moisture, ash, protein and fat contents of red and black rice from Thailand, Taiwan and China. The results were ranged between 8.58–11.99, 3.15-8.58, 8.81-12.49 and 6.21-17.34 %, respectively

Defatting process provided significantly higher total phenolic content of Homnin rice bran extract and the result was similar to Bravo et al. (2013) who reported that defatted coffee beans showed higher total phenolic content than that of the control. The total phenolic content found in this study were higher than those reported by Sompong et al. (2011) who studied the total phenolic content of red and black rice from Thailand, Sri Lanka and China and found that total phenolic content were ranged between 3.40–6.91, 0.79–2.08 and 2.53 mg gallic acid/g, respectively. They also reported that the total phenolic content of Thailand's rice had total phenolic content higher than Sri Lanka's rice and China's rice.

The effects of defatting process on the total flavonoids and anthocyanin resulted in higher total flavonoids and anthocyanin than those of the controls. The resulting trend was similar to those determined for the total phenolic content that the values were significantly different ($P<0.05$) and the defatted Homnin rice bran provided the highest total flavonoids of 14.04 mg CE/g DM and anthocyanin 3.65 mg C3G/g, respectively.

The obtained results clearly demonstrated that the defatted Homnin rice bran increased the total phenolic content and total flavonoids of the extract and the total phenolic content and total flavonoids of Homnin rice bran extracts were significantly higher than those of the controls ($P<0.05$), eventhough the solubility of phenolic compounds in the oil is poor (Maier et al., 2009)

Table 11 Properties of Homnin rice bran and rice bran extracts

Properties of rice bran	Non defatted (control)	Defatted	p-value
Moisture (%)	10.55±0.13	11.63±0.07	0.009*
Ash (%)	8.39±0.44	9.37±0.03	0.067
Crude fiber (%)	7.51±0.37	8.15±0.05	0.098
Crude protein (%)	1.03±0.02	1.07±0.02	0.001*
Crude fat (%)	14.89±0.09	1.33±0.15	0.000*
Properties of rice bran extracts			
Yield (%)	8.52±0.14	8.41±0.10	0.064
Total phenolic content mg GAE/g	34.41±0.09	39.06±0.13	0.000*
Total flavonoid mg CE/g	11.87±0.09	14.04±0.15	0.000*
Anthocyanin mg C3G/g	3.56±0.04	3.65±0.06	0.012*
DPPH (μmol Trolox equivalents/g)	31.42±0.69	36.87±0.31	0.002*
ABTS (μmol Trolox equivalents/g)	25.53±0.51	26.43±0.77	0.028*
FRAP (μmol Trolox equivalents/g)	162.89±1.54	169.56±1.57	0.000*

The antioxidant activities namely DPPH, ABTS and FRAP values of defatted Homnin rice bran extract was higher than those of the control (non-defatted)($p<0.05$) when using solvent extraction and similar phenomenon was also observed in the total phenolic content, total flavonoids and anthocyanin.

The possible explanation could be due to the higher amount of total phenolic content, total flavonoids obtained after defatting process (Xu et al., 2010) while Pérez-Jiménez et al. (2008) found that total antioxidant capacity of the non-defatted sample was much lower than that of defatted fraction and Bravo et al. (2013) also found that the defatted spent coffee extract showed higher antioxidant capacity than that of the not defatted sample. Yue et al. (2008) observed that the defatted soybean flour extract showed higher scavenging capacity than that of the soybean oil and gum since more hydrophilic compounds might be highly concentrated in defatted soy flour extract. Another possible reason to explain the higher antioxidant activity of the defatted Homnin rice bran is that the DPPH, ABTS and FRAP assays are usually

used to measure the antioxidant activity of hydrophilic compounds so that the observed activities of the control were lower.

Correlation between phytochemical and antioxidant activity

The correlation analysis between total phenolic content, total flavonoids, anthocyanin and antioxidant activities obtained from the Homnin rice bran extract are given in Table 12.

In our study, a strong and positive significant correlation among total phenolic content (TP), total flavonoids (TF), anthocyanin (AT) and antioxidant activities (DPPH, ABTS and FRAP) of Homnin rice bran extract was found. The highest correlation coefficient was found between total phenolic content and total flavonoids (0.995, $P<0.01$), and the lowest one was between anthocyanin and FRAP (0.982, $P<0.01$). Anthocyanins are the major compound of flavonoids and flavonoids are the major compounds contributing to total phenols in rice (Min et al., 2012). The correlation data between total flavonoids, anthocyanin, antioxidant activities (DPPH, ABTS FRAP and) and total phenolic content were 0.995, 0.989, 0.991, 0.992 and 0.994, respectively. The correlation of antioxidant activity between DPPH and ABTS, FRAP showed a good correlation (0.992, $P<0.01$ for ABTS and 0.991, $P<0.01$ for FRAP). The correlation study confirms that the total phenolic content are likely to contribute to the radical scavenging activity of the Homnin rice bran extract. The strong correlation between total phenolic content and antioxidant activity are in agreement with other reports (Xu et al., 2010).

Therefore defatting process was selected for studying the effects of solvent type and citric acid concentration on phytochemicals and antioxidant activities of Homnin rice bran.

Table 12 Pearson's correlation coefficients of total phenolic content (TP), total flavonoids (TF), anthocyanin (AT) and antioxidant activities of defatted Homnin rice bran

	TP	TF	AT	DPPH	ABTS	FRAP
TP	1					
TF	0.995**	1				
AT	0.989**	0.994**	1			
DPPH	0.991**	0.986**	0.985**	1		
ABTS	0.992**	0.988**	0.986**	0.992**	1	
FRAP	0.994**	0.985**	0.982**	0.991**	0.987**	1

** Correlation is significant at the 0.01 level (2-tailed)

Section 2 Effects of solvent type and citric acid concentration on bioactive compounds and antioxidant activities of Homnin rice bran extract

1. Effects of the solvent type and citric acid concentration on the yields and antioxidant activity of DHRB

In this experiment, the effects of solvent type on the yields of DHRB extract were summarized in Table 13.

The solvents used for the extraction of DHRB resulted in statistically significant different yields ($P<0.05$). The DHRB extract yields using different solvents with various citric acid concentrations were between 2.41 to 43.72%. The yields of the DHRB extracted by using methanol were higher than those of water and ethanol, and the extraction yields rose with the increasing concentration of citric acid from 0 M to 0.1 M ($P<0.05$). Although, the highest yield was observed when using 0.1 M. of citric acid with water and methanol, there was no significant difference ($P\geq0.05$). Since, the viscosity of the solvent affected the extraction's efficiency, the viscosity of methanol was lower than that of water and ethanol (0.59, 0.89 and 1.07 centipoises) (Alam et al., 2018). Therefore, higher extraction yields in general were obtained by the less viscous solvents (Wijekoon et al., 2011).

The antioxidant activity of the extracts from DHRB determined as ABTS, DPPH and FRAP are presented in Table 13. It was found that both the solvent type and the citric concentration affected the ABTS value of the DHRB extract significantly ($P<0.05$). Therefore, it was likely that the efficiency of the antioxidant activity was then increased with increasing citric acid concentration, which was the same trend as the increase of total phenolic contents (Figure 12).

Table 13 Yields and antioxidant activities of DHRB extracts by using different extracting solvents

Solvent	Yield of powder extract (%)	Antioxidant activity (μmol Trolox equivalents/g)		
		ABTS	DPPH	FRAP
Water	22.73±0.51 ^{d}	24.18±1.44 ^{bc}	29.47±0.58 ^{b}	153.40±2.72 ^{c}
0.05 M of citric acid with water	32.95±2.32 ^{b}	23.41±0.39 ^{bc}	23.42±0.14 ^{d}	92.65±1.92 ^{f}
0.1 M of citric acid with water	41.34±2.56 ^{a}	22.84±0.59 ^{c}	22.47±0.36 ^{e}	65.14±1.36 ^{h}
Methanol	8.77±0.69 ^{f}	25.73±0.57 ^{a}	30.56±0.27 ^{a}	160.89±0.72 ^{b}
0.05 M of citric acid with Methanol	25.41±1.00 ^{c}	25.76±0.55 ^{a}	29.82±0.48 ^{b}	189.21±0.66 ^{a}
0.1 M of citric acid with Methanol	43.72±1.29 ^{a}	25.76±0.55 ^{a}	29.02±0.82 ^{c}	140.57±0.47 ^{d}
Ethanol	2.41±1.03 ^{g}	18.63±0.21 ^{d}	9.26±0.32 ^{g}	155.75±2.71 ^{c}
0.05 M of citric acid with Ethanol	15.40±0.27 ^{e}	24.57±1.18 ^{ab}	8.63±0.17 ^{g}	98.67±0.26 ^{e}
0.1 M of citric acid with Ethanol	22.18±1.69 ^{d}	25.78±1.05 ^{a}	11.29±0.23 ^{f}	70.48±0.91 ^{g}

*Different letters within the same row indicate statistical differences (one-way ANOVA and Duncan test, $P< 0.05$). Values are mean ± S.D of triplicate determinations

Table 13 shows the efficiency of the antioxidant activity of the extract of DHRB. The results reveals that the DPPH values were significantly influenced by the type of solvent and the concentration of citric acid ($P<0.05$). It has also been noticed that the efficiency of antioxidants was decreased when using water with a higher citric acid concentration, whereas the extraction with ethanol provided a higher antioxidant efficiency when using citric acid at a higher concentration. It can be noted that at atmospheric pressure, the dielectric constant of water more than ethanol, an index of polarity, and it is in favor of dissolving of the polyphenols. Hence, increasing the polarity solvent mixture acts as a balancing factor and improves the solubility of polyphenols evidently which provided higher antioxidant activity (Cuevas et al., 2014). Besides, the decrease of DPPH values of DHRB when using water with higher acid concentration may be due to the free form of phenolic compounds obtained from water extraction were destroyed by the acid (Ammar et al., 2015; Bridgers et al., 2010; Rayle, & Cleland, 1992).

The efficiency tests for the antioxidant activity of the extracts from DHRB by using a FRAP assay also found that using different solvents with different citric acid concentration affected the FRAP values ($P<0.05$). In addition, the extraction with water, ethanol and methanol would likely diminish the antioxidant activity when a higher citric acid concentration was used due to the above mentioned reasons.

Considering the antioxidant activity values obtained from the DHRB extract from the three assays, the results showed that the solvent type and the citric acid concentration had a great impact on the overall antioxidant activity. Methanol could be used to extract phenolic compounds and also antioxidants better than water and ethanol, respectively. Alcohol is normally used in antioxidant extraction and is better than water because of its smaller molecular size, less viscosity and stronger polarity, as it could spread into a plant's cells faster and provide a higher extraction capacity. Antioxidants in most plants are normally polar substances and due to the polar nature of the water molecule itself, antioxidants are generally able to dissolve in water. The antioxidant activity of the phenolic compounds depended on a number of hydroxyl categories and properties to catch electrons among carboxylic acid in a molecule capable of providing a decrease in hydrogen (Moongngarm, 2012), as well as functional groups; such as, groups bearing polar molecules. The obtained results

when using water for the extraction of antioxidants had the same trend as the number of phenolic compounds (Figure 12). The antioxidants in rice are mainly phenolic compounds and are immobilized in cellulose, lignin and proteins; such as, ferulic acid which contains 93% of the total volume (Jirum, & Srihanam, 2011; Li, et al., 2012).

The acidic extraction could somehow facilitate the release of phytochemicals by breaking the plant's cells' walls and the performance of the extraction would depend upon the solvent and its concentration (Rayle, & Cleland, 1992; Vadivel, & Brindha, 2015). Moreover, the DHRB extract obtained from different solvents would be composed of several chemical compounds which exhibited different antioxidant activity. Extraction with water tended to decrease the antioxidant activity when using a higher concentration of citric acid, while using alcohol with higher citric acid provided higher antioxidant activity. As such, the results of this present study were in accordance with Jianmei et al. (2004) who reported the antioxidant extraction of peanut skin using water and 80% of ethanol concentration and found that extraction by using water gave better antioxidant activity than that of ethanol. Furthermore, the ABTS values were 4.10 and 3.39 μmol of the Trolox equivalents per 1 gram of the dried sample, respectively. Pinelo et al. (2004) extracted antioxidants from the bark of almonds with water, methanol and ethanol and found that the methanolic extract had better antioxidant activity than that of water and ethanolic extracts, respectively. Moreover, Anwar et al. (2013) investigated the methanolic and ethanolic extracts for the antioxidant activity of cauliflowers, which were dried using a different drying condition and found that the methanolic extract gave better antioxidant activity.

2. Effects of the solvent type and citric acid concentration on the total phenolic content of DHRB

Figure 12 shows that the type of solvent and concentration of citric acid significantly affected the amount of the phenolic compounds in DHRB ($P<0.05$). The methanolic extract gave the highest phenolic compound followed by the water and ethanolic extracts, respectively. By using methanol and ethanol for the extraction, it could be shown that the number of phenolic compounds were likely to rise with an increasing citric acid concentration while the extraction using water presented a lower number of phenolic compounds but when using a lower citric acid concentration, the obtained phenolic compounds were higher. Consequently, it seemed that alcohol and

water were opposed to each other in interacting with the citric acid concentration. DHRB extracted by methanol with 0.1 M of citric acid concentration had the highest phenolic compounds of 86.63 mg of gallic acid/g and the highest phenolic compounds extracted by water only and ethanol with 0.1 M of citric acid were 52.54 and 13.35 mg of gallic acid/g, respectively. Almost all of the phenolic compounds in plants are mainly in the form of a water-soluble form (Moongngarm, 2012), and the extraction of phenolic compounds often uses organic solvents since they can be dissolved by the same principles. Additionally, the organic solvents used for the extraction of phenolic compounds usually included methanol and ethanol (Jirum, & Srihanam, 2011), and ethanol was normally used because it is safer than other types of organic solvents. Thus, the obtained results were consistent with the experiments of Arab et al. (2011) who studied the type of solvent used in the extraction of phenolic compounds from two rice cultivars (Fajr and Tarem) and found that the methanolic extract gave the highest phenolic compounds followed by ethanolic and ethyl acetate extract, respectively. Tan et al. (2013) studied the use of water and methanol in the extraction of phenolic compounds from rice (temukut) and found the methanolic extract provided a higher phenolic compound than that of water. Pinelo et al. (2004) conducted the extraction of phenolic compounds from pine sawdust using different solvents; i.e., water, methanol and ethanol and found that methanolic extract had the highest phenolic compounds followed by ethanolic and water extract, respectively. Jianmei et al. (2004) extracted phenolic compounds from the skin of peanuts using water, methanol and 80% of ethanol concentration and found that the methanolic extract provided the highest phenolic compounds followed by ethanolic and water extracts as 90.1, 89.9 and 56.7 mg of gallic acid/g, respectively. In addition, the results from this present study were consistent with Bahar et al. (2009) who studied the effect of ethanol with various amounts of 30% of citric acid on the extraction of phenolic compounds from olives and found that using 10 ml of citric acid gave the highest phenolic compounds and when using a higher amount of citric acid, the phenolic compound was reduced. This was because phenolic compounds normally found in most plants comprise three different groups; i.e., a free form, conjugated form and bound form. The bound form is the group, which is mainly found in the layer of lignin extracted by using acidic or alkaline hydrolysis. The free form is the second group, but

the structure is rarely stable so when using an acidic extraction of phenolic compounds, the structure would be partially destroyed. However, when using acid at a higher concentration, the plant's cells' walls were ruptured; hence, the more active ingredients were released. The results of this present study revealed that each solvent had a certain citric acid concentration for the optimum extraction condition as well (Adom, & Liu, 2002; Choi et al., 2007; Zhou et al., 2004).

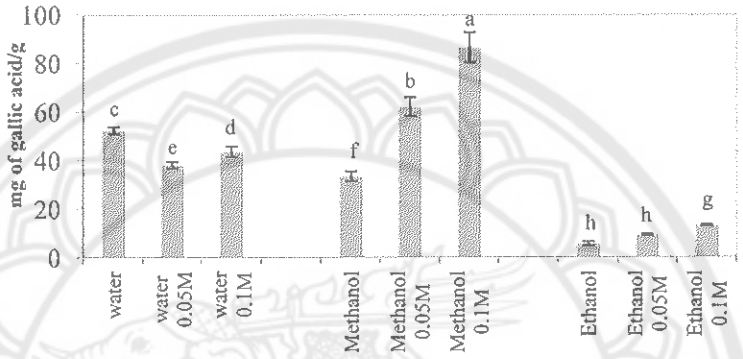

Figure 12 Total phenolic contents of DHRB extracts by using different extracting solvents

Note: *Different letters indicate statistical differences (one-way ANOVA and Duncan test, $P < 0.05$).

3. Effects of the solvent type and citric acid concentration on the total flavonoid content of DHRB

The study found that the type of solvents and the concentrations of citric acid statistically affected the amount of total flavonoids extracted from DHRB ($P<0.05$). The treatments extracted by using methanol gave the highest total flavonoids followed by those with water and ethanol, respectively. The extraction using methanol and ethanol tended to increase the total flavonoid contents when increasing the citric acid concentration while the total flavonoid contents of the water extract was decreased with an increase of the citric acid concentration. The extraction using methanol with 0.1 M of citric acid concentration gave the highest total flavonoid content of 14.67 mg catechin equivalents/g, and other solvents provided a total flavonoid content in the

range between 2.52-13.66 mg catechin equivalents/g. The obtained results were the same as the phenolic contents, as the total flavonoid antioxidants were in the phenolic compound group (Liu, 2004).

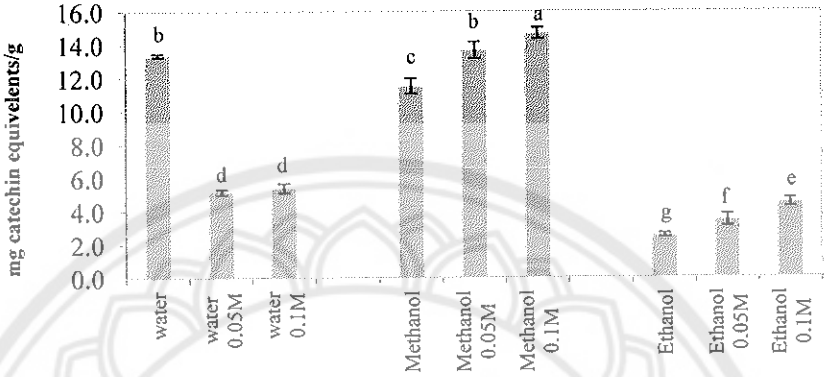

Figure 13 Total flavonoid contents of DHRB extracts by using different extracting solvents

Note: *Different letters indicate statistical differences (one-way ANOVA and Duncan test, $P< 0.05$).

4. Effects of the solvent type and citric acid concentration on the anthocyanin content of DHRB

The content of the anthocyanins extracted from DHRB is shown in Figure 14. The extraction conditions showed a statistically significant effect on the anthocyanin content ($P<0.05$) in which the methanolic extract had the highest anthocyanin content followed by the water and ethanolic extracts, respectively. As previously mentioned, methanol had a lower viscosity than ethanol and water, so it could better disperse into the samples and eluted more anthocyanins, which were more soluble in the polar solvents (Rezaie et al., 2015). The methanolic extract with 0.1 M. of citric acid provided the highest anthocyanin content of 23.01 mg/g, and the anthocyanin contents obtained from using other solvents were found in the range of 3.69-22.42 mg C3G/g. The number of anthocyanins was significantly increased when using acidified alcohol with a higher citric acid concentration while using water as a

solvent that tended to lower the anthocyanin contents even when acidified with citric acid. Furthermore, in using a higher citric acid concentration in the water extraction, the obtained anthocyanin content was significantly higher ($P<0.05$). This was because anthocyanin is more stable in an acid solution (Fuleki, & Francis, 1968) and has the ability to bind to free radicals in the body like vitamin C, Vitamin E and beta-carotene, which are several times (Chen et al., 2006) a more powerful natural antioxidant (Lee, 2010). In addition, using acids to assist the phytochemical extraction would help digest the cell walls of the plant samples; hence, anthocyanin could be released very effectively in higher amounts. The results of this present study were consistent with the study of Li et al. (2012) who reported the use of microwave assisted extraction of anthocyanins from grape peels with citric acid. The results showed the factors that mostly affected the anthocyanin content were the concentration of citric acid, the extraction time, the power of the microwave, and the ratio between the sample and the solvent, respectively. Moreover, the anthocyanin content rose with the increasing citric acid concentration.

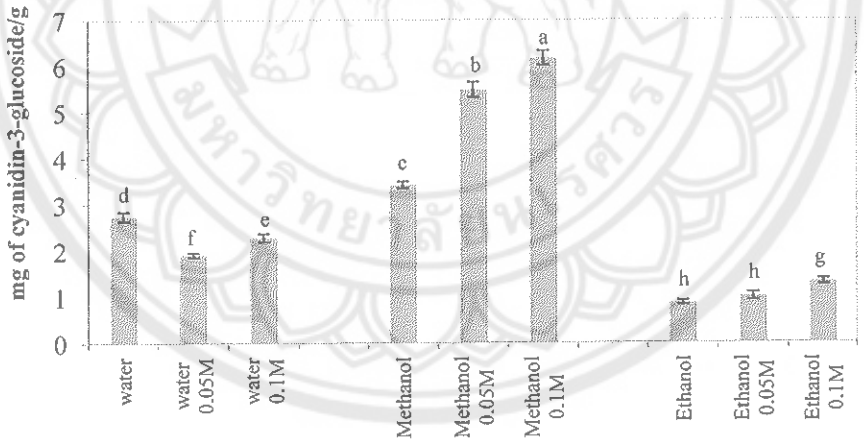

Figure 14 Anthocyanin contents of DHRB extracts by using different extracting solvents

Note: *Different letters indicate statistical differences (one-way ANOVA and Duncan test, $P< 0.05$).

5. Correlation between phytochemicals and antioxidant activity

The correlation analysis between total phenolic content, total flavonoids, anthocyanin and antioxidant activities obtained from the DHRB extract are given in Table 14.

Table 14 Pearson's correlation coefficients of total phenolic content (TP), total flavonoids (TF), anthocyanin (AT) and antioxidant activities of DHRB extract

	TP	TF	AT	DPPH	ABTS	FRAP
TP	1					
TF	0.996**	1				
AT	0.990**	0.991**	1			
DPPH	0.989**	0.988**	0.992**	1		
ABTS	0.982**	0.987**	0.991**	0.993**	1	
FRAP	0.990**	0.989**	0.988**	0.992**	0.992**	1

** Correlation is significant at the 0.01 level (2-tailed)

The total phenolic content, total flavonoids and anthocyanin of the DHRB extracts also exhibited a significant correlation ($P<0.01$) with antioxidant activities. Strong correlation between total phenolic content and antioxidant activity values were found in various conditions. The results presented in this study demonstrated that total phenolic content played an important role in the antioxidant activity and total flavonoids and anthocyanin and antioxidant activity assays (DPPH, ABTS and FRAP) were highly correlated with total phenolic content. The correlation data between total flavonoids, anthocyanin, antioxidant activities (DPPH, ABTS and FRAP) and total phenolic content were 0.996, 0.990, 0.989, 0.982 and 0.990, respectively. When comparing between two correlation data from Table 12 and 14, it was found that the correlation of total phenolic content, total flavonoids, anthocyanin and antioxidant activities from different solvent extract on showed stronger significant correlation than those of the DHRB extracts since total phenolic content contributed in the DHRB

byproducts exhibited stronger correlation with these parameters than those of the DHRB extraction with different solvent extraction.

Therefore water was selected for using as an extracting solvent in the optimization process by microwave assisted extraction (MAE).

Section 3 Application of RSM for optimization of DHRB extraction using MAE with parameters i.e. power energy, citric acid concentration and extraction time on phytochemicals and antioxidant activities of the extract

The experimental data from central composite design demonstrates that the power energy (X1), citric acid concentration (X2) and extraction time (X3) had revealed quadratic effect on total phenolic (Y1), total flavonoid (Y2), anthocyanin (Y3), DPPH (Y4), ABTS (Y5) and FRAP (Y6) (Table 15). The observed values of total phenolic content, total flavonoids and anthocyanin of DHRB extracts varied from 21.97 to 86.78 mg GAE/g DM, 15.00 to 78.33 mg CE/g DM and 4.16 to 16.98 mgC3G/g DM, respectively. The antioxidant activities of the DHRB extracts determined by the DPPH, ABTS and FRAP methods were between 40.27 – 17.22 μmol TE/g DM, 32.89 – 55.99 μmol TE/g DM and 75.15 – 162.31 μmol TE/g DM, respectively.

The experimental data allowed the development of mathematic equations resulting the predicted results. The second order models and regression coefficients of the intercept, linear, quadratic and interaction terms of model had significant effects of $P<0.0001$, $P<0.001$, $P<0.01$ or $P<0.05$ (Table 16). For all responses, the quadratic polynomial models were significant with *P*-values for $P<0.0001$.

The analysis of variance (ANOVA) to assess the goodness of fit of all parameters of the models and to estimate the statistic significant of the factor and interactions between terms is shown in Table 16. The R-squared (R^2) values were in the range between 0.962 to 0.989 and were in agreement with the Adjust R-squared (Adj R^2) values in the range between 0.927 to 0.968. Both R^2 and Adj R^2 values indicated that the general availability and accuracy of the polynomial model were adequate (Han et al., 2016).

Table 15 Experimental design of central composite design (CCD) of TP, TF, DPPH, FRAP and ABTS of DHRB extracts

Run no.	Factors			Responses					
	(X_1) [a] Power energy (Watt)	(X_2)[b] Citric acid concentration (mol/dm^3)	(X_3)[c] Extraction time (secound)	TP (mg GAE/ g DM)	TF (mg CE/ g DM)	AT (mg C3G/ g DM)	DPPH (μ mol TE/g DM)	ABTS (μ mol TE/g DM)	FRAP (μ mol TE/g DM)
1	-1	1	-1	26.97	25.00	6.40	59.54	35.14	98.85
2	0	-1.682(α)	0	50.48	41.67	10.94	85.54	43.45	117.08
3	0	0	-1.682(α)	45.34	30.82	9.58	80.21	45.89	115.54
4	1	-1	1	62.34	52.37	13.99	85.58	42.57	134.54
5	-1	-1	-1	42.90	25.00	7.71	68.58	48.07	96.77
6	1.682(α)	0	0	80.76	57.67	16.19	95.03	49.84	139.38
7	0	0	1.682(α)	43.40	23.57	8.36	66.36	40.89	120.08
8	-1	-1	1	32.64	15.00	4.16	44.89	32.89	82.46
9	1	1	1	63.59	61.73	14.35	95.56	52.24	145.92
10	-1	1	1	21.97	20.19	5.17	45.11	33.27	87.62
11	1	1	-1	50.06	48.33	10.74	90.25	43.22	129.23
12	0	1.682(α)	0	38.25	34.07	9.82	82.38	41.32	127.31
13	1	-1	-1	55.26	47.47	11.65	86.97	45.85	120.77
14	-1.682(α)	0	0	36.30	26.57	5.57	40.27	35.57	75.15
15	0	0	0	84.79	67.30	16.25	99.36	55.99	159.77
16	0	0	0	85.40	70.83	17.02	102.69	55.74	162.31
17	0	0	0	83.32	71.11	17.22	99.83	56.07	158.77
18	0	0	0	86.59	78.33	16.42	101.97	55.82	162.00
19	0	0	0	85.17	75.00	16.98	103.25	55.44	157.85
20	0	0	0	86.78	74.67	16.89	104.26	55.39	160.15

[a] Level of power energy: -α(298), -1(400), 0(550), 1(700), +α(800)

[b] Level of citric acid concentration: -α(0.033), -1(0.050), 0(0.075), 1(0.100), +α(0.117)

[c] Level of extraction time: -α(13), -1(40), 0(80), 1(120), +α(147)

Table 16 Second order polynomial equations and regression coefficients and analysis of the models for six response variables

Coefficient	Responses					
	TP	**TF**	**AT**	**DPPH**	**ABTS**	**FRAP**
b_0	85.42^{d}	72.76^{d}	16.81^{d}	101.93^{d}	55.75^{d}	160.1^{d}
X_1	13.29^{d}	12.96^{d}	3.31^{d}	17.01^{d}	4.28^{d}	19.97^{d}
X_2	-3.74^{d}	0.19^{ns}	-0.20^{ns}	-0.06^{ns}	-0.67^{d}	3.24^{d}
X_3	0.15^{ns}	-0.64^{ns}	-0.06^{ns}	-4.21^{d}	-1.44^{d}	0.92^{ns}
X_1^2	-9.99^{d}	-10.15^{d}	-2.18^{d}	-12.37^{d}	-4.69^{d}	-18.91^{d}
X_2^2	-15.00^{d}	-11.66^{d}	-2.35^{d}	-6.61^{d}	-4.80^{d}	-13.64^{d}
X_3^2	-15.00^{d}	-15.43^{d}	-2.85^{d}	-10.38^{d}	-4.45^{d}	-15.19^{d}
X_{12}	2.83^{b}	0.63^{ns}	-0.03^{ns}	2.76^{b}	2.45^{d}	1.58^{a}
X_{13}	4.48^{d}	4.14^{ns}	1.34^{d}	5.26^{d}	2.85^{d}	7.00^{d}
X_{23}	1.46^{a}	1.71^{ns}	0.45^{a}	2.00^{a}	3.20^{d}	0.75^{ns}
Regression model						
F-value	326.17	27.97	165.10	244.24	1341.50	596.27
P -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Lack of fit						
F -value	3.03^{ns}	3.32^{ns}	2.67^{ns}	1.00^{ns}	1.70^{ns}	0.86^{ns}
P -value	0.125	0.107	0.153	0.500	0.287	0.565
R^2	0.987	0.962	0.963	0.976	0.989	0.978
R^2-*adj*	0.964	0.927	0.957	0.951	0.968	0.956

[ns] Not significant

[a] Significant at $P < 0.05$

[b] Significant at $P < 0.01$

[c] Significant at $P < 0.001$

[d] Significant at $P < 0.0001$

The lack of fit indicates the variation of the data around the fitted model. If the model will be significant, it does not fit the data well (Ghafari et al., 2009; Han et al., 2016). The P-value for lack of fit presents in Table 16, showing the non-significant, implying model had a significant correlation between the responses and variables. Therefore, the six responses suggested quadratic polynomial models could be adequately used to predict models. These results were corresponding with the finding of González-Centeno et al. (2014) who reported the significant regression and non-significant lack of fit of model would be fitted to the experimental data which were suitable to predict within the system.

The effects of power energy, citric acid concentration and extraction time on TP, TF, AT, DPPH, ABTS and FRAP values of DHRB extract are depicted with the three dimension response surface plots. Fig.15-20 presents three dimension response surface showing interaction of two variables, while another variable was fixed constant at their respective zero level. The results indicated that power energy, citric acid concentration and extraction time had a significantly effected ($P<0.01$) by the quadratic terms of TP, TF, AT, DPPH, ABTS and FRAP.

Response surface of TP, TF and AT

The response surface of TP, TF and AT as presented in Table 14 demonstrates that power energy (X_1), citric acid concentration (X_2) and extraction time (X_3) had a quadratic effect. The experimental results were attained second-order polynomial equation of TP, TF and AT as follows:

$$Y_1 = 85.42 + 13.29X_1 - 3.74X_2 + 0.15X_3 - 9.99X_1^2 - 15.00X_2^2 - 15.00X_3^2 + 2.83X_1X_2 + 4.48X_1X_3 + 1.46X_2X_3 \quad (3)$$

$$Y_2 = 72.76 + 12.96X_1 + 0.19X_2 - 0.64X_3 - 10.15X_1^2 - 11.66X_2^2 - 15.43X_3^2 + 0.63X_1X_2 + 4.14X_1X_3 + 1.71X_2X_3 \quad (4)$$

$$Y_3 = 16.81 + 3.31X_1 + 0.20X_2 - 0.06X_3 - 2.18X_1^2 - 2.35X_2^2 - 2.85X_3^2 - 0.03X_1X_2 + 1.34X_1X_3 + 0.45X_2X_3 \quad (5)$$

As depicted in Table 15, each parameter had more significant influence on the TP, TF and AT contents. All of equations were extremely significant (P<0.0001) and the lack of fit was non-significant (P>0.05). Hence the results indicated that the equations were reasonable. ANOVA of model regression coefficients was significance. The effects of power energy (X_1) citric acid concentration (X_2) from linear, quadratic (X_1^2, X_2^2 and X_3^2) and interaction (X_1X_2, X_1X_3 and X_2X_3) of TP were significant (P<0.05), while the effects of citric acid concentration (X_2) extraction time (X_3) of TF and AT model from linear and interaction were non-significant but all quadratic parameters were significant (P<0.05). The R^2 for all response TP, TF and AT were 0.987, 0.962 and 0.963, respectively, indicated that the model could explain 98.7, 96.2 and 96.3% variability of the response variables. This results was in accordance with González-Centeno et al. (2014) and Sai-Ut et al. (2015) who found that the R^2 values had to be higher than 0.80 which indicated a good correlation between responses and independent variable of model. The Adj R^2 of TP, TF and AT (0.964, 0.927 and 0.957) were agreed with the R^2. The P-value for lack of fit shows non-significant, implying the effects of independent variables on the TP, TF and AT were described by the obtained model.

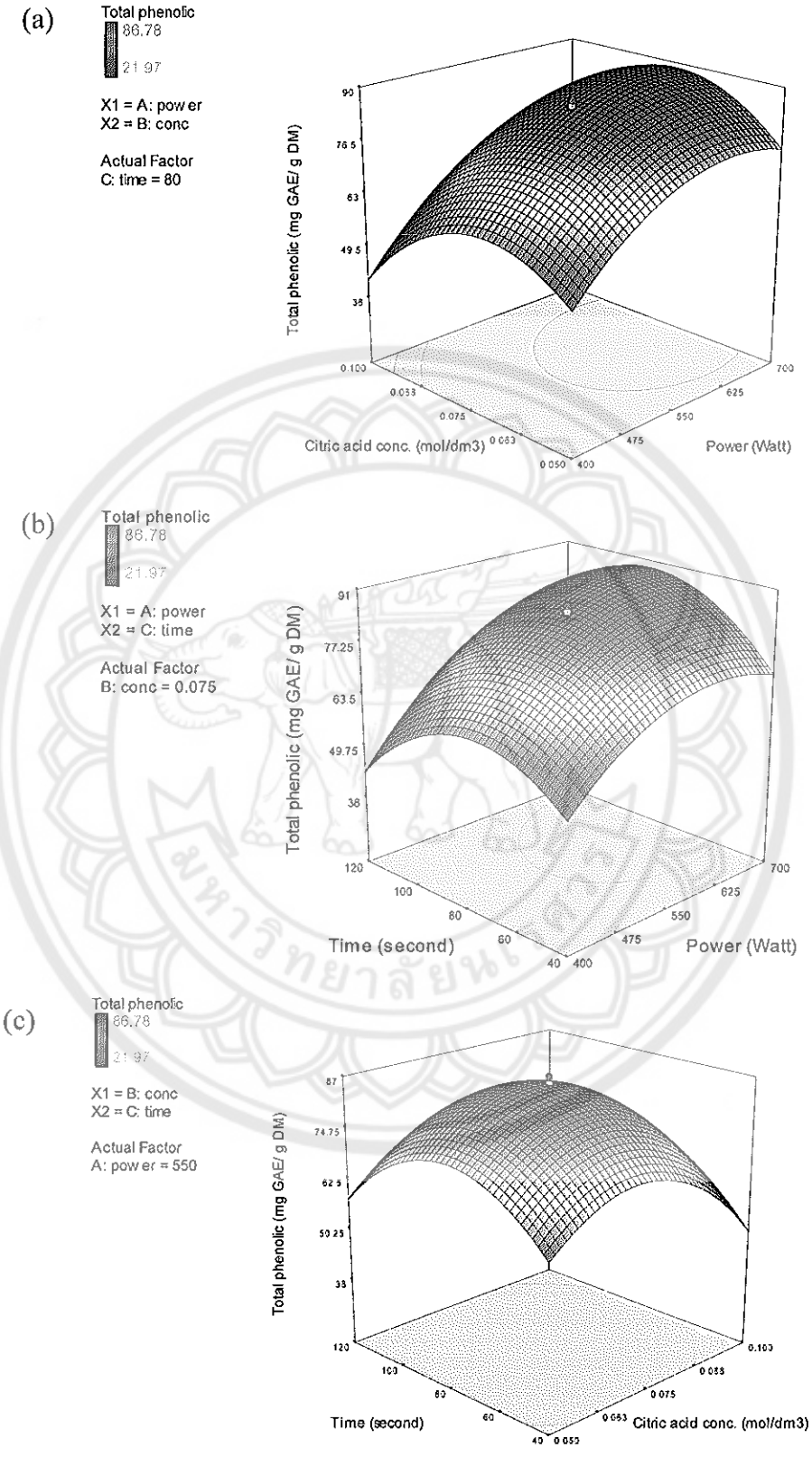

Figure 15 Response surface plots of DHRB showing the effects of power energy, citric acid concentration and extraction time on total phenolic content

The response surface three dimension plots are the representation of regression equation indicating the relationship between dependent and independent variables. The response surface plots are different shapes indicating whether the variables or mutual interactions are significant or not (Liu et al., 2013). The response surface plots based on equations 3, showing the effects of two variables within the experiment and another one variable was kept constant at their optimum values are presented in Fig. 15. The effects of power energy and citric acid concentration on the TP, TF and AT contents are shown in Fig. 15a, 16a and 17a, respectively. Fig. 15a, 16a and 17a demonstrates the power energy (X_1), the citric acid concentration (X_2) and their reciprocal interaction on TP, TF and AT contents, where the extraction time (X_3) was fixed at 80 seconds. The results indicated that TP, TF and AT increased slowly with increasing citric acid concentration while the increase of power energy resulted the rapidly increase of TP, TF and AT content. However, beyond 0.075 mol/dm^3 and 625 watt, the TP content decreases slightly. The response surface three dimension plots in Fig. 15b, 16b and 17b, which kept the citric acid concentration at zero level (0.075 mol/dm^3), showed that the TP, TF and AT contents increased with increasing extraction time at the initial stage and then slightly decreased of trend, while the increase of power energy resulted the rapidly increase of TP, TF and AT content. However, beyond 80 second and 625 watt, the TP, TF and AT content was decreased slightly. As shown in Fig. 15c, 16c and 17c, the TP, TF and AT contents were increased when citric acid concentration increased from 0.050 to 0.075 mol/dm^3 and the extraction time from 40 to 90 second, respectively and then they began to decrease afterwards.

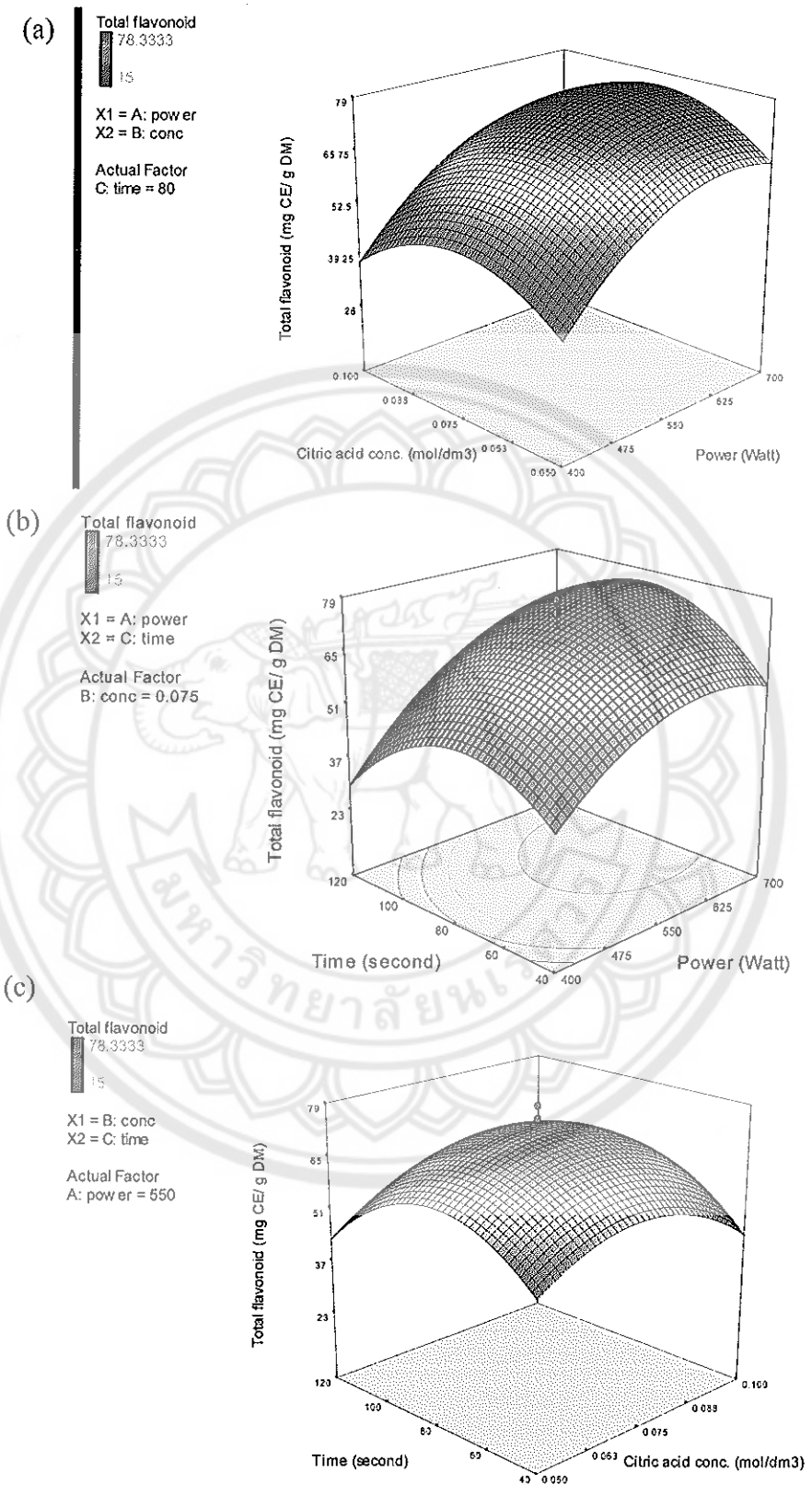

Figure 16 Response surface plots of DHRB showing the effects of power energy, citric acid concentration and extraction time on total flavonoid content

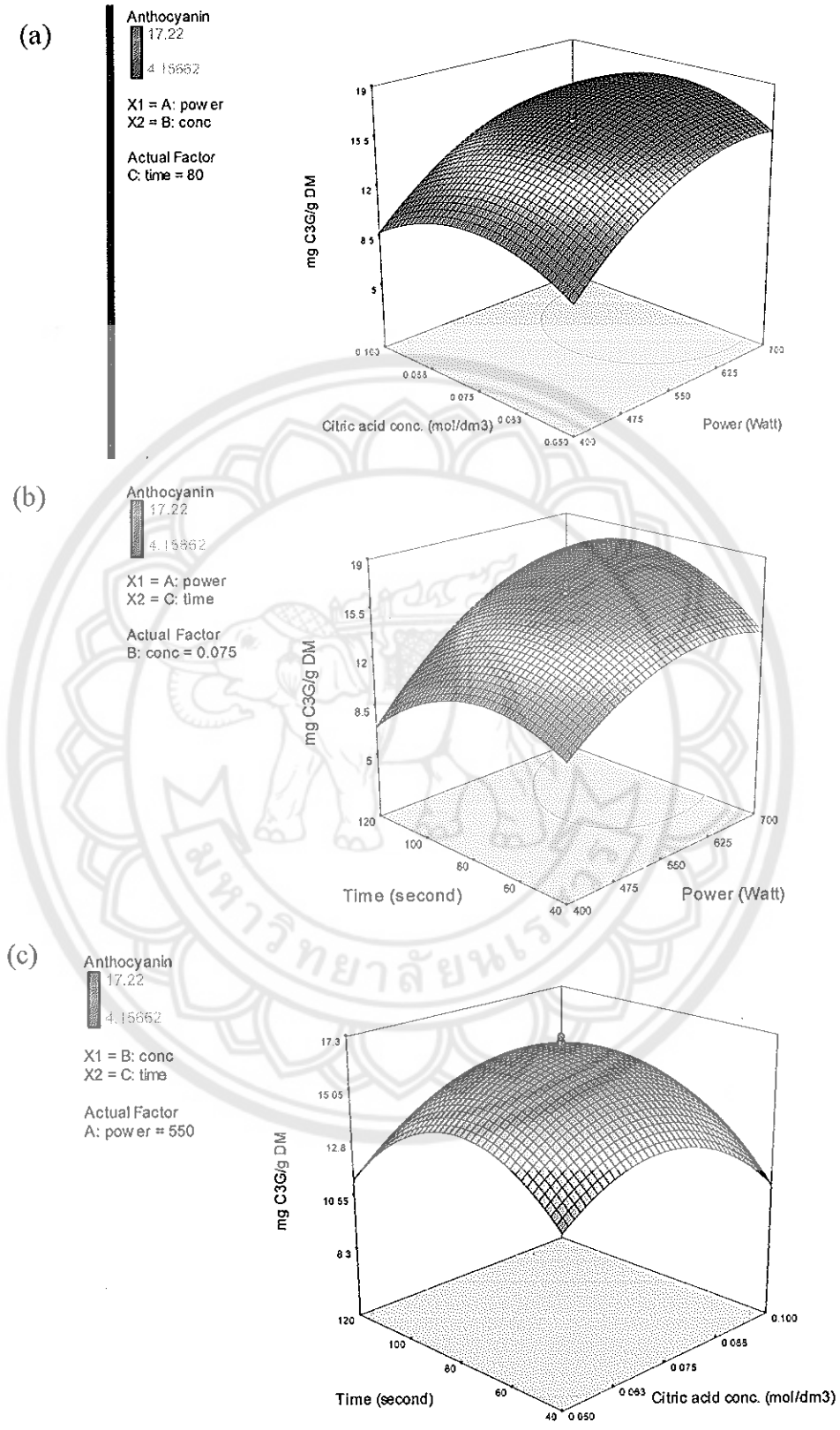

Figure 17 Response surface plots of DHRB showing the effects of power energy, citric acid concentration and extraction time on anthocyanin content

The similar results was also reported in the extraction of anthocyanin content from grape peel by Li et al. (2012), found that increasing power energy and citric acid concentration resulted in higher anthocyanin. MAE utilizes the energy of microwaves to cause molecular movement and rotation of liquids with a permanent dipole leading to a very fast heating of the solvent and the sample, offering advantages such as improved efficiency and reduced extraction time (Sun et al., 2007). This result was agreed with the opinion of Li et al. (2012) pointed out that higher energy level could be enough to rupture of the plant material structure and release the chemicals contained therein. In addition, the factors that mostly affected the anthocyanin content were the concentration of citric acid, the extraction time, the power of the microwave, and the ratio between the sample and the solvent, respectively. Citric acid concentration affecting anthocyanin extraction contents which may be attributed to the change of polarity of solvent as citric acid concentration was changed. More polar phenolic compounds may be extracted according to "like dissolves like" principle and solvent would extract compounds which have similar polarity with solvents (Chew et al., 2011; Gong et al., 2012). Basically, phenolic could easily be dissolved from material cells when solvent polarity are similar to phenolics (Sai-Ut et al., 2015). In addition, using acids to assist the phytochemical extraction would help digest the cell walls of the plant samples; hence, anthocyanin could be released very effectively in higher amounts.

When increasing citric acid concentration, the pH of solvent was lower and the TP, TF and AT were decreased. The result were similar to Bahar et al. (2009) studied the effect of solvent and citric acid on the extraction of phenolic compounds from olives and found that when using a higher amount of citric acid, the phenolic compound was reduced. This was due to phenolic compounds normally found in most plants comprise three different groups; i.e., a free form, conjugated form and bound form. The bound form is mainly found in the layer of lignin extracted by using acidic or alkaline hydrolysis, while the free form is the group that the structure is rarely stable so that when using acidic extraction of phenolic compounds, it would be partially destroyed. However, when using acid at a higher concentration, the plant cell walls were ruptured, hence the more active ingredients were released.

From the investigation of influence of extraction time on the TP, TF and AT content found that TP, TF and AT were increased in the early stage until they reacted the maximum release and then they were decreased since longer extraction causes more degradation of the extract due to phenolics could more react with surrounding environment such as heat, oxygen and light (Chew et al., 2011).

Response surface of antioxidant activity

The effect of independent variables i.e., power energy (X_1), citric acid concentration (X_2) and extraction time (X_3) on antioxidant activity obtained from DRHB with MAE were investigated. Antioxidant activity of DRHB determined by DPPH, ABTS and FRAP methods ranged between 40.27-104.26 μmol TE/g DM, 32.89-56.07 μmol TE/g DM and 75.15-162.31 μmol TE/g DM, respectively. The ANOVA indicated that three quadratic polynomial equations (DPPH, ABTS and FRAP) were highly significant ($P<0.0001$) while lack of fits from three equations were not significant ($P>0.05$), indicating the models were good fitted to the experimental data. These results were confirmed by coefficient of determination (R^2) of DPPH, ABTS and FRAP models, which were 0.976, 0.989 and 0.978, respectively, which explained good regression values of all variances in the data. The quadratic polynomial equations for antioxidant activities were as follows (6) - (8).

$$Y_4 = 101.93 + 17.01X_1 - 0.06X_2 - 4.21X_3 - 12.37X_1^2 - 6.61X_2^2 - 10.38X_3^2 + 2.76X_1X_2 + 5.26X_1X_3 + 2.00X_2X_3 \quad (6)$$

$$Y_5 = 55.75 + 4.28X_1 - 0.67X_2 - 1.44X_3 - 4.69X_1^2 - 4.80X_2^2 - 4.45X_3^2 + 2.45X_1X_2 + 2.85X_1X_3 + 3.20X_2X_3 \quad (7)$$

$$Y_6 = 160.18 + 19.97X_1 + 3.24X_2 + 0.92X_3 - 18.91X_1^2 - 13.64X_2^2 - 15.19X_3^2 + 1.58X_1X_2 + 7.00X_1X_3 + 0.75X_2X_3 \quad (8)$$

The relationship between the extraction factors and antioxidant activities investigated by three dimensional response surface plots, are presented in Fig. 18-20.

The three dimension response surface plot of DPPH, ABTS and FRAP assay were similar to the same trend of TP, TF and AT. The response surface plots depicted in Fig. 18a, 19a and 20a, indicating the effects of two independent variables on DPPH, ABTS and FRAP while the other factor was set at zero level. The response surface plots showed that DPPH, ABTS and FRAP increased slowly with increasing citric acid concentration as well as extraction time at the initial stage and then the antioxidant activities slightly decreased, while the increase of power energy resulted the rapidly increase of DPPH, ABTS and FRAP. However, beyond 625 watt and 0.075 mol/dm^3, the DPPH, ABTS and FRAP decreased slightly. Our results were in accordance with Bachir Bey *et al*, (2014) who reported the polarity played more important role in antioxidant activity. This might be ascribed to the fact that citric acid is a strong organic acid, and there are three H^+ which can be ionized, so the pH value is much lower in the solution with higher citric acid concentration. Microwave can make those polar ions move in the solution, and then heat the solution and there is much more H^+ in low pH solution, therefore, the low pH solution has higher temperature than the high pH solution (Li et al., 2012). It was noticed that both citric acid concentration and power energy were independent upon DPPH, ABTS and. FRAP.

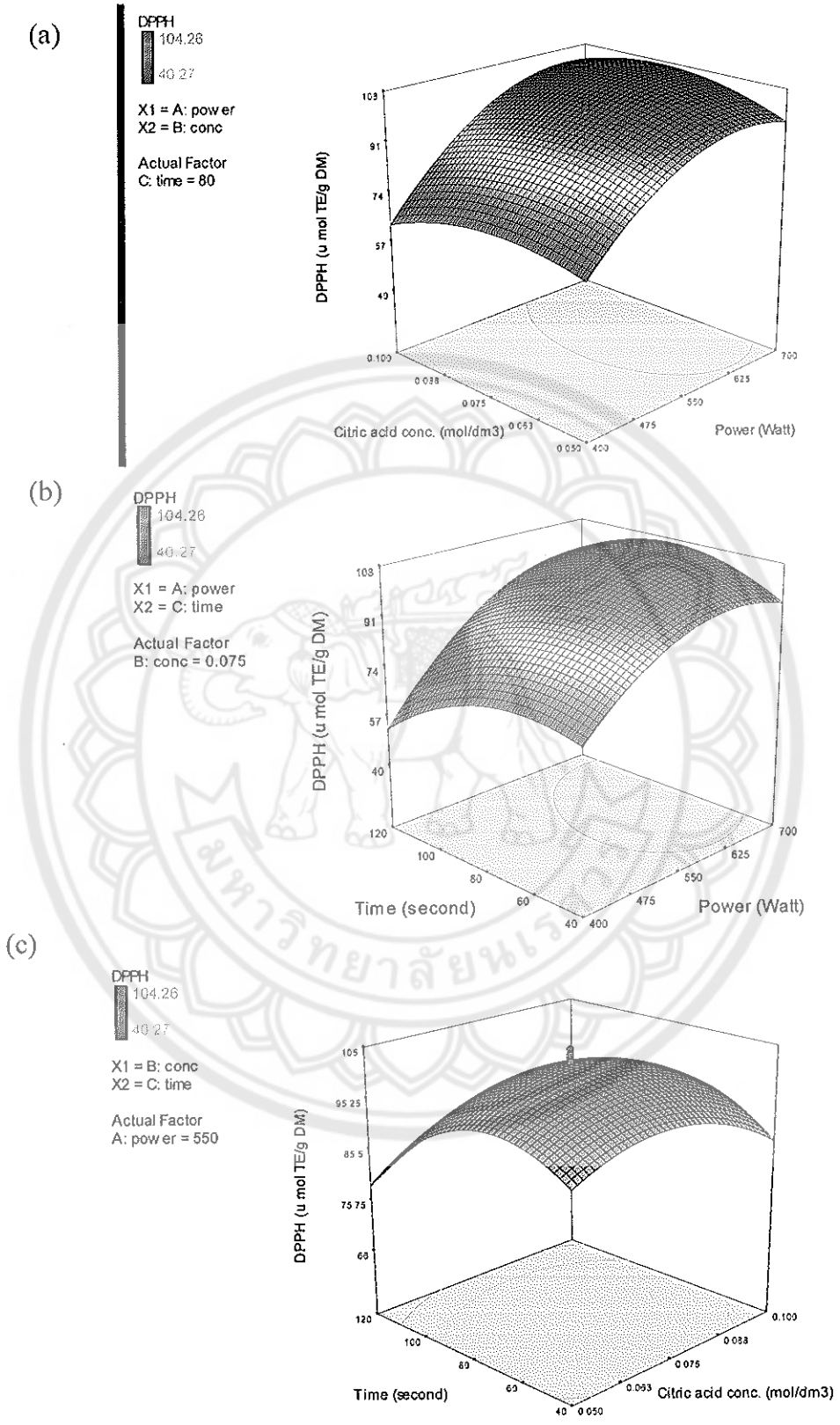

Figure 18 Response surface plots of DPPH of DHRB as affected by power energy, citric acid concentration and extraction time

The plots illustrated in Fig. 18b, 19b and 20b indicating the effects of two independent variables on DPPH, ABTS and FRAP while the other factor was set at zero level. The response surface plots showed that DPPH, ABTS and FRAP increased slowly with increasing extraction time at the initial stage and then the antioxidant activities slightly decreased., while the increase of power energy resulted in rapidly increase of DPPH, ABTS and FRAP. However, beyond 625 watt and 80 second, the DPPH, ABTS and FRAP were decreased slightly.

The plots illustrated in Fig. 18c, 19c and 20c, the response surface plots showed that DPPH, FRAP and ABTS increased with increasing citric acid concentration as well as extraction time at the initial stage and then the antioxidant activities slightly decreased. The DPPH, ABTS and FRAP were increased when citric acid concentration increased from 0.050 to 0.075 mol/dm^3 and the extraction time from 40 to 75 second, respectively and then they began to decrease afterwards. González-Centeno et al. (2014) reported the longer contact time between solvent and solid during extraction improved the diffusion of the compounds, however the prolong extraction time would lead to decrease the antioxidant activity of DHRB due to the equilibrium between the solute in the solute matrix and in the bulk solution (Silva et al., 2007) as well as the oxidation of phenolic compounds by prolonging the exposure to environment factors such as heat, light and oxygen (Juntachote et al., 2006).

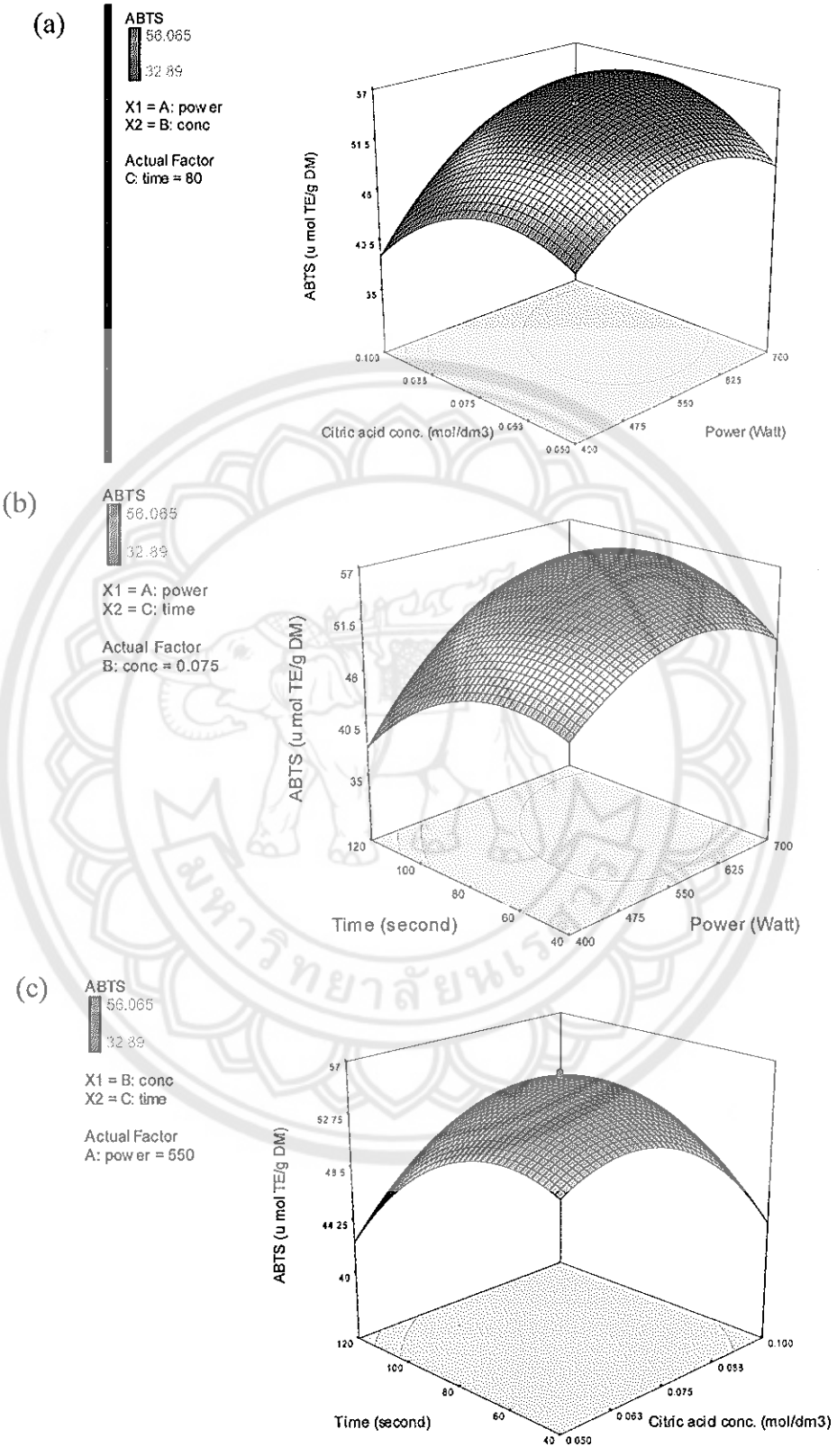

Figure 19 Response surface plots of ABTS of DHRB as affected by power energy, citric acid concentration and extraction time

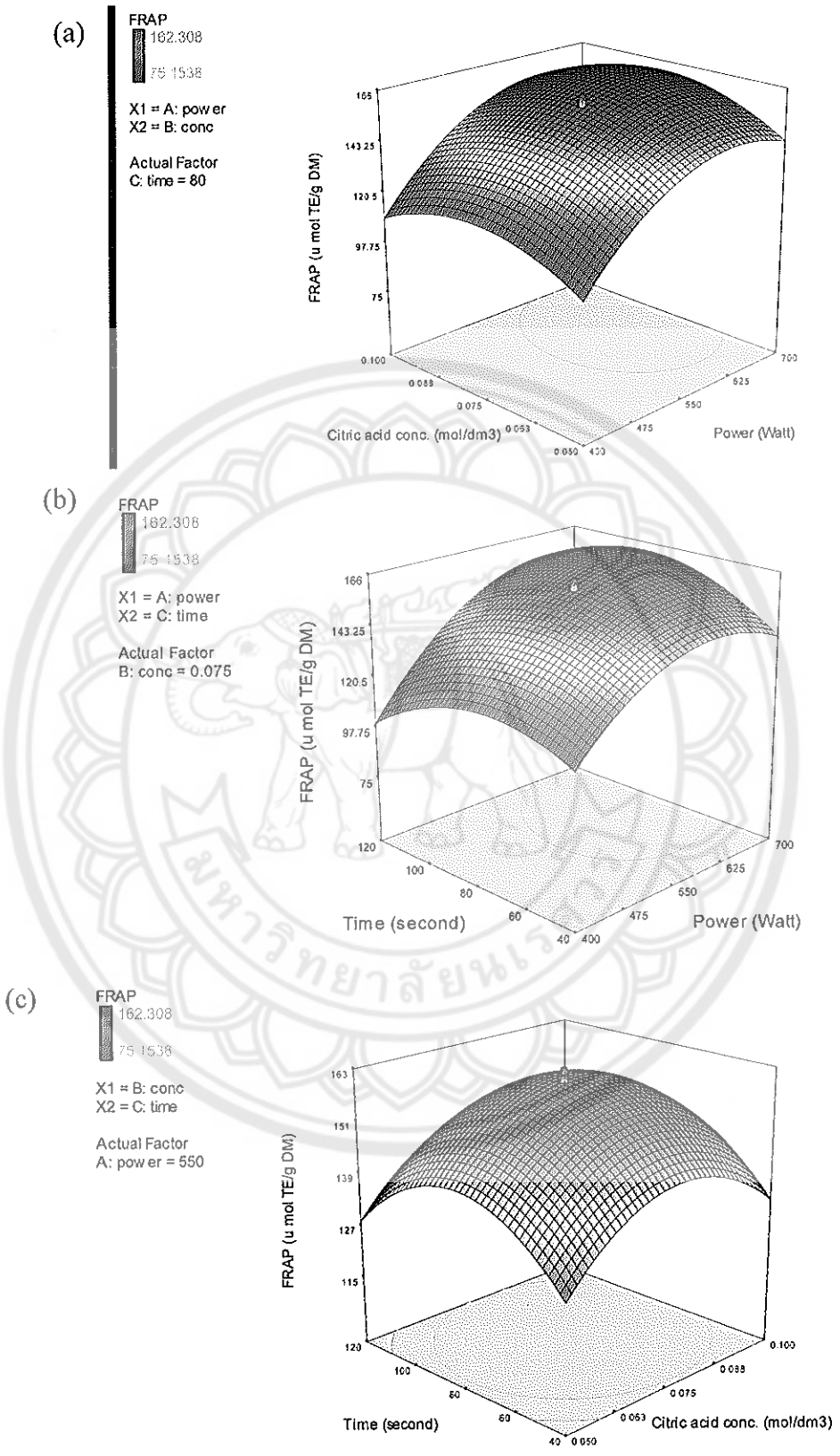

Figure 20 Response surface plots of FRAP of DHRB as affected by power energy, citric acid concentration and extraction time

Optimum conditions from DHRB and model validation

The RSM procedure is used to determine the experimental factors and levels which would allow to obtain an extract with high phytochemicals and antioxidant activities. The predictive ability of the models was examined by extraction at optimum conditions. Table 16 shows the optimum extracting condition which the three factors obtained from the model were following: 648 watt of power energy, 0.076 mol/dm^3 of citric acid concentration and extraction time of 83 seconds and the experimental values of 90.59 mg GAE/g DM (Y_1), 77.53 mg CE/g DM (Y_2), 18.82 mg C3G/g DM (Y_3), 109.35 µmol TE/g DM (Y_4), 58.09 µmol TE/g DM (Y_5), 168.26 µmol TE/g DM (Y_6), were obtained. To compare the predicted results which were close to the predicted values of 89.89 mg GAE/g DM (Y_1), 76.98 mg CE/g DM (Y_2), 18.08 mg/g DM (Y_3), 107.72 µmol TE/g DM (Y_4), 56.60 µmol TE/g DM (Y_5), 165.63 µmol TE/g DM (Y_6) by the regression models. Validation step was done to ensure that the predicted results were not biased towards the practical value with each response to obtain maximum values (Yim et al., 2012). The predicted values match well with the experimental values obtained by using optimum extracting conditions which the validation between the predicted and experimental values were within an acceptable error range as depicted by average mean deviation with the E% ranged between -1.85 to 3.27% (Table 16), which clearly showed that the model fitted the experimental data and therefore optimized the DHRB extract.

Table 17 Validation of predicted and experimental values of the TP, TF, AT, DPPH, FRAP and ABTS values from DHRB extract with different extraction conditions

Responses	Extracting conditions			
	Worse[a]	High[b]	Medium[c]	Optimum[d]
Predicted value				
TP	44.51	63.92	85.42	89.89
TF	29.49	54.53	72.77	76.98
AT	8.15	14.24	16.81	18.08
DPPH	69.84	95.32	101.94	107.72
ABTS	48.13	52.49	55.75	56.60
FRAP	97.63	145.90	160.18	165.63
Experimental value				
TP	46.02±0.49	65.08±0.43	86.59±0.85	90.59±0.46
TF	30.08±0.56	56.03±0.34	72.61±0.46	77.53±0.55
AT	8.02±0.43	14.62±0.66	17.06±0.52	18.82±0.49
DPPH	70.68±1.06	97.16±1.79	100.26±2.52	109.35±2.62
ABTS	47.26±1.13	53.69±1.76	56.95±1.80	58.09±1.42
FRAP	99.26±2.20	148.02±1.49	162.35±2.20	168.26±2.33
E%				
TP	3.27	1.78	1.35	0.77
TF	1.96	2.69	0.21	0.71
AT	-1.57	2.63	1.45	3.95
DPPH	1.18	1.89	-1.67	1.49
ABTS	-1.85	2.24	2.10	2.57
FRAP	1.66	1.43	1.34	1.56

[a] Worse(run 5): $X_1 : X_2 : X_3$ as 400 : 0.05 : 40

[b] High (run 9) : $X_1 : X_2 : X_3$ as 700 : 0.10 : 120

[c] Medium (run 15) : $X_1 : X_2 : X_3$ as 550 : 0.075 : 80

[d] Optimum : $X_1 : X_2 : X_3$ as 648 : 0.076 : 83

The obtained experimental values of power energy was in the same range as Chen et al. (2016) who reported the optimum power energy of 704 watt for extraction of antioxidant from tangerine peels, in addition Oussaid et al. (2018) reported the values of 600 watt for power energy and 69 second for extraction of phenolic compound from *Scirpus holoschoenus* L., respectively. The increase of power energy level beyond 500 watt could have speed-up the mass transfer of intracellular bioactive compounds. However, excessive microwave power could lead to the degradation of the total flavonoid content responsible for the antioxidant activity (Dahmoune et al., 2014; Spigno and De Faveri, 2009).

Therefore, power energy 648 watt, citric acid concentration 0.076 mol/dm^3 and extraction time 83 seconds gave the highest total phenolic content, total flavonoids and anthocyanin, hence the highest antioxidant activities were obtained so that it was selected to be used in the next experiments.

Section 4 Effect of encapsulation on phytochemicals and antioxidants activity of defatted Homnin rice bran extract

The physical properties of DHRB extract powder i.e. moisture content, water activity (a_w) and color during storage are shown in Table 18. The moisture content (MC) of DHRB extract powder ranged between 3.60 and 3.85%, while, water activity was in range between 0.234 to 0.242. The initial color parameters (L* (lightness), a* (redness) and b* (yellowness)) for encapsulation of DHRB extract powder were 66.67, 66.95, 70.16 and 67.40, respectively. Our results were in accordance with Suravanichnirachorn et al. (2018) who reported that the moisture content and water activity of mao (*Antidesma bunius* L.) powder ranged between 3.53 to 6.96 % and 0.149 to 0.360, respectively.

The initial values showed that the combination of MD and WPI as encapsulating agents gave higher lightness value compared with other agent ($P<0.05$). Lightness in food related with many factors, including the concentration and type of pigment present, water content and extract type (Viuda-Marton et al., 2010). For red-green coordinate, redness show that MD gave significantly higher red (a*value) color ($P<0.05$) than that of the combination of MD:SPI, MD:SPI:WPI and MD:WPI. The a* of DHRB extract powder ranges between 7.65 and 19.28. For the yellow color, MD:

SPI: WPI gave higher yellow (b*value) color than those of MD:SPI, MD:WPI and MD, respectively (Table 18). The results showed that encapsulating agents significantly affected the powdered color. The lightness, redness, and yellowness values of encapsulated DHRB extract powder were significant different ($P<0.05$). The solubility of powders in water at 25 ◦C was in the range between 16.08–21.14%. The sample prepared with MD (20% w/v) as a carrier material was significantly different ($P<0.05$) from those prepared from the mixture of MD with SPI and WPI. This behavior is associated with the interaction of phenolic compound with the different carriers on the microencapsulation process. The powders containing only 20% MD showed the highest solubility due to the high polarity of this polysaccharide (Zhang et al., 2014). The powders prepared with MD: SPI, MD: WPI and MD:WPI:SPI had less solubility due to the hydrophilic and hydrophobic interaction of phenolic compound with the protein. The hydrophilic region of the molecule of phenolic compound, especially anthocyanin is characterized by hydroxyl groups, which form hydrogen bonds with carbonyl groups and amine of protein. Moreover, hydrophobic region of the anthocyanin molecule constituted by the benzene ring interacts with nonpolar regions of proteins such as the amino acids groups. In the presence of water, nonpolar regions of proteins and anthocyanins tend to associate through van der Waals interactions decreasing the nonpolar surface areas exposed to water. The solubility of the system decreases due to migration of water molecules nonpolar regions to the solvent (Zhang et al., 2014).

Table 18 Physiochemical properties of encapsulated DHRB extract powder

Samples	DHRB extract powder properties					
	MC[ns]	a_w[ns]	L*	a*	b*	Solubility (%)
MD 20	3.60±0.15	0.234±0.004	66.77±0.42[b]	19.28±0.10[a]	2.23±0.40[b]	21.14±0.29[a]
MD 15: SPI 5	3.82±0.27	0.240±0.003	66.95±0.53[b]	15.26±0.05[b]	2.64±0.22[b]	16.08±0.85[b]
MD 15:WPI 5	3.85±0.29	0.242±0.005	70.16±0.75[a]	7.65±0.16[d]	2.26±0.36[b]	17.85±0.52[b]
MD 10:SPI 5: WPI 5	3.81±0.22	0.242±0.004	67.40±0.48[b]	8.47±0.15[c]	6.30±0.36[a]	16.50±1.52[b]

Different alphabetic uppercase letters within column indicate significant differences ($P<0.05$) for each measured parameter.

MD 20 = Maltodextrin 20% w/v of the extracts

MD 15: SPI 5 = Maltodextrin 15% w/v+ Soy protein isolate 5% w/v

MD 15: SPI 5 = Maltodextrin 15% w/v+ Whey protein isolate 5% w/v

MD 10: SPI 5:WPI5 = Maltodextrin 10% w/v+ Soy protein isolate 5% w/v +Whey protein isolate 5% w/v

[ns] Not significantly different.

Effects of encapsulating agent on the phytochemicals and antioxidant of DHRB extract powder

Table 19 shows that the encapsulating agents significantly affected ($P<0.05$) the amount of the TP, TF and AT in DHRB extract powder. The DHRB aqueous extracts before encapsulation had higher TP, TF and AT values (90.59 mg GAE/g DM, 77.53 mg CE/g DM. and 18.82 mg C3G/g DM, respectively) compared to the powders produced by freeze-drying. During freeze-drying, polyphenol degradation may have occurred due to freezing and dehydration stresses which may have been generated during the encapsulation process, as well as the grinding after lyophilization (Abdelwahed et al., 2006). During the grinding of the lyophilized material, the surface exposed to oxygen increased, leading to the oxidation of the phenolic compounds and antioxidants. The TP, TF and AT values of the powders obtained by freeze-drying ranged from 39.77 to 56.55 mg GAE/g DM, 25.63 to 30.39 mg CE/g DM and from 10.02 to 16.31 mg C3G/g DM, respectively. However, in our study, the highest TP, TF, and AT values obtained by freeze-drying using the mixture between maltodextrin and whey protein as a coating agent (Table 19). These results could be attributed to the capacity of whey proteins to interact with various coating agents, including maltodextrin and forming colloidal particles which encapsulated polyphenols. Furthermore, due to the light and oxygen, the degradation of some sensitive phenolic compounds could have produced these results (Jia et al., 2016; Schmitt, & Turgeon, 2011). In summary, the use of the polysaccharide-protein proved to be the most efficient coating agent for encapsulation of DHRB aqueous extracts by freeze-drying.

Table 19 Total phenolic content, total flavonoids and anthocyanin content of DHRB extract powder by using different encapsulating agent

Samples	DHRB extract powder properties		
	TP	TF	AT
	mg of GAE/g	mg of CA/g	mg of C3G/g
MD 20 %	39.77±1.32^{c}	25.63±1.28^{b}	10.25±1.20^{b}
MD 15: SPI 5%	41.91±2.21^{c}	26.38±2.22^{b}	10.02±1.11^{b}
MD 15:WPI 5%	56.55±2.23^{a}	30.39±2.63^{a}	16.31±0.98^{a}
MD 10:SPI 5: WPI 5%	52.24±1.95^{b}	28.06±2.31^{ab}	14.13±1.16^{a}

Different alphabetic uppercase letters within column indicate significant differences ($P<0.05$) for each measured parameter

The antioxidant activities of DHRB extract powder were determined by DPPH, ABTS and FRAP assays. Table 20 shows that the encapsulating agents significantly affected ($P<0.05$) the DPPH, ABTS and FRAP in DHRB extract powder. The DHRB aqueous extracts before encapsulation had higher the DPPH, FRAP and ABTS values (109.35, 58.09 and 168.26 μmol TE/g DM, respectively) compared to the powders produced by freeze-drying. The DPPH, ABTS and FRAP values of the powders obtained by freeze-drying ranged from 78.20 to 89.25 μmol TE/g DM, 36.82 to 58.59 μmol TE/g DM and from 95.69 to 119.25 μmol TE/g DM, respectively. However, in our study, the highest DPPH and FRAP values obtained by freeze-drying using the mixture between maltodextrin and whey protein isolate as a coating agent (Table 20). The obtained results when using different coating matrix types for encapsulation of antioxidants were the same trend as the contents of TP, TF and AT (Table 19). The antioxidants in rice are mainly phenolic compounds and immobilized in cellulose, lignin and proteins such as ferulic acid which contains 93% of the total volume (Jirum, & Srihanam, 2011; Li, et al., 2012).

Table 20 Antioxidant activities of DHRB extracts by using different encapsulating agents determined by DPPH, ABTS and FRAP assays

Samples	DHRB extract powder properties		
	DPPH	ABTS	FRAP
	µmol TE/g DM	µmol TE/g DM	µmol TE/g DM
MD 20 %	78.20±3.02[b]	36.82±1.28[c]	95.69±2.30[c]
MD 15: SPI 5%	80.90±2.05[b]	45.04±2.22[b]	97.60±2.41[c]
MD 15:WPI 5%	89.25±2.11[a]	55.35±2.63[a]	119.25±3.98[a]
MD 10:SPI 5: WPI 5%	87.21±1.85[a]	58.59±2.31[a]	102.99±2. 60[b]

Different alphabetic uppercase letters within column indicate significant differences ($P<0.05$) for each measured parameter

Encapsulation efficiency

In this step, the efficiency of different coatings to encapsulate phytochemicals and antioxidant activity of the extract from DHRB was evaluated and compared. Fig. 21 shows the percentage of TP, TF, AT, DPPH, ABTS and FRAP retained within the matrix, and antioxidant activity of the samples after encapsulation compared to the initial values present in DHRB extract. The antioxidant activity was expected to be reduced when compared to the initial antioxidant capacity of the DHRB extract, due to the lower amount of TP, TF, AT, DPPH, ABTS and FRAP presented in the encapsulated sample. The highest EE_{TP}, EE_{TF}, EE_{AT}, EE_{DPPH} and EE_{FRAP} values were obtained by freeze-drying and the mixture between MD and WPI (Table 17) was used as a coating agent. The results revealed that the coating had an important role in the retention of phytochemicals and antioxidant activity within the matrix. Under these conditions, the amount of TP, TF, AT, DPPH, ABTS and FRAP retained in the encapsulated sample from 43.90-62.42%, 33.06-39.20%, 53.24-86.66%, 71.51-81.62, 63.38-100.86 and 56.87-70.87%, respectively. However, these results are in agreement with those reported by Papoutsis et al. (2015), using the mixture between MD and SPI resulted in higher encapsulation efficiency of TP, TF, and FRAP compared with using 20% of MD as wall material. This behavior may be explained by the fact that

encapsulation efficiency is highly dependent on the encapsulated compounds and the coating material used (Rosa et al., 2014). The highest encapsulation efficiency values were observed when using the mixture of MD and WPI, indicating combination of both matrices with respect to the phytochemicals and antioxidant activity. The efficiency of coating agents to encapsulate polyphenols is associated to their solubility in dispersion, structure, and capacity to form films (Tao et al., 2017). The enhancement of encapsulation efficiency with the addition of the soybean protein or whey protein into maltodextrin was probably due to the interaction of the protein with the maltodextrin and the formation of complexes with interfacial and amphiphilic properties (Tao et al., 2017). These results suggest that the encapsulation efficiency of DHRB aqueous extracts enriched with polyphenols is affected by both the coating agent as well as the encapsulation technique.

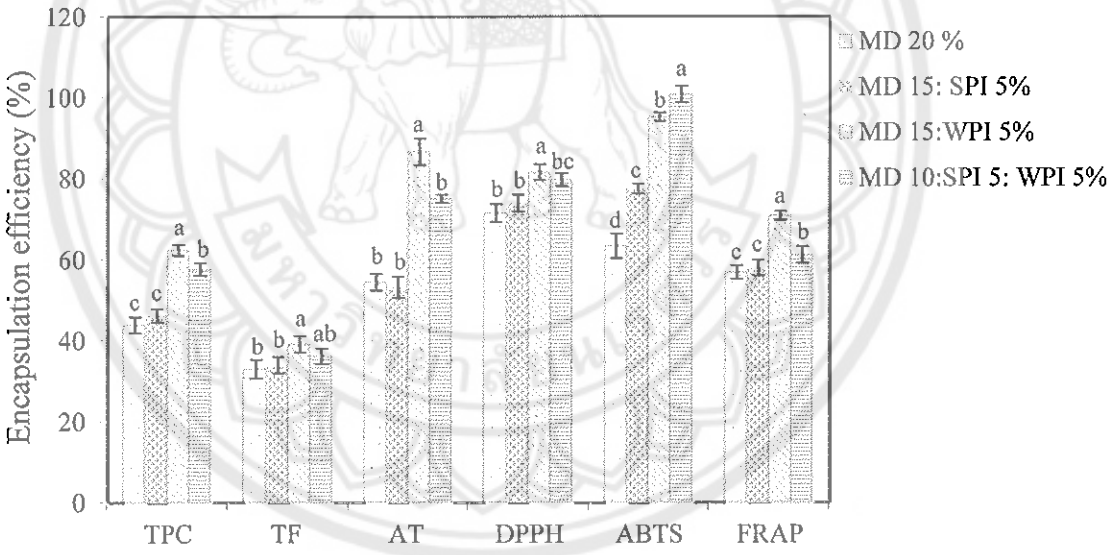

Figure 21 Encapsulation efficiency (EE) of DHRB extract powder when using different coatings to encapsulating phytochemicals and antioxidant activity

Section 5 Stability of defatted Homnin rice bran extract powder on phytochemicals and antioxidant activity

Effect of storage on the phytochemicals and antioxidant stability of DHRB extract powder

The stability of the TP of DHRB extract powder was evaluated during 7 weeks storage at 25 °C (Fig. 22). The retention rate of TP decreased significantly during storage in all samples. The stability of the microcapsules prepared by using different encapsulating agents was significantly different ($P<0.05$). After 7 weeks of storage at 25 °C, the TP retention of the four microcapsule powders ranged from 18.85-67.74%, while the retention of TP coating with MD was 18.85%. These observations indicated that the microcapsule formed when using encapsulating agents of TP could effectively reduce the damage of phenolics caused by adverse environmental conditions. These results were in agreement with the previous report of Wang et al. (2016), in which the microencapsulation techniques significantly improved the stability of tea polyphenols. Furthermore, there were differences in the stability of the four microcapsules due to the different encapsulating agent combinations. The powder prepared by MD:SPI:WPI had the best stability, and the retention rate of TP was 67.74% after storage at 25 °C for 7 weeks. The stability of the powder prepared from MD:WPI, was better than that of samples prepared from MD:SPI and MD, respectively.

According to the results of Fig. 22, the stability of TP from DHRB extract powder was related to the microencapsulated efficiency of microcapsules. The higher the microencapsulated efficiency, the better the stability of the powder and the smoother the surface of the particles, the phenolic substances of the microcapsules may be less likely to be damaged by the external environment (Li et al., 2018). The enhancement of encapsulation efficiency with the addition of the SPI or WPI into MD was probably due to the interaction of the protein with MD and the formation of complexes with interfacial and amphiphilic properties (Tao et al., 2017).

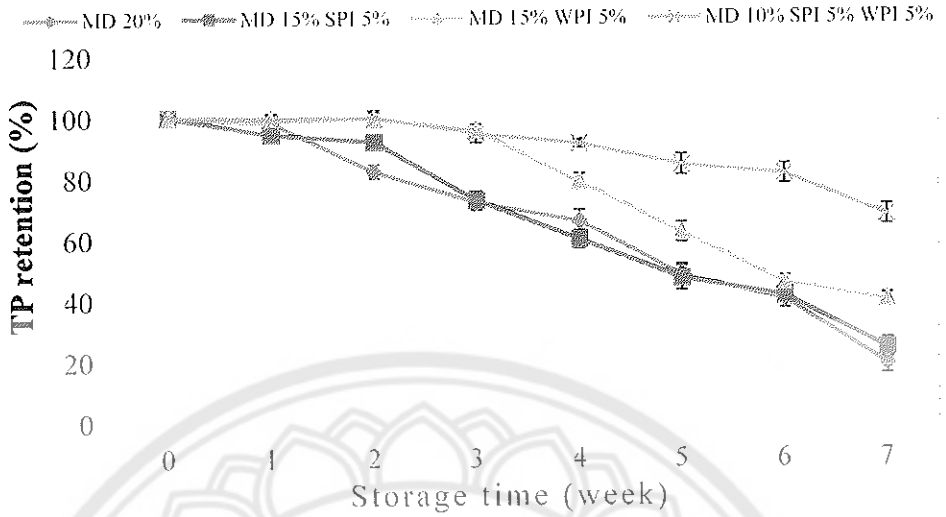

Figure 22 TP retention of DHRB extract powder during storage at 25°C

The retention rate of TF of DHRB extract powder different coating materials were evaluated during storage (Fig. 23). The retention of TF decreased significantly during storage ($P<0.05$). The stability of the powder prepared by different encapsulating agents was significantly different ($P<0.05$). TF of DHRB extracts powder shown 17.53, 17.96, 20.06 and 23.01 mg CE/g from MD, MD:SPI, MD:WPI and MD:SPI:WPI, respectively. After 7 weeks of storage, the TF retention show that 27.50, 45.29, 62.06 and 70.22 % from MD, MD:SPI, MD:WPI and MD:SPI:WPI, respectively. The powder prepared by MD:SPI:WPI had the best stability, and the retention rate of TF was 70.22% at the end of storage. The stability of the powder prepared from MD:WPI, was better than that of products prepared from MD:SPI and MD, respectively. The stability of TF during storage depends on its capacity to polymerize and react with other phenolic compounds, besides other conditions that might be exposed during their conservation (Brownmiller et al., 2008).

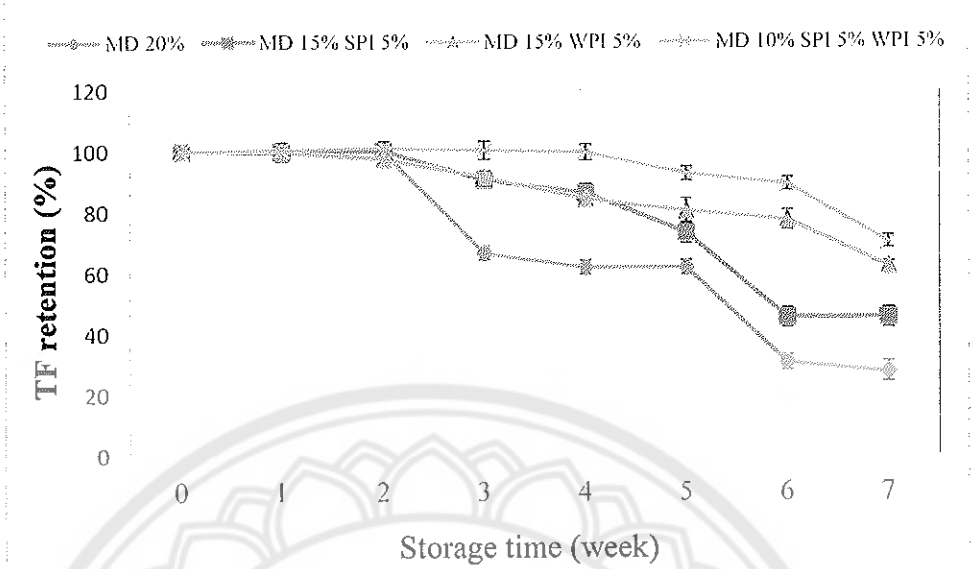

Figure 23 TF retention of DHRB extract powder during storage at 25°C

The stability of the AT of DHRB extract powder was evaluated during 7 weeks storage at 25 °C (Fig. 24) and the retention rate of AT decreased significantly until the end of storage. At the beginning (week 0-week 2) of the storage period, slightly significant difference was observed ($P < 0.05$) among the four coatings materials and then the retention was gradually decreased until week 7. After 7 weeks storage at 25 °C, the AT retention of the four microcapsule powders raged from 19.36-70.36. The powder prepared by MD: SPI: WPI had the highest stability, and the retention of AT was 70.36% at the end of storage. Burin et al. (2011) evaluated Cabernet Sauvignon anthocyanin stability in relation to storage and also showed that light is an important accelerating factor in the degradation of anthocyanins. In addition, Janna et al. (2007) reported that when exposed to light at 25 °C, anthocyanins showed a significant decrease in the pigment content, with a reduction of more than 50% on the third day of exposure. Moser et al.(2017), evaluated the storage stability of phenolic compounds in encapsulated red grape juice using different whey protein or soy protein blends with maltodextrin and found that flavonols were limited in their ability to form copigment complexes with anthocyanins because the competing stabilizing action of maltodextrin and the presence of aromatic amino acid residues that can also act as copigments. In addition, anthocyanidins have varying sensitivity to

temperature increase, with cyanidins being more sensitive to thermal decomposition than delphinidin. Besides decomposition, anthocyanins may also polymerize upon prolonged storage (Takeungwongtrakul et al., 2015). An increase in polymeric pigments and losses of monomeric anthocyanins may be due to several factors, including residual enzymatic activity or condensation reactions between the anthocyanins and other phenolic compounds, such as flavan-3-ols (Moser et al., 2017).

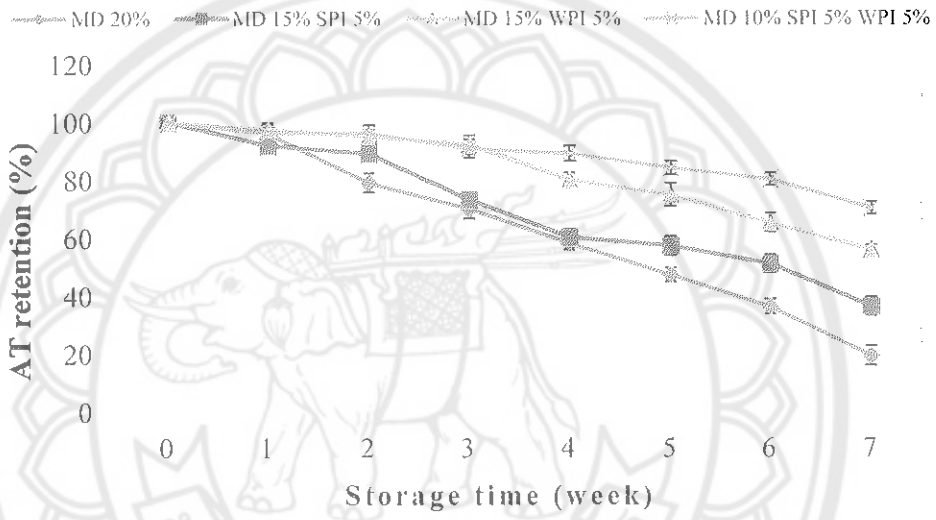

Figure 24 AT retention of DHRB extract powder during storage at 25°C

In this part, the storage time of DHRB extract powder was studied for 7 weeks at 25°C and the DPPH, ABTS and FRAP were determined during the storage. The results of DPPH, ABTS and FRAP are summarized in Figure 25, 26 and 27, respectively. It was shown that, the values of DPPH, ABTS and FRAP decreased with time of storage ($P<0.05$). All samples, the retention rate of DPPH, ABTS and FRAP decreased significantly during storage. At the beginning (week 0-week 2) of the storage period, slightly significant difference was observed ($P < 0.05$) and this tendency kept continue until 7 weeks. Furthermore, the stability of the microcapsules was due to different encapsulating agent and their combinations. The powder prepared by MD:WPI:SPI had the best stability, and the retention rate of DPPH, ABTS and FRAP were 75.18, 69.72 and 80.64% after storage at 25 °C for 7 weeks. The stability

of the powder prepared from MD: WPI was better than those prepared from MD and MD: SPI, respectively.

The tendency of DPPH, ABTS and FRAP during storage at 25 °C for 7 weeks was similar to those obtained from TP, TF and AT as previously discussed (Fig. 22-24). A similar study reported by Jimenez-Gonzalez et al. (2018) showed that the antioxidant capacity of encapsulates of *Renealmia alpinia* extract tended to decrease after 7 days of storage at 4 and 25 °C with the three types of coating agents (maltodextrin, gum arabic and maltodextrin mixed with gum arabic) were used even though no significant difference was observed between the 7 and 14 days. However, capsules with maltodextrin preserved a constant antioxidant activity at 4 °C for 28 days and this tendency was similar to that obtained from the total phenolic content and anthocyanins. According to Nori et al. (2011) encapsulated propolis extract employing soy protein isolated and pectin as coating agents had relatively high encapsulation efficiency and the researcher confirmed that encapsulation provided not only protection against degradation of phenolic and flavonoid compounds presented in free propolis, but also preservation of their antioxidant properties.

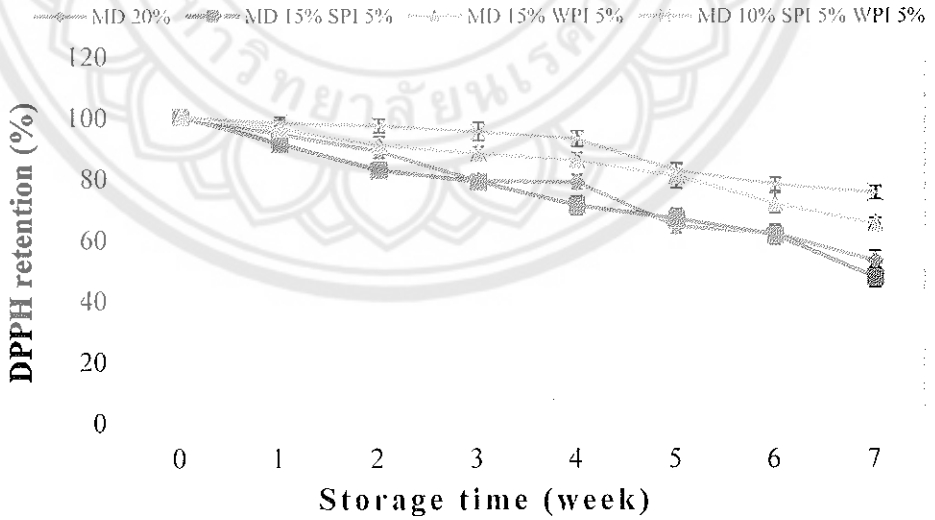

Figure 25 DPPH values of DHRB extract powders with different coating agents during storage at 25°C

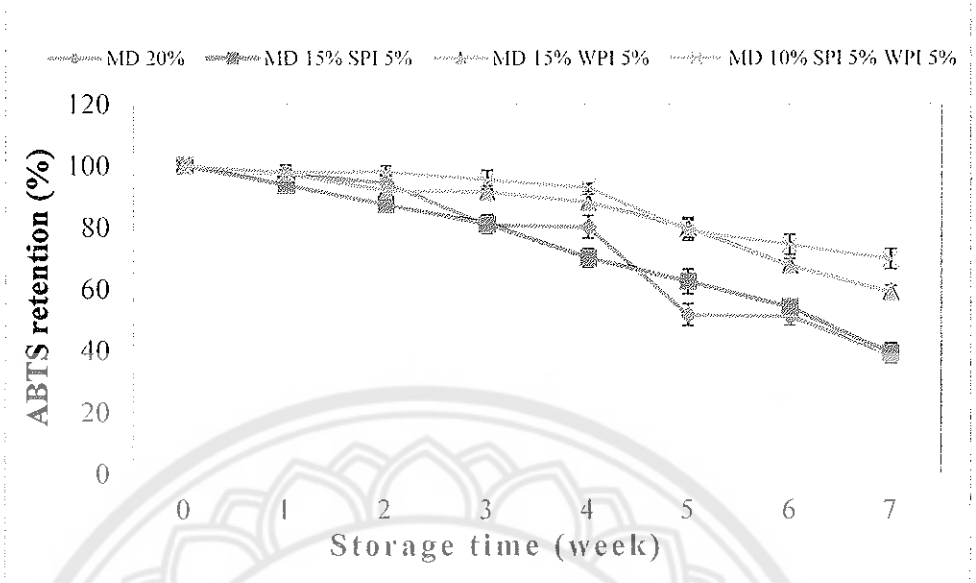

Figure 26 ABTS values of DHRB extract powders with different coating agents during storage at 25°C

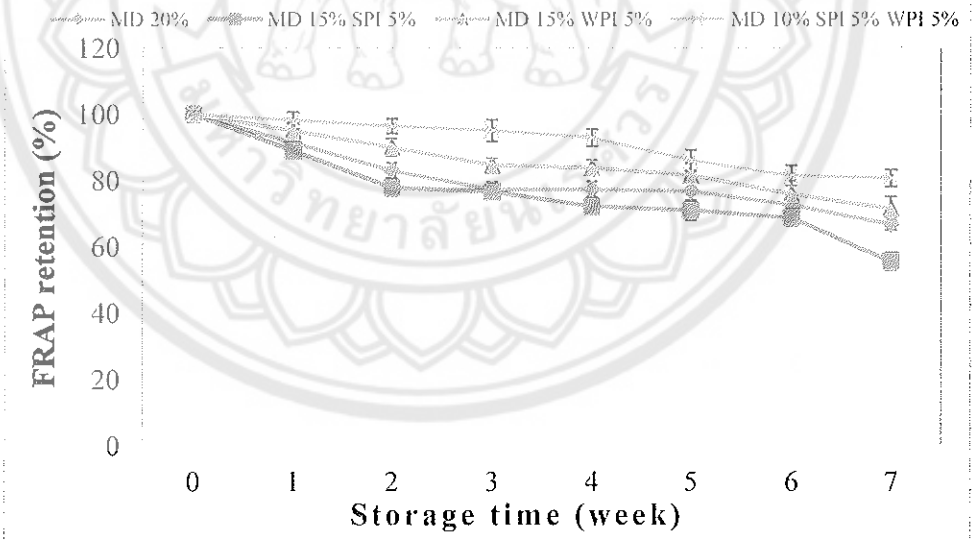

Figure 27 FRAP values of DHRB extract powders with different coating agents during storage at 25°C

Effects of pH on phytochemicals and antioxidant stability of DHRB extract powders

To study the effects of pH on phytochemical and antioxidant stability of DHRB extract powder, various pH solutions were prepared to mimic common beverages: pH 2.5 (orange juice or soft drinks), 4.5 (yogurt) and 6.5 (milk). Fig. 28-29 shows the stability of phytochemicals (TP, TF and AT) and antioxidant activity (DPPH, ABTS and FRAP) at various pH values after testing for 1 hour. The phytochemicals and antioxidant activity at pH 2.5, 4.5 and 6.5 were observed to be significantly ($P < 0.05$) degraded relative to DHRB extract powder under the same conditions. TP, TF and AT determined in the DHRB extract powders with different coatings were different with respect to treated different pH. DHRB extract powder at acidic pH 4.5 exhibited the highest TP, TF and AT, which the powder coating with MD, MD:SPI, MD:WPI and MD:SPI:WPI were between 143.36-161.26 mg of GAE/g, 55.25-59.84 mg of CE/g and 20.01-25.54 mg of C3G/g, respectively, and there was significant decrease under neutral conditions (pH 6.5). Even when the pH was reduced to 2.5, it still maintained TP, TF and AT from 127.40-140.85 mg of GAE/g, 51.19-54.02 mg of CE/g and 17.36-17.96 mg of C3G/g, respectively.

Flavonoids and anthocyanin were unstable when the pH were increased which all samples were degraded when pH changed from 4.5 to 6.5. Zhu et al. (1997) reported that tea catechins are extremely unstable in alkaline solution ($pH > 8$) and were degraded completely in a few minutes while they were stable in acidic solution ($pH < 4$). Similarly, Su et al. (2003) also found that catechin was more stable at acidic pH than at alkaline pH. Catechin is prone to oxidative degradation and polymerisation under high-pH conditions. This might be due to acid acting as a reductant that can recycle the catechin into free radical form (Su et al., 2003).

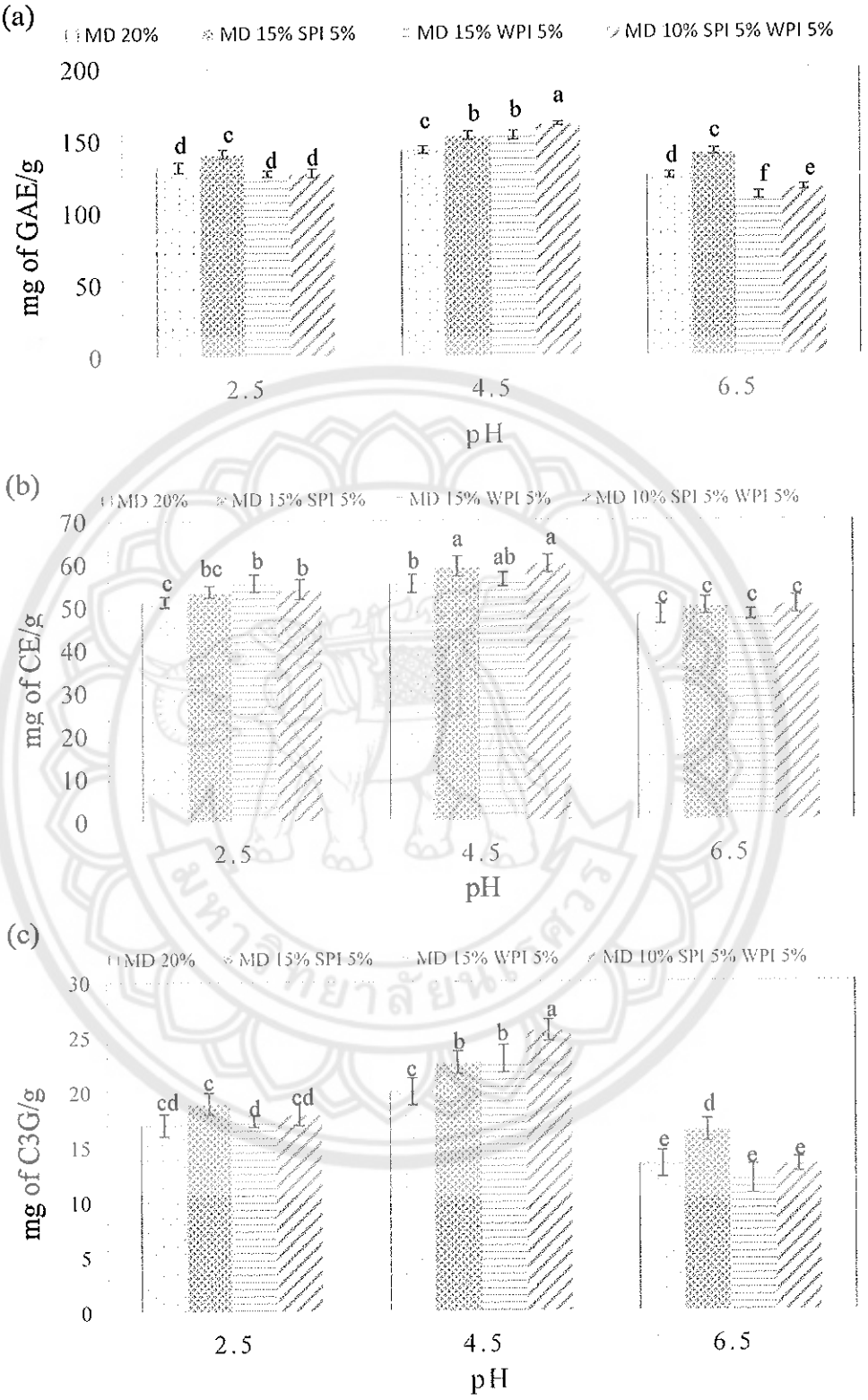

Figure 28 TP, TF and AT values of DHRB extract powders with different coating agents and different pH

The antioxidant potential of DHRB extract powder at various pH values were determined on the basis of the scavenging activity of the stable free radicals DPPH (29a) and ABTS (29b) and reducing ability by FRAP assay (29c). The antioxidant activity of DHRB extract powders after treated different pH (DPPH, FRAP and ABTS) tended of increase with increasing pH from 2.5 to 4.5 and then it was decreased when increasing pH to 6.5. The obtained results were in the samples way as the change of phytochemicals (Fig. 28).

When considering, the DPPH radical scavenging activity of the DHRB extracts, it was greatly affected by the neutral condition. When the pH was increased to 6.5, the DPPH sharply declined compared with that under high acidic pH (2.5) condition, while the Fe^{2+}-chelating ability and ABTS ability showed a similar trend. There are several factors that could account for the loss of antioxidant activity under neutral and alkaline conditions. The possible loss of activity could be due to the activation energy of phenolic compound degradation which varies with the pH. Different pH values will affect the actual degradation pathway (Bell, & Labuza, 1991).

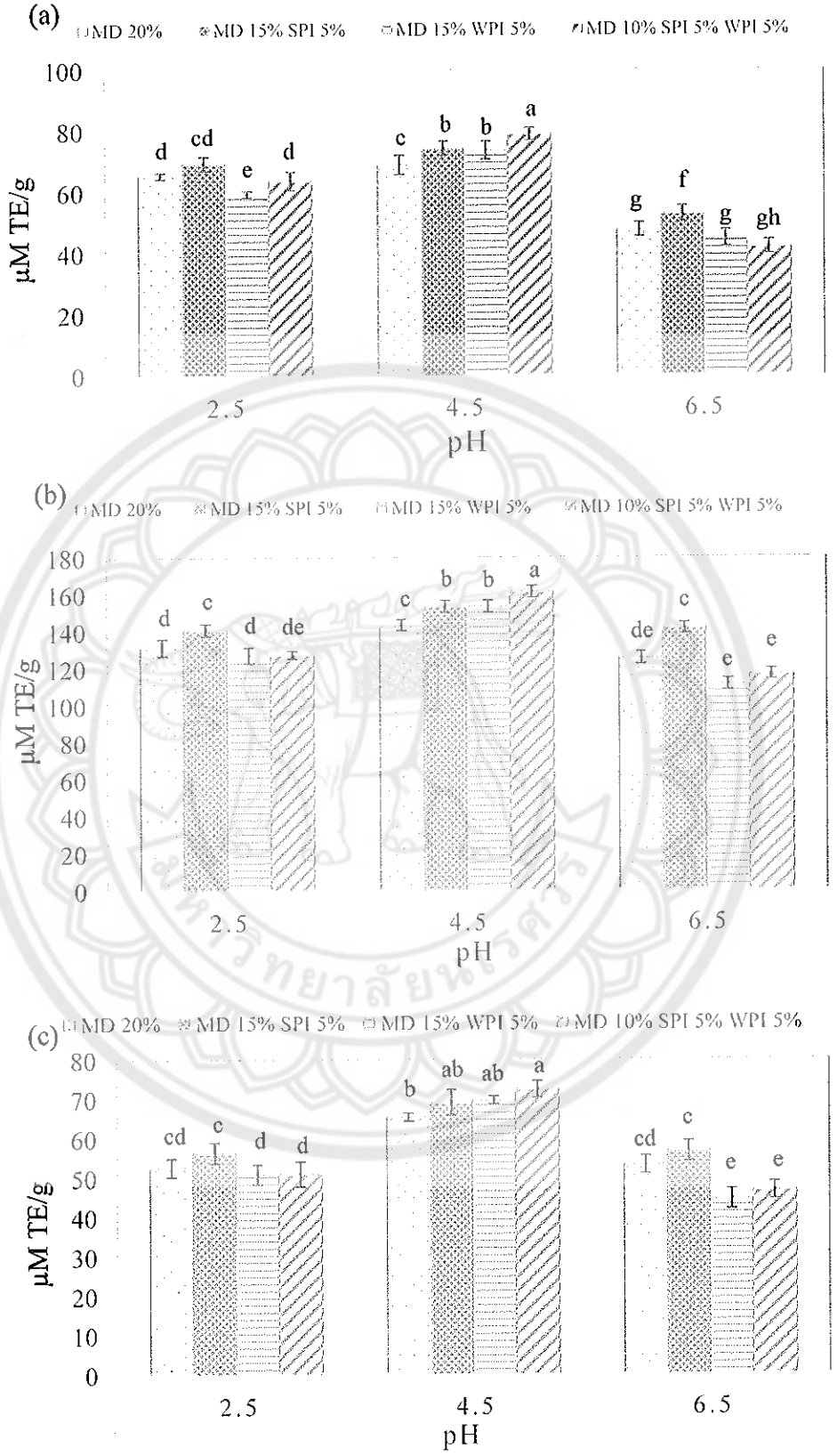

Figure 29 DPPH, ABTS and FRAP values of DHRB extract powders with different coating agents and different pH

Effects of heating on phytochemicals and antioxidant stability of DHRB extract powders

TP, TF and AT determined from different DHRB extract powders showed significant difference with respect to heating time. The DHRB extract powder in aqueous exposed to 80°C for 90 minutes had a stated decrease in total phenolics. Significant losses ($P < 0.05$) in the TP, TF and AT were determined in the samples compared to the initials and the amount of loss increased with increasing heating time. The DHRB extract powders coating with the combination of MD: SPI: WPI had higher TP, TF and AT than those of other samples. The highest retention rate of TP was 82.41% after storage at 80 °C for 90 minutes. The retention rate of TP of the powder prepared from MD: WPI, was better than that of products prepared from MD: SPI and MD, respectively. After 90 minutes heating at 80 °C, the TP retention of the four different microcapsule powders range from 39.51-82.41%. Wang, & Ryu (2013) reported that phenolic compounds are less resistant to the heat, and heating over 80°C may destroy or alter their natural properties. Phenolics are soluble in water but susceptible to thermal process (Nemś et al., 2015; Friedman, 1997) also found during cooking is the main cause of the losses of phenolics. The reduction of total phenolics may be attributed either to the decomposition of phenolic compounds under the high extrusion temperature or the alteration of molecular structure of phenolic compounds that may lead to reduction in the chemical reactivity (Sharma, Gujral, & Singh, 2012).

TF and AT had similar trend as TP since anthocyanins are the major compound of flavonoids and flavonoids are the major compounds contributing to phenolics (Min et al., 2012). The mechanism of flavonoids thermal degradation was explained by Scibisz et al. (2012) that under the influence of heat, glycosidic bonds in dye molecules undergo hydrolysis leading to unstable aglycones, which easily oxidize and forming brown, high molecular weight compounds. Hou et al. (2013) suggested that anthocyanin stability is related to their structures and copigmentation capacity. Four anthocyanin structures exist in equilibrium: flavylium cation, quinonoidal base, carbinol pseudobase and chalcone. Stability of anthocyanins can be increased with intermolecular copigmentation. Grain extracts, with high anthocyanin content, contain mixtures of different compounds that may serve as copigments for intermolecular association with anthocyanins.

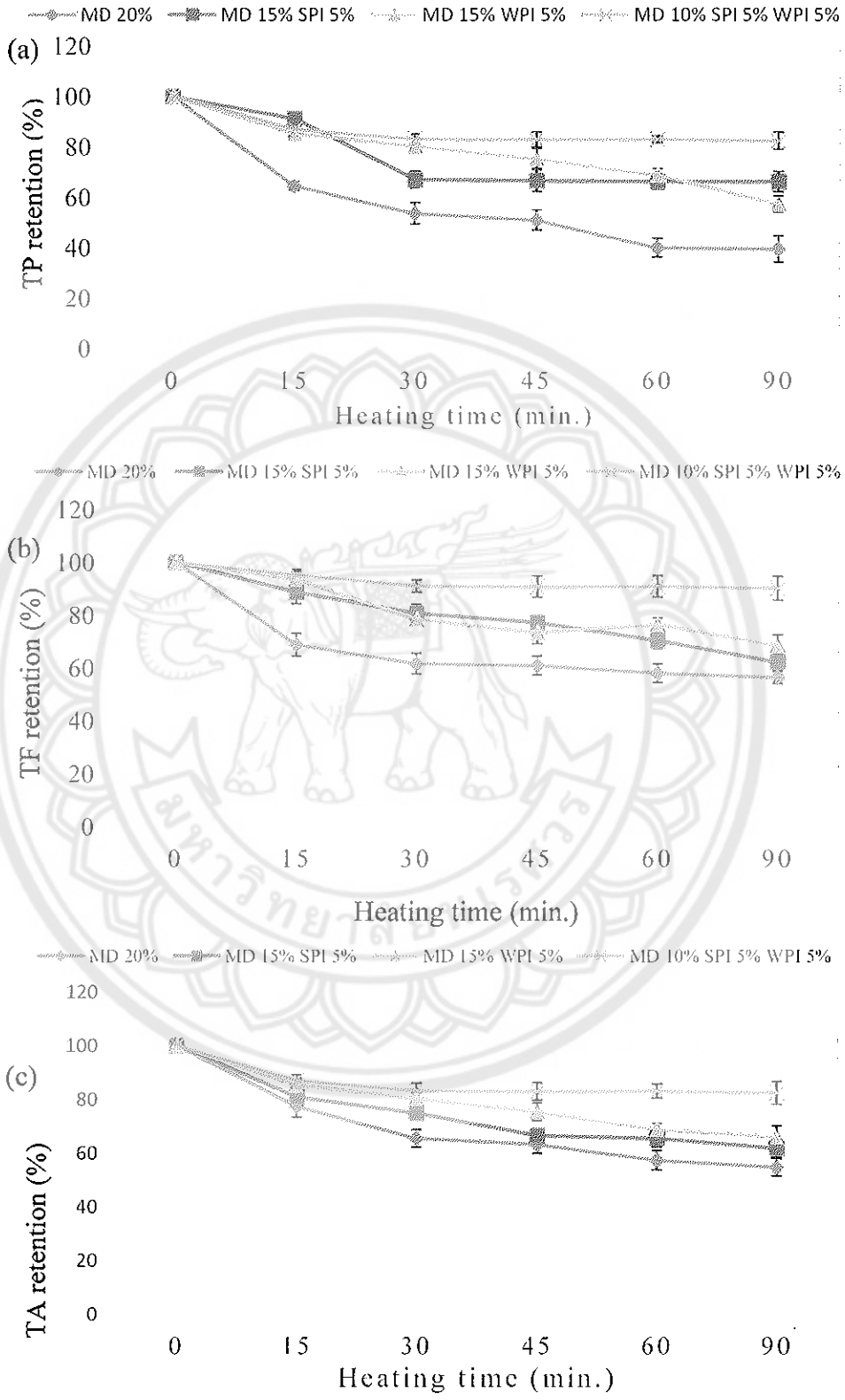

Figure 30 TP, TF and AT values of DHRB extract powders with different coating agents and during heating at 80°C

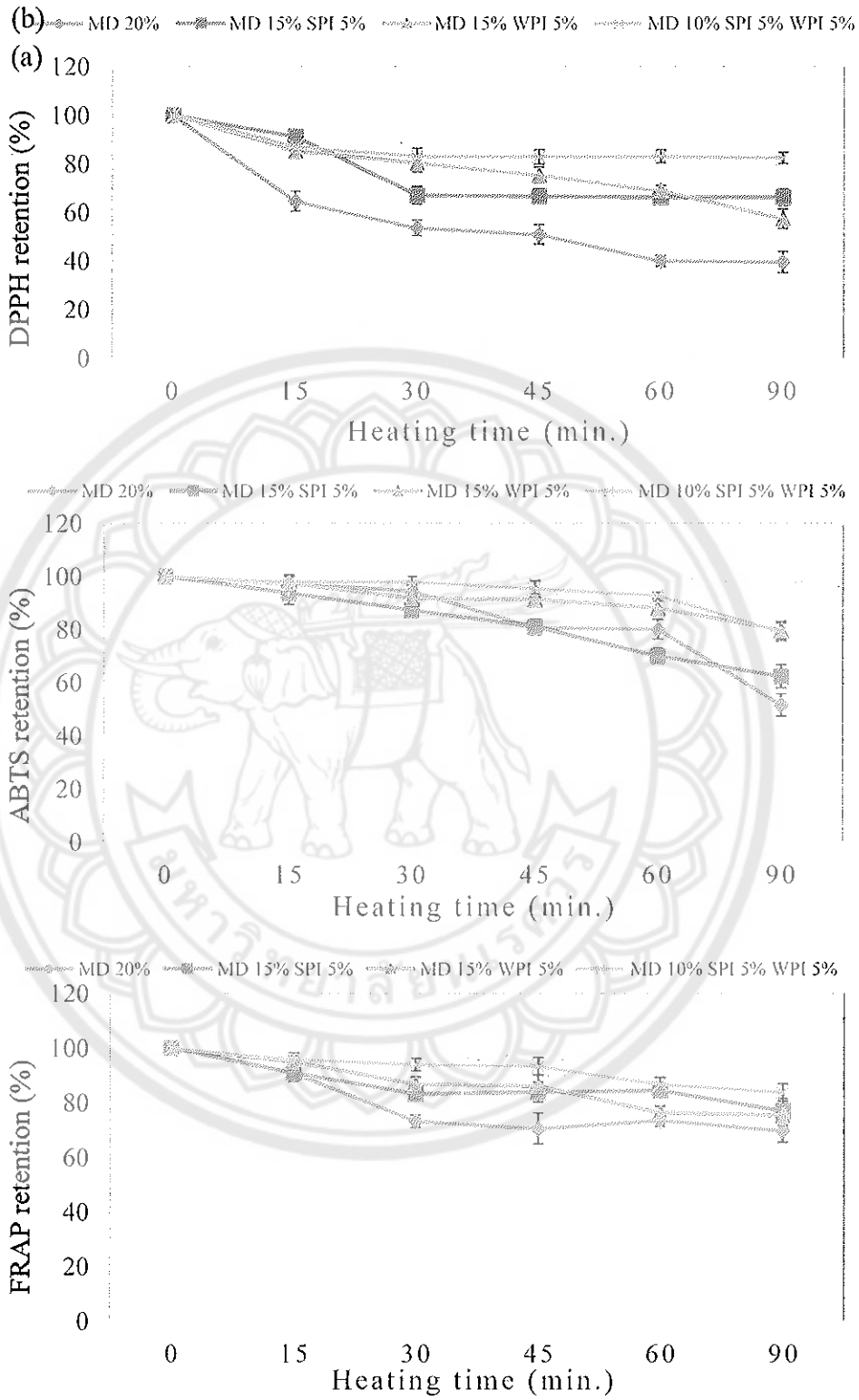

Figure 31 DPPH, ABTS and FRAP values of DHRB extract powders with different coating agents and during heating at 80°C

The DPPH, ABTS and FRAP were used to collectively evaluate the antioxidant capacity of DHRB extract in aqueous at 80°C for 90 minute (Cavalcanti et al., 2011). As shown in Fig. 31, DPPH, ABTS and FRAP of DHRB solutions at pH 5.0 significantly decreased after thermal treatment at 80 °C for 90 min. The average MD, MD:SPI, MD:WPI and MD:SPI:WPI of 39.51 to 81.25%, 64.38 to 80.21%, and 69.64 to 83.65%, of DPPH, ABTS and FRAP of DHRB solutions were retained after heating at 80 °C for 90 minutes, respectively, while the maximum retentions when using MD:SPI:WPI were 82.41%, 79.02%, and 83.65%, respectively. The decreased retentions of antioxidant properties of DHRB solutions likely due to the thermal decomposition of DHRB solutions. The observations in Fig. 31 were in accordance with the preservation of antioxidant capacity of anthocyanins by yeast mannoprotein after thermal treatment at pH 7.0 (Wu et al., 2015). A relationship between TP content and antioxidant activity has been described. Thus, the antioxidant effect of DHRB solutions could be related to its phenolic constituents. The antioxidant activity of DHRB solutions may also interfere with the propagation reactions besides inhibiting the enzymatic systems involved in initiation reactions or they can act as the scavengers of free radicals, hydrogen donation, metallic ion chelation or even acting as substrate of radicals such as superoxide or hydroxyl (Viuda-Martos et al., 2010). The phenolic compounds are believed to be major phytochemical constituents responsible for antioxidant capacity from plant (Udomkun et al., 2016). In this study, antioxidant activity as defined by DPPH, ABTS and FRAP were related with TP TF and AT and the results indicated that antioxidant activity of DHRB extract during storage were directly affected by phenolic compound content.

Similar results were reported Nemś et al. (2015) reported the basic factors that decrease the total phenolic and antioxidant activity in food products are the conditions of the thermal processes and pressure. Many studies reported the process of heating has affected the concentration of total polyphenols and antioxidant activity of products (Gordon, & Kourimská, 1995; Kita et al., 2013).

Effects of sucrose concentration on phytochemicals and antioxidant stability of DHRB extract powder

TP, TF and AT determined in the DHRB extract powder using different coatings agent were significant when reacted with different sugar concentrations. Heating the extract containing sugar solution in water bath at 90°C for 2 hours caused a slightly increase in total phenolics while TF and AT also showed the same trends.

TP of DHRB extract solutions which the powder coating with MD, MD:SPI, MD:WPI and MD:SPI:WPI were 58.42, 60.78, 51.71 and 64.99 mg GAE/g DM respectively. TP was increased significantly with increasing sucrose concentration. When sugar concentration increased from 0-20% TP of DHRB extract solution was increased about 2.63, 2.22, 5.96 and 8.89 % for the powders prepared from MD, MD:SPI, MD:WPI and MD:SPI:WPI, respectively, while TF was increased about 9.36, 20.61, 19.69 and 20.58 % and AT was 0.52, 8.64, 15.30 and 25.40 %, respectively.

In the DHRB extract solution found that TF and AT had the same trend to TP. The TF reached the level of 22.96 and 33.63 mg CE/g DM, respectively, while AT the level of 19.23 and 30.12 mg C3G/g DM, respectively.

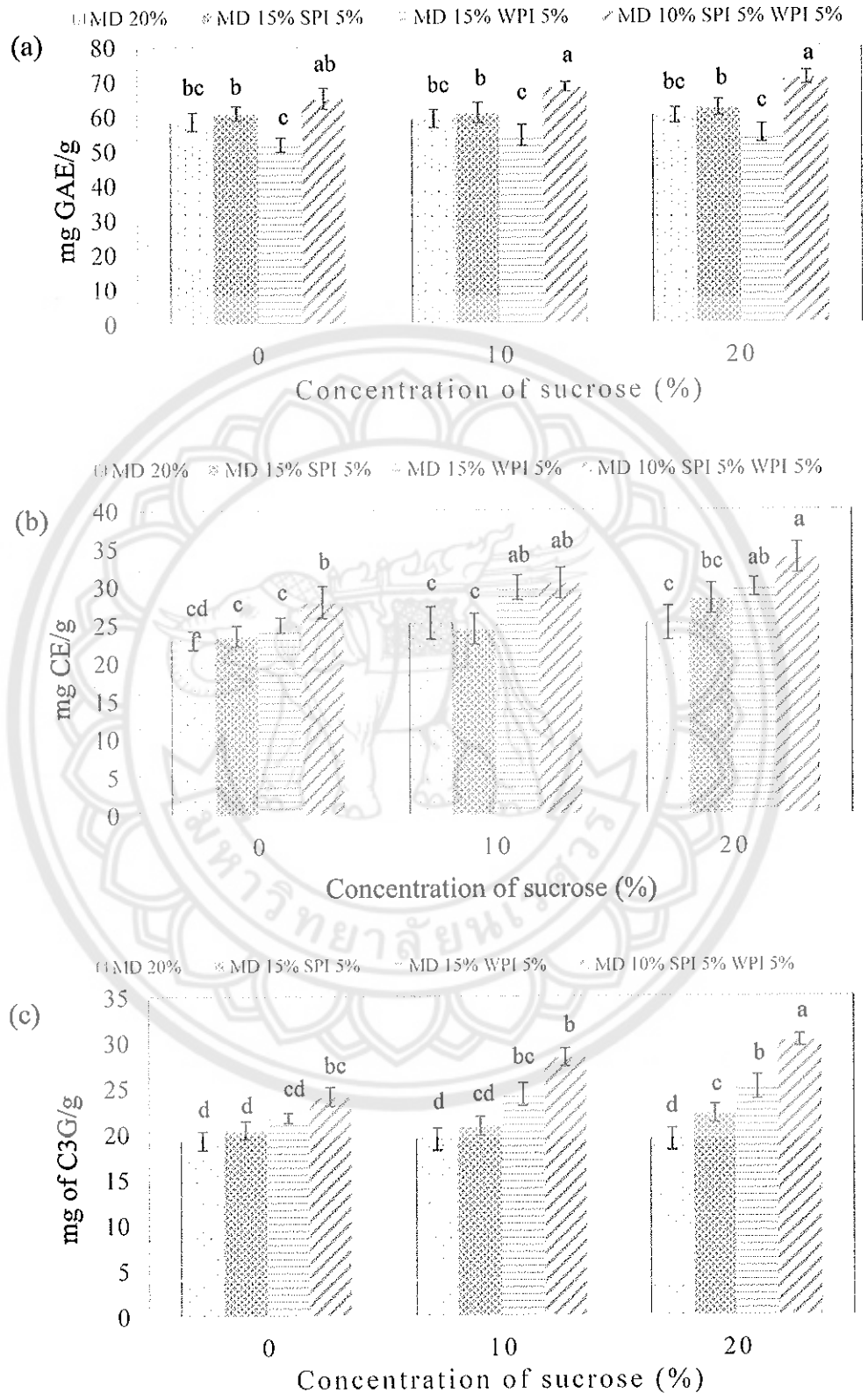

Figure 32 TP, TF and AT values of DHRB extract powders with different coating agents and sucrose concentration

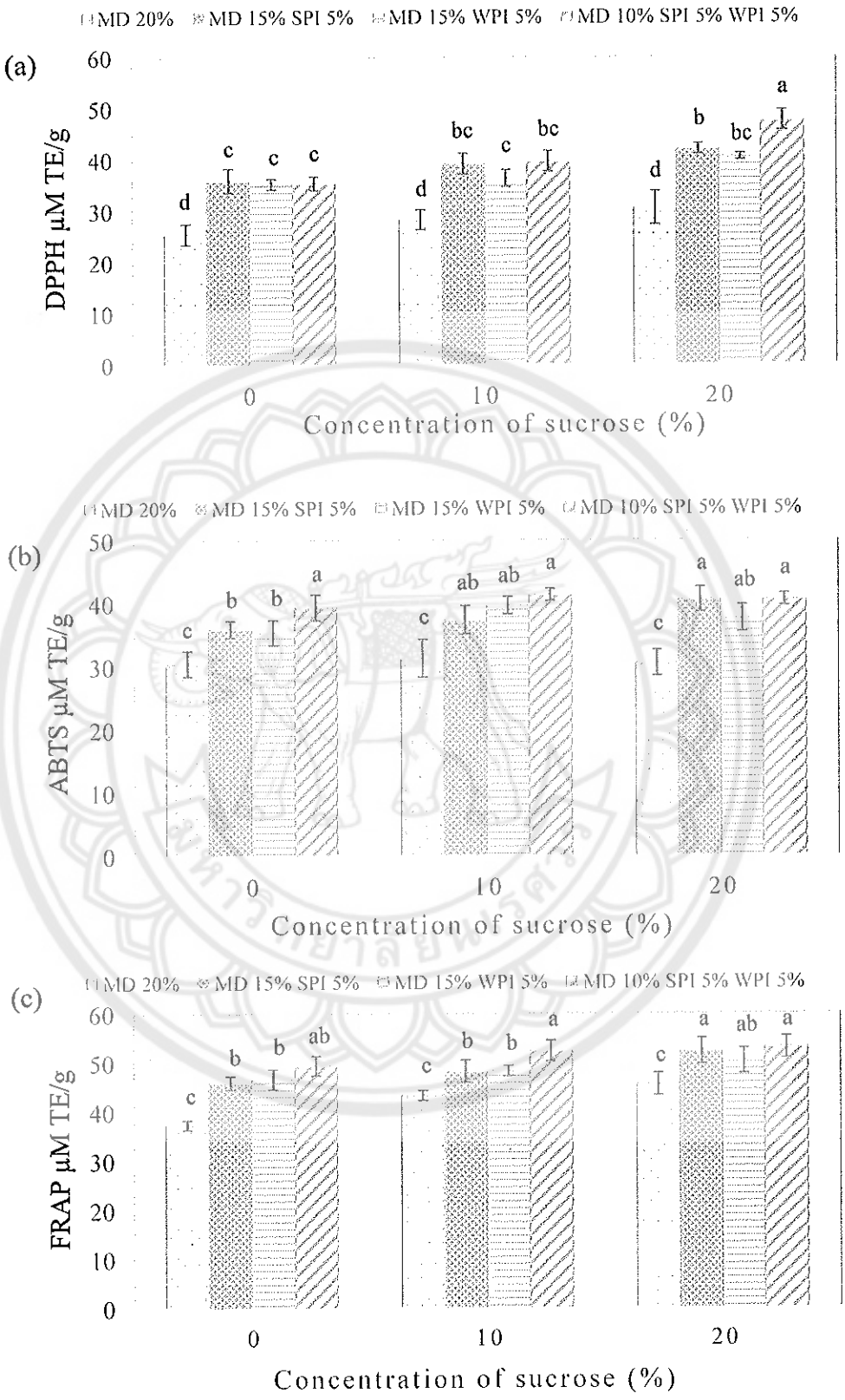

Figure 33 DPPH, ABTS and FRAP values of DHRB extract powders with different coating agents and sucrose concentration

The antioxidant activity of DHRB extract powder coating with different agents in different sugar concentration solution were expressed as DPPH, ABTS and FRAP (Fig 33).

Increasing sucrose concentration from 0 - 20 % significantly provided the increase of DPPH of DHRB extract powder ($P<0.05$). DPPH of DHRB extract powder solutions without sugar were 25.52, 35.94, 35.27 and 35.39 μmol TE/g DM for MD, MD:SPI, MD:WPI and MD:SPI:WPI, respectively. DPPH was increased significantly about 19.59, 16.92, 14.88 and 34.30%, respectively. The highest DPPH was obtained from DHRB extract solution which the powder was coated with MD:SPI:WPI in the 20% sucrose concentration.

ABTS and FRAP values also show the same trend as DPPH. This was especially evident with the treatment of 20% sucrose, where DHRB extract solutions had significantly higher ABTS and FRAP than other treatments. DHRB extract powder in the sucrose solutions also had significantly higher reducing power than the control, The reason may due to the sucrose browning products which may increase antioxidant capacity. The increase of the antioxidant activity during heating caused from other complexes derived from caramelization, like 5-HMF (Granados et al., 1996) or disaccharide (Ratsimba et al., 1999) instead of furfural were reported, In addition, the antioxidant might be associated with 3-methyl-butanal, furfural, benzaldehyde and benzene acetaldehyde that they were highly increased after the Maillard reaction. Generally, heterocyclic compounds such as furans, pyrroles, and thiazoles, were found to contribute to strong antioxidant activity (Eiserich et al., 1992).

CHAPTER V

CONCLUSION AND RECOMMENDATIONS

Conclusion

Phytochemicals and antioxidant activity of Homnin rice bran (defatted and non defatted) extracted with solvent were determined. The results showed that the defatted Homnin rice bran increased the TP, TF, AT and antioxidant activity (DPPH, ABTS and FRAP). The defatted rice bran extract provided the highest TP, TF, AT of 39.06 mg GAE/g, 14.04 mg CE/g DM, 3.65 mg C3G/g, respectively. The defatted Homnin rice bran extracts had the highest antioxidant activity values of 31.42, 25.53 and 162.89 μmol TE/g DM for DPPH, ABTS and FRAP assays, respectively.

The effects of solvent type and citric acid concentration on bioactive compounds and antioxidant activities of Homnin rice bran extract showed that the TP and TF as well as the efficiency of antioxidant activity had been affected by the solvent types and citric acid concentration used for antioxidant extraction from colored rice, and the DHRB methanolic extract with 0.1 mol/dm^3 of citric acid gave the highest antioxidants. The extraction using water and ethanol without acid found a similar efficiency of antioxidant activity. However, using citric acid in the extraction showed a trend of decreasing antioxidant activity and the anthocyanin content from DHRB extracted by using acidified water tended to increase with an increasing citric acid concentration.

This study reveals that the optimum extraction condition which the three factors obtained from the model were following: 648 Watt of power energy, 0.076 mol/dm^3 of citric acid concentration and extraction time of 83 second and the experimental values of TPC, TF, AT and antioxidant activity (DPPH, ABTS and FRAP) were 90.59 mg GAE/g DM, 77.53 mg CE/g DM, 18.82 mg C3G/g DM, 109.35 μmol TE/g DM, 58.09 μmol TE/g DM, 168.26 μmol TE/g DM, respectively. The predicted values matched well with the experimental values which the validation between the predicted values and experimental were within an acceptable error range

as depicted by average mean deviation with the E% ranged from -1.85 to 3.27. Therefore, it was selected for the encapsulation studies.

Coating agents had affected on the encapsulation efficiency of powder extract under the different environmental stresses. The results indicated that extensive the phytochemicals and antioxidant activity were reduced when compared to the initial antioxidant capacity of the DHRB extract. Under these stressed conditions, the retentions of TP, TF, AT, DPPH, ABTS and FRAP retained in the encapsulated sample were in the range of 43.90-62.42%, 33.06-39.20%, 53.24-86.66%, 71.51-81.62%, 63.38-100.86% and 56.87-70.87%, respectively. The stability of Homnin rice bran extract powder was evaluated during 7 weeks storage at 25 °C and the retentions rate of TP, TF, AT and antioxidant activities (DPPH, ABTS and FRAP) decreased significantly during storage ($P<0.05$). The encapsulation using MD:SPI:WPI had the highest stability, and the retentions of TP, TF, AT and antioxidant activities (DPPH, ABTS and FRAP) were 67.74%, 70.22%, 70.36%, 75.18%, 69.72 and 80.63%, respectively.

The study of the stability of DHRB extract powder found that pH and heating affected phytochemicals and antioxidants activity, while sucrose had slightly affected. DHRB extract powder at acidic pH (4.5) exhibited the highest TP, TF, AT, DPPH, ABTS and FRAP.

The study of the stability of DHRB extract powder under various environments found that the feasibility of DHRB for applying in food product at following condition: slightly acid at pH about 4.6, sugar concentration was between 0 – 20% and the heating treatment should not over 80°C for 15 min. The foods that are suitable to apply the DHRB extract were including yoghurt, fruit juices and some acidified herbal drinks.

Recommendations

For improvement

The quality control of Homnin rice bran is necessary because the amount of phytochemicals is varied according to the storage Homnin time of rice bran. Therefore, the pretreatment of Homnin rice bran and raw material should be used. For

example, the method used to inactivate enzyme before storage, the low temperature storage and the selection of the package are needed to be considered.

The DHRB extract powder by using microwave assist extraction was done in laboratory scale and should be adapt for the continuous extraction process.

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