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Free Radical Scavenging Activity of Riceberry and Pathum Thani Rice Extracts for Developing Face Powder

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Abstract

Thai rice today is not limited to staple food, but it has been transformed into a variety of medicinal and beauty products, as part of a growing industry with a bright future. We measured total phenolic content, total flavonoid content, free radical scavenging activity, lycopene, β-carotene, color and SPF of raw materials in face powder. Riceberry extract sample contained phenolic and flavonoid with the highest level at 26.46±0.008 mg GAE/g of extract and 101.63±0.034 mg Rutin/g of extract. Free radical scavenging activity was measured by DPPH assay: the Riceberry extract sample contained antioxidants that indicated the EC_{50} at 108.31±0.024 μg/mL. All raw materials showed SPF, which could be used as foundation data for making face powder. However, wood apple powder has the highest SPF for a material, that can be used as a raw material, for further development of face powder.

Introduction

Rice is Thailand's main economic crop; it has been developed to meet the people's basic needs. The development is consistently extended from local wisdom, inherited from the past generations, and with new technology and innovation in producing rice that meets modern consumer needs. Riceberry is a newly registered rice variety from Thailand in 2002, which was derived from a cross-breed between Jao Hom Nin (JHN), the local non-glutinous purple rice, and Khoa Dawk Mali 105 (Jasmine rice), the Thai Hom Mali rice, by the Rice Science Center, Kasetsart University, Thailand. After 4 years of careful selection for nutritional properties, anthocyanin stability, physical and cooking properties, the outcome was a deep purple whole grain rice, that was soft and palatable. Riceberry has come a popular brown rice, known for health promoting properties. Attracting people to consume more brown rice is significant contributor to solving food-related chronic diseases like diabetes, heart disease, high blood chloresterol, obesity and cancers (Suttiarporn et al., 2016; Leardkamolkarn et al., 2011). Pathum Thani rice is a new strain of fragrant rice, developed by a rice institute in Pathum Thani Province, it's kernel and fragrance is so similar to Thai Hom Mali Rice Kernel, that consumers are often unable to tell it from the latter. Pathum Thani rice is mainly grown in the central provinces, which have abundant water. Two or three crops of Pathum Thani rice can be harvested each year, and it is considered as a replacement for Thai Hom Mali rice (Sreethong et al., 2018).

The current consumer trends stimulate strong

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interests and preferences in choosing cosmetics that contain natural extracts, because many natural extracts are now comparable to synthetic substances, obtained from laboratories including ability to nourish and protect the skin, antioxidant ability and the aging rejuvenation ability. Consumers are excited by these features and demand them, so cosmetic products, containing natural extracts, are therefore extremely interesting and in high demand by consumers. Free radicals refer to molecules that are unstable and sensitive to chemical reactions and encourage continuous destruction of other molecules in chain reactions. Free radicals are therefore toxic to the body cells. If there are many, it can be dangerous. They will destroy the cell membranes and other structures. In the short term, free radicals cause inflammation and tissue destruction while, in the long term, they affect the degeneration or aging of cells. At present, both domestic and international studies have shown that free radicals are associated with the occurrence of many non-communicable chronic diseases, especially cancer, which is one of the top causes of death for globally (Freddie et al., 2018). Free radicals are created both by the body's metabolic processes and in unhealthy conditions, for example, illnesses or polluted environments. These abnormal condition will cause the body to accumulate more free radicals. Therefore, it is vital for the body to find a way to prevent from being destroyed by free radicals. Antioxidant compounds promote health by protecting the cells of the body from damage caused by free radicals and reactive oxygen species (Pukumpuang & Seansrimon, 2020). They are various substances or enzymes with a low concentration that can delay or prevent the oxidation of substrates, which are sensitive to the reaction. These substrates include almost all substances in the body, for example, proteins, fats, carbohydrates and DNA. However, to some certain extent, the imbalance between free radicals and antioxidants in favor of free radicals results in oxidative stress (Khanthapok & Sukrong, 2019). This affects living cells, in various way, e.g. oxidation of DNA proteins, carbohydrates and destruction of molecules with S-H bonds and cell membranes, that have detrimental effects on cells and cell destruction. This directly leads to aging and some chronic diseases, such as stroke, autoimmune disease, diseases caused by the blood, returning to the organs, that have been constricted, due to short-term stroke, and cancer (Flachenecker, 2012). Antioxidants prevent formation of free radicals by inhibiting free radical chain reactions, stop new free radicals forming, repair damage to the body cells destroyed by free radicals, eliminate and replace the damaged molecules (Lobo et al., 2010). Fig. 1 showed the DPPH assay, an odd electron displays a strong absorption band at a wavelength of 519 nm, which loses absorption once the odd electron is paired off by a hydrogen or electron-donating antioxidant (Ningjian & David, 2014).

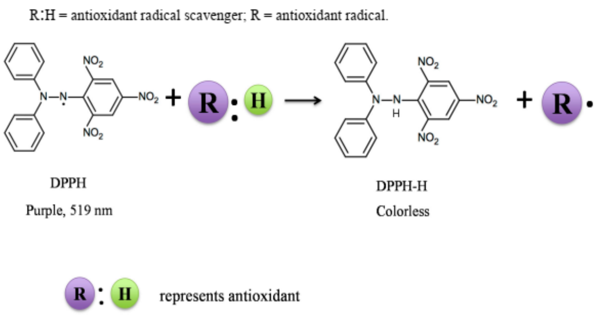


Fig. 1 Reaction mechanism of DPPH with antioxidant (Ningjian & David, 2014)

"Phenolic" refers to molecules containing at least one phenol group and many phenolic substances are found in plants. The total phenolic content varies with type of plant, planting method, degree of ripeness, processing and storage. Heat, used in the processing, reduces the amount of phenolic compounds. Many phenolic compounds have antioxidant properties. The hydroxyl group of the phenol, can be replaced by other cluster functions, *e.g.* flavonoids, lignins, cinnamic acid and coenzyme-Q (Moeiklang & Ruangviriyachai, 2014). Phenolic compounds have chemical formulas ranging from simple structural groups, the phenolic acids, to structural polymers, *e.g.* lignin. The largest group is the flavonoid compounds. Phenolic compounds found in plants often embedded in the sugar molecules in the form of glycosides. The most common type of sugar in the phenolic molecules is glucose and it was found that this perhaps is a recomposition between the phenolic compounds, or phenolic compounds with other compounds such as organic acids including in the molecules of the protein alkaloids and terpenoids (Moeiklang & Ruangviriyachai, 2014). They have been classified into six subgroups in Fig. 2 (Ali & Neda, 2011).

Carotenoid are yellow, orange or red pigments, commonly found in plants, algae, animals and microbes (Costache et al., 2012; Dhir et al., 2013). Carotenoid in plants can be found in different parts, *e.g.* fruits, flowers and roots. Carotenoids are highly effective antioxidants especially β-carotene, lycopene, and lutein which are

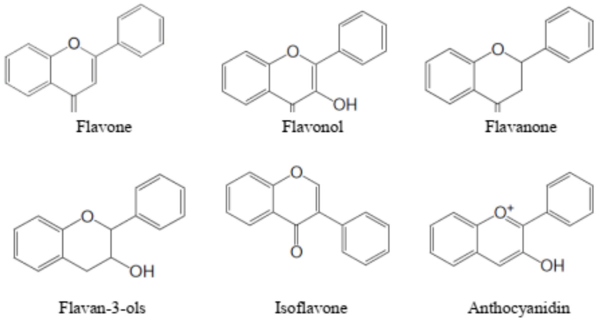


Fig. 2 Structure of some phenolics acids (Ali & Neda, 2011)

used as supplements to help prevent heart disease, cancer, aging and skin disorders caused by sunlight (Sadighara et al., 2016; Stahl & Sies, 2012; Aust et al., 2003; Fazekas et al., 2003). However, carotenoids are also anti-inflammatory and empowering the removal of foreign bodies from the human body. Thus carotenoids are important for the promotion of human health, *e.g.* β-carotene, lutein, cisanthin, lycopene and astaxanthin (Jia et al., 2011).

The sunlight reaching the surface of the earth consists of a broad spectrum, but, the radiation affecting the skin is quite obvious and labeled UV, which is divided into 2 types according to the wavelength: UV-A -in the range 320-400 nm and UV-B-in the range 290-320 nm (Ulrike et al., 2006). UV has positive effects on the skin, e.g. stimulating vitamin D production and skin pigmentation to protect the skin from sunlight (Anitha, 2012). However, UV is also somewhat harmful, in that UV-A can penetrate the skin to the dermis and destroy the collagen tissue and elastic fibers, which causes premature aging for skin cells, while UV-B, if consistent exposure, will cause the skin to swell, become red, swell and peel, causing sunburn, and it may develop skin cancer with long term exposure. The Sun Protection Factor (SPF) measures the effectiveness of sun protection, it tells consumers the length of exposure of the skin to sunlight, that the skin will tolerate without burning. For example, people normally exposed to sunlight for 10 minutes will feel burning pain or skin burn. However, after applying cosmetics to the skin, with SPF 15 sunscreen, exposure can be prolonged by 15 times or 150 minutes, without skin burn. Therefore, if consumers know and understand sunscreen products, they can more accurately choose the right sunscreen items and achieve the highest efficiency in protecting the skin from UV.

Thus, here we measured total phenolic content, total flavonoid content, free radical scavenging activity, lycopene, β-carotene, color, SPF, morphology and elemental composition for use as foundation data to use these raw materials, for developing effective face powder formulae.

Materials and methods

1. Extraction preparation

10 g Riceberry powder (or Pathum Thani rice powder) was soaked in 100 mL 95 % ethanol in a closed container, left it for 24 hr, then filtered with vacuum filter on Whatman filter paper No.1 and evaporated ethanol in a vacuum rotary evaporator at 75˚C.

2. Total phenolic content

Following a Folin-Ciocalteu assay method, modified from Butsat & Siriamornpun (2010), 100 mL Riceberry extract sample (or Pathum Thani rice extract sample) with a concentration of 2 mL per mL and 500 µL of 10% Folin Ciocalteu reagent were transferred to a test tube. It was shaken and left at room temperature for 1 min. After that, 1 mL 20 % w/v Na_2CO_3 was added shaken and left in a dark room for 60 min. Absorbance was measured at a 760 nm, using a UV-Vis spectrophotometer. Three replicates were measured. Total phenolic content was determined by comparing absorbances to the gallic acid calibration graph. Results were reported as mg gallic acid equivalent to 1 g of extracts (mg GAE/g of extract).

3. Total flavonoid content

Following a method, modified from Prabnok et al. (2016), 1 mL Riceberry extract sample (or Pathum Thani rice extract sample) with concentration of 2 mg/mL was pipetted into a test tube and added 4 mL distilled water, shaken and added 300 µL 5% $NaNO_2$. The mixture was shaken again and left for 5 min at room temperature. After that, 300 µL 10% $AlCl_3$ was added and left at room temperature for 6 min. Then, 225 µL of 1 M NaOH and 775 µL distilled water were added. The mixture was shaken and measured the absorbance at wavelength 510 nm using UV-Vis spectrophotometer. The results were reported in mg Rutin/g of extract.

4. DPPH radical scavenging assay

Following a method, modified from Butsat & Siriamornpun (2010), 1 mL of Riceberry extract sample (or Pathum Thani rice extract sample), with concentrations of 31.25, 62.50, 125.00, 250.00 and 500.00 µg/mL was pipetted into a test tube and 3 mL 0.2 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) was added. It was shaken and left in the dark for 30 min. Then, it was measured the absorbances at 517 nm by a UV-Vis spectrophotometer, using 95 % ethanol as blank and using 0.1 mM DPPH as control. The fraction of DPPH inhibition was calculated by plotting a graph of the relationship between % DPPH inhibition and sample concentration to indicate efficient concentration 50 (EC_{50}), which represents the antioxidant content that reduces DPPH concentration to 50 % by using butyl hydroxytoluene (BHT) as a standard substance. Three replicates were measured. The free radical scavenging activity was calculated as follows:

$$\text{Free radical scavenging activity (\%)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

where Abs_{sample} is the absorbance of DPPH with sample and $Abs_{control}$ is the absorbance of DPPH without sample. A linear regression was calculated from activity vs concentration to find EC_{50} in μg/mL.

5. Lycopene and beta-carotene content

Following a method, modified from Nagata & Yamashita (1992), 1 g of gac fruit (or wood apple) powder was added 20 mL 4:6 acetone: hexane and stirred for 15 min. Absorbances were measurement at 453, 505, 645 and 663 nm. The following equations were used to find concentration from absorbances:

$$\text{Lycopene (mg/100 mL)} = -0.0458Abs_{663} + 0.204Abs_{645} + 0.372Abs_{505} - 0.0806Abs_{453}$$
$$\beta\text{-carotene (mg/100 mL)} = 0.216Abs_{663} - 1.22Abs_{645} - 0.304Abs_{505} + 0.452Abs_{453}$$

6. Color measurement

Colors were measured in the CIELab L* a* b* space, when L* represents "lightness", ranging from 0 to 100, where 0 means low brightness and 100 means high brightness, a* measures green vs red and b* measures blue vs yellow (Mir et al., 2013). Total color differences, ΔE, where computed as:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

where ΔL = L* - L, Δa = a* - a , and Δb = b* - b and L*, a*, b* refer to colors of the reference object and L, a, b refer to colors of the sample.

7. Sun protection factor (SPF)

Following a method, modified from Dutra et al. (2004), 100 g sample was weighed and transferred to a 100 mL volumetric flask, diluted to volume with ethanol, shaken for 5 minutes and filtered through a Whatman No.1 filter paper. The filtrate was collected after rejecting the first 10 mL of filtrate. Then a 5.0 mL aliquot was transferred to a 50 mL volumetric flask and diluted to volume with ethanol. Subsequently, a 5.0 mL aliquot was transferred to a 25 mL volumetric flask and the volume completed with ethanol. The filtered solution was put in a quartz cell and the absorbance was measured from 290 to 320 nm at 5 nm steps. The measurement was repeated three times. SPF was calculated from the absorbances (Mansur et al., 1986):

$$SPF = CF\ x \sum_{290}^{320} EE\ (\lambda) x\ I(\lambda) x\ Abs(\lambda)$$

where *EE*(λ) is an erythemal effect spectrum, *I*(λ) mean solar intensity spectrum, *Abs*(λ) means absorbance of sunscreen products and *CF* means correction factor (=10).

8. Morphology and elemental composition

Morphology and elemental composition of samples were checked by scanning electron microscopy (SEM); model LEO 1455 VP, Germany, coupled with energy dispersive X-ray (EDX Oxford, ISIS 300, England) analyzer.

9. Formula for face powder from rice and natural substance

Samples of two types of broken rice were ground, until they passed a 200 mesh filter by peeling off the rice shells. The gac fruit powder was made from the flesh covering its seeds. The wood apple powder was derived from wood apple tree branches. Samples were prepared using mixtures listed in Table 1, ground, mixed together, and packed in containers.

Table 1 Percentage of the components in the form of face powder formula

Component (%)	Formula 1	Formula 2
Talcum	-	60
Pathum Thani rice	22	-
Riceberry	6	-
Wood apple	3	-
Gac fruit	44	-
Zinc oxide	3	5
Zinc stearate	3	10
Magnesium carbonate	3	10
Kaolin	3	5
Calcium carbonate powder	13	10
Total	**100**	**100**

Results and discussion

Table 2 showed that Riceberry extract sample had total phenolic content at 26.46±0.008 mg of gallic acid/g of extract, while Pathum Thani rice extract sample had 3.67±0.010 mg. The higher phenolic content for Riceberry extract sample was likely due to the darker color of Riceberry seed, showing that it containing more flavonoids. For flavanoid content, the Riceberry extract sample had 101.63±0.034 mg rutin/g and Pathum Thani extract sample had 56.04±0.004 mg of rutin/g.

Table 2 Total phenolic and flavonoid content of rice extract sample

Sample	Total phenolic (mg of gallic acid/g of extract)	Total flavonoid (mg of rutin/g of extract)
Pathum Thani rice	3.67±0.010	56.04±0.004
Riceberry	26.46±0.008	101.63±0.034

Fig. 3 illustrates calculation of the efficient concentration 50 (EC_{50}) of Riceberry extract sample, based on the DPPH assay, using the plot of % free radical scavenging activity *versus* concentration. As expected, free radical scavenging activity increased with sample concentration-dose-dependent curve. Table 3 indicates that the antioxidant activities, EC_{50}, were 108.31±0.024 for Riceberry extract sample and 430.46±0.062 µg/mL for Pathum Thani rice extract sample. This confirms reports from Chakuton et al. (2012), Chen et al. (2012) and Moko et al. (2014), who found that colored rice had higher amounts of phenolic compounds and antioxidant activity than colorless rice. That is, the extracts with a low EC_{50} hold good free radical scavenging activity.

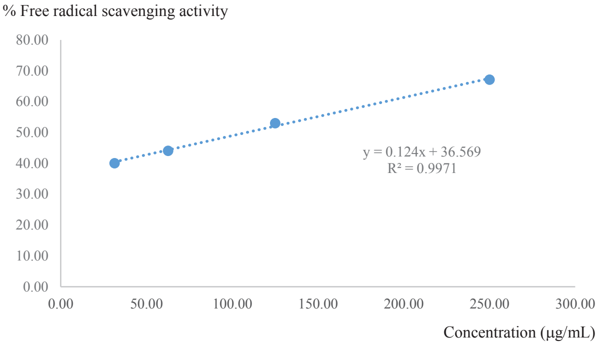


Fig. 3 The relationship between concentration and % free radical scavenging activity of Riceberry extract sample

Table 3 Antioxidant activity of rice extract sample

Sample	EC_{50} (g/mL)	Linear regression	R^2
Butylated hydroxytoluene (BHT)	128.68±0.024	y = 0.124x + 34.034	0.9971
Pathum Thani rice	430.46±0.062	y = 0.108x + 3.337	0.9983
Riceberry	108.31±0.024	y = 0.124x + 36.569	0.9971

Table 4 shows free radicals capturing ability, measured by DPPH assay method. Gac fruit had the highest EC_{50} at 290.39±0.032 µg/mL, lycopene at 1.1099±0.099 mg/100 mL and β-carotene at 0.6292±0.081 mg/100 mL on a dry basic. Gac fruit had the clear pigment at L* = 56.70±0.02, a* is 42.77±0.02, b* = 40.94±0.05. SPFs in Table 5 showed that wood apple offered the highest of SPF at 6.78±0.097.

Table 4 Antioxidant activity, Lycopene and β-carotene of natural extract

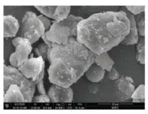
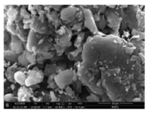
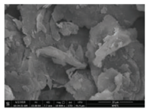
Properties	Gac fruit	Wood apple
Antioxidant activity (g/mL)	290.39±0.032	55.67±0.045
Lycopene (mg/100 mL dry basic)	1.1099±0.099	0.0346±0.000
β-carotene (mg/100 mL dry basic)	0.6292±0.081	0.0400±0.000

Table 5 Sun protection factor of samples

Sample	Riceberry	Phatum Thani	Gac fruit	Wood apple
SPF	1.83±0.083	1.12±0.159	1.58±0.155	6.78±0.097

Table 6 indicates that face powder made from rice and natural extract offers ΔE of color closer to commercial face powder No.3 than talcum face powder. Face powder from rice and natural extracts offered better SPF than talcum powder, which means that rice and natural extracts can protect from UV even though the sun protection value is not very high, compared to those of synthetic sunscreens in the market. However, natural extracts also contained useful antioxidants, which may be used in combination with synthetic sunscreen, to enhance sunscreen efficiency and reduce the use of synthetic sunscreen. Analysis for metals content showed no heavy metals according to TIS 981-2013 standards, which prescribes no more than 20, 5, 1 and 0.05 ppm of lead, arsenic, mercury and barium by weight. The SEM images indicated that the powder formula was similar to that of the commercial formula which had round particles, while the talcum powder formula showed sheet-like particles.

Table 6 Chemical composition and morphology of samples

Item	Commercial face powder No.3	Formula 1	Formula 2
Color			
L*	62.61±0.00	75.73±0.02	92.81±0.02
a*	13.66±0.02	8.09±0.06	-1.48±0.01
b*	23.86±0.02	13.50±0.04	2.06±0.01
ΔE	-	17.62±0.05	40.20±0.01
SPF value	39.39±0.00	1.19±0.06	0.60±0.12
Element by weight (ppm)			
• Lead	nd	nd	nd
• Arsenic	nd	nd	nd
• Mercury	nd	nd	nd
• Barium	nd	nd	nd
Morphology			

Remark: nd = not detected

Conclusion

We showed that face powders based on natural ground rice with added natural ingredients provided a useful level of sun protection, additional antioxidants and free radical scavenging, yet contained no toxic heavy metals, thus were beneficial and safe. Riceberry and Phatum Thani rice both showed significant levels of phenolic content-26.46±0.008 mg GAE/g and 3.67±0.010 mg GAE/g; flavonoids-101.63±0.034 mg Rutin/g and 56.04±0.004 mg Rutin/g-and antioxidants at 108.31±0.024 µg/mL and 430.46±0.062 µg/mL. SPFs were Riceberry -1.83±0.083, Pathum Thani rice-1.12±0.159, gac fruit -1.58±0.155 and wood apple-6.78±0.097.

Thus natural extracts are highly appropriate for use as an ingredient in cosmetic products; further, they show unique local identity and pride for the community, that, could earn more income and promote career choices for people in it. Antioxidants in these natural extracts can additionally offer rejuvenation of the cells and reduce the use of synthetic sunscreens. Therefore, they could be developed further for future research projects.

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