Antioxidant activities and sensory properties of rice bran with marigold tea

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Abstract

Bioactive compounds and antioxidant activities of the rice bran with marigold tea formulated by mixing different proportions of rice bran and marigold were investigated. Comparative results of sensory evaluation were conducted by Thai panelists (n=30) and Austrian panelists (n=30), respectively. The results showed that rice bran with marigold tea had high values of antioxidant activities as measured by DPPH radical scavenging (95-97%), ferric reducing/antioxidant power (FRAP) (80-84 μmol $FeSO_4$/g dry sample), total phenolic content (TPC) (29-49 mgGAE/g dry sample), and total flavonoids content (TFC) (61-69 mgRE/g dry sample). The predominant phenolic acids in rice bran with marigold tea were gallic, ferulic and sinapic acid and the main flavonoids were quercetin and rutin. A proportion of 50% rice bran: 50% marigold showed the highest sensory scores of all formulas for Thai panelists (n=30), while Austrian panelists have evaluated a proportion of 30% rice bran: 70% marigold with the highest score. Austrian panelists seemed to dislike the odor of the product when the proportion of rice bran was increased. This study demonstrated that rice bran with marigold tea could be considered as alternative beverage for health benefits. However the optimum formula was dependent on consumers' preference.

Keywords: antioxidant activity, sensory evaluation, rice bran, marigold, tea

1. Introduction

Tea is one of the most popular beverages in the world. Numbers of scientific evidence indicated that tea extracts have antioxidant properties and health benefits that include reducing the risk of chronic diseases such as coronary heart disease, hypertension, cancers, and arthritis

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(Hodgson and Croft, 2010; McKay et al., 2010; Chen and Dou, 2008; Ahmed, 2010). Most of the beneficial effects come from antioxidants present in a variety of teas. Beside tea which commonly refers to the product from tea plant (*Camellia sinensis*), a number of plants can be used as herbal teas because of their phytochemicals and antioxidant properties. Antioxidants can repress free radicals and prevent cells against oxidative stress, therefore preventing cell damages and diseases (Seifried et al., 2007). Consumers have increased interest in drinking herbal teas by reason of the belief that they have less side effects comparing to synthetic medicine (Pinitsoontorn et al., 2012). In Thailand, herbal teas from medicinal plants are produced and marketed by several herbal companies. Most herbal teas are made from only one kind of herb although some may be blended.

Rice bran, as a rice by-product has been claimed to be a good source of protein, fat and antioxidants, but is currently under-utilized, in spite of its high potential as a raw material for the preparation of functional foods or nutraceuticals. Rice bran is the outer layer of brown rice kernel, which is removed while milling brown rice to white. The bran is primarily composed of pericarp, aleurone and subaleurone layers of the kernel and typically includes the embryo or germ and small amounts of starchy endosperm (Tahira et al., 2007). Rice bran powder is high in protein, fiber and bioactive compounds (Saunders, 1990), offers benefits like lowering of blood cholesterol (Chotimarkorn and Silalai, 2008; Kahlon et al., 1994) and decreases the incidence of artherosclerosis disease (Saunders, 1985) and has a laxative effect (Saunders, 1990).

Marigold (*Tagetes erecta* L.) is a herb of ancient medicinal repute. It grows as a wild and common garden plant throughout Europe, North America and Asia. It has long been used as a food and animal feed. Marigold petal has also been used as medicinal plant in infusions and unguents. It has been reported to have therapeutic activities, such as anti-mutagenicity, anti-inflammatory, anti-tumourogenic, antiviral and immunostimulating effects (Gonzalez de Mejia et al., 1997; Hamburger et al., 2003). The pharmacological activities of marigold are related to the content of several classes of secondary metabolites such as flavonoids, carotenoids, tannins, sterols, saponins, triterpene alcohols, polysaccharides, a bitter principle, mucilage and resin (Jacobs et al., 1994; Piccagliy et al., 1998). Marigold flowers are used as tea, food colorant and ingredient in cooking. They may be used in form of fresh petals or as a dried powder, which can be made into tea, spice and medicine (tinctures, ointments and creams) (Gonzalez de Mejia et al., 1997).

The objectives of this study were to produce a tea product by blending rice bran powder and marigold flower and to investigate its antioxidant activities and sensory properties. This attempt was pursued in order to utilize the nutritious rice bran, which is likely to be ignored and considered as a waste product.

2. Materials and Methods

2.1 Sample preparation

Paddy-rice samples (Khao Dowk Mali (KDML) 105 variety) were obtained from northeastern Thailand. The grains were milled to separate the husks from the brown rice. Then the brown rice was polished to obtain the bran. The bran was dried by far-infrared radiation. Marigold (*T. erecta* L.) flowers were bought from the market from Maha Sarakham Province, Thailand, in June 2013. Marigold flowers were cleaned and the petals were separated. The raw marigold petals were washed and kept at room temperature to drain. Afterwards, the petals were dried by combined far-infrared radiation with hot-air convection. Dried rice bran and marigold were mixed together in various ratios (Table 1). The samples were stored at -20°C prior to analysis. Moisture was determined by drying 5±0.001 g powder at 110°C to constant mass. All analytical results were expressed on a dry matter basis and performed in triplicate.

Table1. Description of formulas of rice bran with marigold tea samples.

Formula	Rice bran (% by weight)	Marigold (% by weight)
1	30	70
2	40	60
3	50	50
4	60	40
5	70	30

2.2 Chemicals and reagents

The compounds 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), 2,4,6-tripiridyl-s-triazine (TPTZ), Folin–Ciocalteu's reagent, standards of phenolic compounds (gallic acid (GA), protocatechuic acid (PCCA), *p*-hydroxybenzoic acid (*p*-OH), vanilic acid (VA), chorogenic acid (ChA), caffeic acid (CFA), syringic acid (SyA), *p*-coumaric acid (*p*-CA), ferulic acid (FA), sinapic acid (SNA), rutin, myricetin, quercetin, apigenin and kaempferol were obtained from Fluka Co. (Neu-Ulm, Germany).

Oryzanol (food grade, 99.9% purity) was obtained from Tsuno Rice Fine Chemicals Co., Ltd. (Wakayama, Japan). Acetic acid, methanol, acetonitrile and other solvents and reagents used in the HPLC analysis were purchased from Merck Co. (Darmstadt, Germany). All chemicals and reagents used in the study were of analytical grade.

2.3 Assessment of antioxidant activity

2.3.1 Sample extraction

The extracts prepared from the rice bran with marigold teas were made by boiling the test material in distilled water for 5 min. The ratio between sample and extraction medium was 1:25 (w/v). The mixtures were then filtered through filter paper (Whatman No. 1) (Dasgupta and De, 2004) and the filtrate used for analyzing antioxidant activity *in vitro*. All analyses were performed in triplicate.

2.3.2 DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity of the extracts was measured as described by Butsat and Siriamornpun (2010) with some modifications. Briefly, sample extract (0.1 mL) was mixed with 1.9 mL of a solution of 0.1 mM DPPH in ethanol. The mixture was vortexed (1 min), left to stand at room temperature in dark (30 min) and then the absorbance of this solution was read at 517 nm. The percent inhibition activity was calculated as [(Ao-Ae)/Ao]x100 (Ao= Absorbance without extract; Ae = absorbance with extract).

2.3.3 Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay is a method of measuring the ability of reductants (antioxidants) to reduce Fe^{3+} to Fe^{2+}. The formation of blue coloured Fe^{2+}-TPTZ complex (Fe^{2+} tripyridyltriazine) increases the absorbance at 593 nm. The method of Kubola and Siriamornpun (2008) was used with some modifications. The FRAP reagent was freshly prepared by mixing 100 mL of acetate buffer (300 mM, pH 3.6), 10 mL TPTZ solution (10 mM TPTZ in 40 mM/HCl), 10 mL $FeCl_3 \cdot 6H_2O$ (20 nM) in a ratio of 10:1:1 and 12 mL distilled water, at 37^{o}C. To perform the assay, 1.8 mL of FRAP reagent, 180 µL Milli-Q water and 60 µL sample, standard or blank were then added to the same test tubes, and incubated at 37^{o}C for 4 min; absorbance was measured at 593 nm, using the FRAP working solution as a blank. The reading of relative absorbance should be within the range 0–2.0; otherwise, the sample should be diluted. In the FRAP assay, the antioxidant potential

of sample was determined from a standard curve plotted using the $FeSO_4 \cdot 7H_2O$ linear regression equation to calculate the FRAP values of the sample.

2.4 Determination of total flavonoid content

Total flavonoid content (TFC) was determined using the colorimetric method described by Abu Bakar et al. (2009) with slight modification. Briefly, 0.5 mL of the extract was mixed with 2.25 mL of distilled water in a test tube followed by addition of 0.15 mL of 5% $NaNO_2$ solution. After 6 min, 0.3 mL of a 10% $AlCl_3 \cdot 6H_2O$ solution was added and allowed to stand for another 5 min before 1.0 mL of 1M NaOH was added. The mixture was mixed by vortex mixer. The absorbance was measured immediately at 510 nm using spectrophotometer. Results were expressed as mg rutin equivalents in 1 g of dried sample (mg RE/g).

2.5 Identification and quantification of phenolic compounds

2.5.1 Phenolics extraction

The phenolic compounds in test samples were extracted using a modification of the procedure described by Uzelac et al. (2005). Each sample (5 g) was mixed with 50 mL methanol/HCl (100:1, v/v) which contained 2% tert-butyl hydroquinone, in an inert atmosphere (N_2) during 12 h at 35°C in the dark. The extract was then centrifuged at 4000 rpm/min, and the supernatant was evaporated to dryness under reduced pressure (35–40°C). The residue was redissolved in 25 mL of water/ethanol (80:20, v/v) and extracted four times with 25 mL of ethyl acetate. The organic fractions were combined, dried for 30–40 min with anhydrous sodium sulphate, filtered through a Whatman-40 filter, and evaporated to dryness under vacuum (35–40 °C). The residue was redissolved in 5 mL of methanol/water (50:50, v/v) and filtered through a 0.45 m filter before injection (20 L) into the HPLC aperture.

2.5.2 Determination of total phenolic content

The total phenolics content (TPC) was determined using the Folin–Ciocalteu reagent as followed by Kubola et al. (2011). Briefly, 300 µL of extract was mixed with 2.25 mL of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min; 2.25 mL of sodium carbonate (60 g/l) solution was added to the mixture. After 90 min at room temperature, absorbance was measured at 725 nm using

a spectrophotometer. Results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g).

2.5.3 HPLC-DAD system for analysis of phenolic compounds

HPLC analysis was performed using Shimadzu LC-20AC pumps, SPD-M20A Diode-array detection; chromatographic separations were performed on a LUNA C-18 column (4.6 x 250 mm i.d., 5 µm). The composition of solvents and gradient elution conditions were described previously by Uzelac et al. (2005). The solvent system used was a gradient of mobile phase A containing 3% acetic acid in water; solution B contained a mixture of 3% acetic acid, 25% acetonitrile and 72% water. The following gradient was used: 0–40 min, from 100% A to 30% A - 70% B with a flow rate 1 mL/min; 40–45 min, from 30% A - 70% B to 20% A - 80% B with a flow rate 1 mL/min; 45–55 min, from 20% A - 80% B to 15% A - 85% B with a flow rate 1.2 mL/min; 55–57 min, from 15% A - 85% B to 10% A - 90% B with a flow rate 1.2 mL/min; 57–75 min 10% A - 90% B with a flow rate 1.2 mL/min. Operating conditions were as follows: column temperature, 40°C, injection volume, 20 µL, UV-Diode Array detection at at 280 nm (hydroxybenzoic acids), 320 nm (hydroxycinnamic acids) and 370 nm (flavonols) at a flow-rate of 0.8 mL/min. Spectra were recorded from 200 to 600 nm. Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with those of standard compounds and were detected using an external standard method.

2.6 Sensory Evaluation

Sensory evaluations of rice bran with marigold teas were conducted by two groups of trained panelists (30 panelists per group). The first group consisting of Mahasarakham University students (Mahasarakham, Thailand), and the second group consisting of University of Natural Resources and Life Sciences students (Vienna, Austria). Teas from different formulas were freshly prepared by infusing 1 g of teas in 250 mL of hot water for 5 min. Sensory attributes (color, odor, taste and overall acceptability) were evaluated by a nine-point hedonic scale where nine was “like extremely”, five “neither like nor dislike” and one “dislike extremely”. Three coded samples were served and water was provided for rinsing between the samples.

2.7 Statistical analyses

Analysis of variance (ANOVA) was performed in a completely randomized design, using Duncan's Multiple Range Test. All determinations were done at least in triplicate. The confidence limits used in this study were based on 95 % (p<0.05).

3. Results and Discussion

3.1 Antioxidant activity

The DPPH radical scavenging, and FRAP assays were used to evaluate the antioxidant capacities of rice bran with marigold tea. DPPH assay was used to evaluate the free radical scavenging ability of the tea extract and the results are shown in Table 2. DPPH radical scavenging activities of rice bran with marigold tea ranged from 95% in formula 5 (rice bran: marigold 70:30) to 97% in formula1 (rice bran: marigold 30:70) and were not significantly different (p>0.05) among all formulas studied. The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe3+–TPTZ) complex to produce a colored ferrous tripyridyltriazine (Benzie and Strain, 1996). The reducing properties are generally associated with the presence of compounds which exert their action by breaking the free-radical chain by donating a hydrogen atom (Gordon, 1990). FRAP values of the extracts of rice bran with marigold tea increased with increasing amount of marigold. Similar results were found in TPC and TFC (Table 2).

TPC was determined in comparison to standard gallic acid and the results were expressed in mg GAE/g dry sample. TPC of the extracts of rice bran rice bran with marigold tea were significantly increased when the amount of marigold increased (Table 2). For extracts of all samples, TFC ranged from 61 mgRE/g dry sample in formula 5 to 69 mgRE/g dry samples in formula 1. Again TFC was increased with increasing amount of marigold (Table 2).

Table 2. Antioxidant activity, TPC and TFC of rice bran with marigold tea samples.

Formulas (rice bran: marigold)	DPPH (% inhibition)	FRAP (μmol $FeSO_4$/g DW)	TPC (mgGAE/g DW)	TFC (mgRE/g DW)
1 (30: 70)	97.35±0.49	84.52±0.36[a]	49.03±0.15[a]	69.45±0.19[a]
2 (40: 60)	96.21±0.51	82.14±0.45[b]	47.08±0.46[b]	68.80±1.17[a]
3 (50: 50)	96.42±0.49	81.79±0.57[bc]	43.61±0.71[c]	68.47±0.83[a]
4 (60: 40)	95.85±0.84	80.72±0.92[cd]	37.09±0.34[d]	67.20±0.25[a]
5 (70: 30)	95.81±0.82	80.34±0.85[d]	29.25±0.38[e]	61.11±2.16[b]

Values are expressed as means ± standard deviation (n=3).

[a, e] Values in the same column followed by different letters are significantly different (p<0.05).

3.2 Identification and quantification of phenolic compounds

Phenolic compounds are the most active antioxidant derivatives in plants which are mostly found in the outer layers of plants, such as the peel, shell and hull, to protect the inner components (Bors et al., 2001). A number of phenolic acids are linked to various cell-wall components, such as arabinoxylans and proteins (Hartley et al., 1990). They are known to be good natural antioxidants which not only donate hydrogen or electrons but are also stable radical intermediates (Maillard et al., 1996). RP-HPLC analysis was used to identify the phenolic compounds of rice bran with marigold tea extracts, in comparison to standard compounds. Phenolic acids are hydroxylated derivatives of hydrobenzoic and hydrocinnamic, which often occur in plants as esters, glycosides and bound complexes (Germano et al., 2006). In the rice bran/marigold tea samples it was possible to identify 4 hydroxybenzoic acids (HBA): gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanilic acid and 6 hydrocinnamic acids (HCA): chorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, and sinapic acid. The distribution of phenolic acids in all formulas is presented in Table 3.

Table 3. Phenolic acids of rice bran with marigold tea (µg/g dry weight).

Phenolic acids	Formulas				
	1	2	3	4	5
Hydrobenzoic acids					
gallic acid	89.99±0.13^{a}	76.02±0.20^{b}	74.14±0.54^{c}	68.04±1.69^{d}	67.11±0.22^{d}
protocatechuic acid	12.19±0.12^{d}	14.84±0.11^{c}	18.22±0.10^{b}	18.58±0.15^{b}	19.38±0.71^{a}
p-hydroxybenzoic acid	4.31±0.05^{a}	3.88±0.12^{b}	3.70±0.09^{c}	3.54±0.01^{d}	3.31±0.04^{e}
vanilic acid	4.06±0.02^{e}	4.15±0.01^{d}	4.53±0.06^{c}	4.63±0.04^{b}	4.74±0.03^{a}
Hydrocinnamic acids					
chorogenic acid	17.58±0.30^{a}	17.36±0.09^{ab}	17.15±0.11^{b}	16.78±0.04^{c}	16.16±0.12^{d}
caffeic acid	14.81±0.37^{a}	14.61±0.63^{a}	13.61±0.38^{b}	12.57±0.33^{c}	11.56±0.16^{d}
syringic acid	9.72±0.04^{a}	8.75±0.05^{b}	7.13±0.05^{c}	5.38±0.17^{d}	5.04±0.05^{e}
p-coumaric acid	9.19±0.21^{a}	8.95±0.17^{ab}	8.89±0.09^{b}	8.75±0.04^{b}	8.31±0.09^{c}
ferulic acid	52.65±0.10^{a}	51.94±0.12^{b}	50.52±0.20^{c}	49.11±0.15^{d}	45.28±0.41^{e}
sinapic acid	53.85±0.79^{a}	43.07±1.14^{b}	41.26±0.68^{c}	39.38±0.60^{d}	37.55±0.29^{e}
Total	268.35±0.35^{a}	243.57±1.67^{b}	239.18±1.45^{c}	226.77±1.48^{d}	218.44±1.08^{e}

Note: formulas is in Table 1. Values are expressed as means ± standard deviation (n=3).

$^{a, e}$ Values in the same row followed by different letters are significantly different (p<0.05).

The main phenolic acids found in all formulas were gallic, ferulic, and sinapic acids. Comparing the phenolic acids of all samples, formula 1 contained the highest levels of total phenolic acid, with a concentration of 268 µg/g (Table 3). For flavonoids, it was possible to identify 5 flavonoids: rutin, myricetin, quercetin, apigenin and kaempferol. The most abundant flavonoids found in all samples of rice bran with marigold tea were quercetin (804-1007 µg/g dry sample) and rutin (113-140 µg/g dry sample) (Table 4).

Table 4. Flavonoids of rice bran with marigold tea (µg/g dry weight).

Formulas (rice bran: marigold)	flavonoid contents (µg/g DW)					
	Rutin	Myricetin	Quercetin	Apigenin	Kaempferol	Total
1 (30: 70)	140.36±0.51[e]	6.06±0.01[a]	1007.80±1.87[a]	Nd	3.14±0.03[a]	1157.36±1.94[a]
2 (40: 60)	135.56±0.50[d]	6.01±0.01[b]	989.46±0.60[b]	Nd	2.87±0.00[b]	1133.90±0.97[b]
3 (50: 50)	128.36±0.40[c]	5.98±0.01[c]	919.35±0.31[c]	Nd	2.68±0.01[c]	1056.37±0.48[c]
4 (60: 40)	121.03±0.46[d]	5.93±0.00[d]	866.19±0.54[d]	Nd	2.26±0.03[d]	995.42±0.64[d]
5 (30: 70)	113.34±0.42[e]	5.90±0.01[e]	804.05±3.29[e]	Nd	1.78±0.01[e]	925.07±3.69[e]

Values are expressed as means ± standard deviation (n=3). Nd, not detected.

[a, e] Values in the same column followed by different letters are significantly different (p<0.05).

3.3 Sensory Evaluation

The sensory scores for color, odor, taste and overall acceptability of rice bran with marigold tea are shown in Table 5 and 6. All rice bran with marigold teas were within the range of 6.0-8.0 (like slightly to like very much). The scores for all characteristics of rice bran with marigold tea were within acceptance values. Table 5 shows the sensory scores of the Thai panelists. Formula 3 (rice bran: marigold, 50:50) had the highest scores for all characteristics, while formula 1 and 5 (rice bran: marigold, 30:70 and 70:30, respectively) had the lowest scores. Most of the panelists stated that they liked the color and odor of the mixed tea when the proportion of rice bran and marigold was 50:50. Additionally, they also commented that adding higher amounts of marigold resulted in a dark yellow color and pungent smell of flowers, while small amounts of marigold caused a pale color. The sensory scores of the Austrian panelists differed to the results of the Thai panelists (Table 6). Formula 1 (rice bran: marigold 30:70) had the highest scores for all characteristics. Sensory evaluations revealed that this rice bran with marigold tea was overall acceptable (score 8 out of 9). Most of Austrian panelist stated that they liked the high intensity of color and odor of marigold.

Table 5. Sensory score of rice bran with marigold tea samples evaluated by Thai panelists (n=30).

Formulas (rice bran: marigold)	Sensory score			
	Color	Odor	Taste	Overall acceptability
1 (30: 70)	6.80±1.32 [bc]	7.13±1.25 [ab]	6.50±1.87 [bc]	6.90±1.58 [c]
2 (40: 60)	7.20±1.40 [b]	7.20±1.32 [a]	7.23±1.65 [ab]	7.37±1.16 [ab]
3 (50: 50)	8.17±0.99 [a]	7.60±1.25 [a]	7.67±1.92 [a]	8.00±1.17 [a]
4 (60: 40)	7.07±1.51 [b]	7.27±1.36 [a]	7.23±1.30 [ab]	7.30±1.47 [ab]
5 (70: 30)	6.83±1.23 [bc]	6.93±1.31 [ab]	6.67±1.21 [bc]	6.77±1.45 [cd]

Values are expressed as means ± standard deviation (n=30).

[a, d] Values in the same column followed by different letters are significantly different (p<0.05).

Table 6. Sensory score of rice bran with marigold tea samples evaluated by Austrian panelists (n=30).

Formulas (rice bran: marigold)	Sensory score			
	Color	Odor	Taste	Overall acceptability
1 (30: 70)	7.57±1.27 [a]	8.10±1.31 [a]	7.73±1.55 [a]	8.07±1.23 [a]
2 (40: 60)	7.27±1.31 [ab]	6.20±1.36 [b]	6.70±1.78 [b]	6.78±1.51 [b]
3 (50: 50)	7.07±1.39 [ab]	6.23±1.25 [b]	6.23±1.59 [b]	6.50±1.28 [bc]
4 (60: 40)	6.57±1.70 [bc]	6.43±1.32 [b]	6.10±1.69 [b]	6.03±1.30 [c]
5 (70: 30)	5.87±1.89 [c]	6.63±1.25 [b]	6.70±1.58 [b]	6.35±1.21 [bc]

Values are expressed as means ± standard deviation (n=30).

[a, c] Values in the same column followed by different letters are significantly different (p<0.05).

4. Conclusions

This study demonstrated that rice bran with marigold teas contained important bioactive compounds such as phenolic acids and flavonoids and had high values of antioxidant activities. The score of sensory evaluation ranged from like slightly to like very much. The evaluation within two different ethnic groups (Asia and Europe), revealed that ethnic origin influenced consumer preferences. Overall, a rice bran with marigold tea could be made from a significant proportion of rice bran with an overall acceptance. It is suggested that such a product could be considered as a good alternative drink for health benefits.

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