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ORIGINAL ARTICLE

Chlorophyll and total phenolic contents, antioxidant activities and consumer acceptance test of processed grass drinks

Wiwat Wangcharoen[1] · **Suthaya Phimphilai**[1]

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Abstract The processed drinks produced from grass of three varieties of rice (Jasmine, Sukhothai 1 and Sukhothai 2) and one each from wheat and barley grasses were analyzed for chlorophyll and total phenolic contents, and antioxidant activities [ABTS radical cation decolorisation assay and ferric reducing/antioxidative power (FRAP) assay]. The consumer acceptance of all the drinks was also evaluated. One serving size (200 mL) of these contained 82–958 µg of chlorophyll, 5.60–26.14 mg Gallic acid equivalent for total phenolic content and their antioxidant activities, ABTS and FRAP values were 17.88–35.18 mg Vitamin C equivalent and 5.66–23.70 mg $FeSO_4$ equivalent, respectively. The overall consumer acceptability of drinks was significantly correlated to their aroma intensity and consumer preference on aroma and flavor. Jasmine and Sukhothai 1 rice grass drinks were most preferred, however, one subgroup preferred grass drink from Sukhothai 1 rice, while the second subgroup preferred the drinks from Sukhothai 2 rice and Jasmine rice. Wheat and barley grass drinks were not preferred by both subgroups.

Keywords Grass drinks · Chlorophyll · Total phenolic content · Antioxidant activities · Internal preference map · Cluster analysis

✉ Wiwat Wangcharoen
wiwat@mju.ac.th

[1] Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai 50290, Thailand

Introduction

Functional beverages are the fastest growing segment in the functional food category (Gruenwald 2009). In Thailand, functional drink market grew from Bt1.8 billion in 2009 to Bt6.6 billion in 2014 (Ketnil 2014). The consumer interest in natural functional drinks, with anti-aging, energy supplying, relaxing, or beauty enhancing effects is increasing. To avoid intake of chemical substances, natural substances from plant, which are preferred than animal sources, have been increasingly used as functional botanical ingredients in beverages (Gruenwald 2009).

The cereal grasses (young leaves of grain-bearing plants), including wheat, barley, alfalfa, rye, oat, and kamut, are interesting ingredients for functional drinks. They contain substantial concentrations of phytochemicals and vitamins (Gruenwald 2009). Especially wheatgrass juice, obtained from young wheat plant was first used for promoting human health by Ann Wigmore, founder of the Hippocrates Health Institute in Boston (Wigmore 1985). It is commonly known as the "green blood" due to its high chlorophyll content (Padalia et al. 2010). Chlorophyll bears structural similarity to hemoglobin and has been found to regenerate or act as a substitution for hemoglobin in hemoglobin deficiency conditions. This might be the reason behind the utility of wheatgrass in clinical conditions like thalassemia and hemolytic anemia (Marwaha et al. 2004; Padalia et al. 2010). In addition, chlorophyll together with the vital enzymes like superoxide dismutase, the plant hormone abscisic acid or dormin, and its alkalinity play their roles in the anticancer function (Livingston 1976; Pokhrel 1999; Mates et al. 2000; Jed et al. 2005; Padalia et al. 2010).

Antioxidants in wheatgrass juice such as (pro) vitamins C, E, β-carotene and zinc are responsible for anti-allergic and anti-asthmatic treatment, while bioflavonoids account for many clinical utilities such as management of inflammatory bowel disease and as a general detoxifier. Wheatgrass juice is safe and the incidence of side effects is very low. It may cause nausea and headache if excessive quantities are consumed. Throat swelling may occur in hyper-sensitive individuals (Padalia et al. 2010). In case of barley grass, there is a report on the contents of vitamin C, total polyphenols, ferulic acid, monosaccharides and amino acids indicating that it is a valuable plant material (Paulíčková et al. 2007). For other species of cereal grasses, more research is needed to uncover additional unknown benefits (Gruenwald 2009).

Rice is an edible cereal grain eaten by more than half of the world's population (Zronik 2006). Thailand is one of the world's biggest rice producers, with paddy output of 19.4 million metric tons in marketing year 2014/2015, while wheat production is insignificant in Thailand due to unfavourable climate conditions, lack of seed development, and unattractive returns (USDA Foreign Agricultural Service 2015). With increasing of functional drink demand in Thailand (Ketnil 2014) and emerging of green botanicals in beverages including cereal grasses (Gruenwald 2009), processed drinks from grasses of rice, wheat and barley have been developed.

This study was aimed to evaluate some chemical properties related to health benefits, including chlorophyll content, total phenolic content and antioxidant activities (improved ABTS radical cation decolorisation assay and ferric reducing/antioxidative power (FRAP) assay), of processed grass drinks produced from three varieties of rice (Jasmine, Sukhothai 1 and Sukhothai 2), wheat and barley. Consumer acceptance of products was studied and the preference direction of this consumer group was identified by internal preference mapping and cluster analysis.

Materials and methods

Chemicals

Acetone, Folin–Ciocalteau phenol reagent, sodium bicarbonate, gallic acid, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium persulphate, vitamin C, 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride, glacial acetic acid, hydrochloric acid, sodium acetate, and ferrous sulphate were obtained from Sigma-Aldrich and all of them were of analytical grade.

Grass production

Seeds were washed and soaked in water for 24 h. Floating seeds were discharged. Soaked seeds were then covered by wet cheesecloth for 12–48 h to let them sprout. Sprouts were placed on the wet soil and were covered by wet cheesecloth for 3 days. After that, they were watered twice a day. Grasses of wheat and barley were harvested after 7 days, while those of rice were harvested after 20 days. Harvesting was done by cutting it about ½ inch from the soil.

Samples

Five processed drinks produced from young grasses of three rice varieties (Jasmine, Sukhothai 1 and Sukhothai 2), wheat, and barley were produced. In brief, the grasses with optimum ages were collected, extracted, and filled in 200 mL clear glass bottles before processing under the same sterilizing condition. The samples were kept at room temperature for a month before all chemical analysis and consumer acceptance test.

Chemical analysis

Chlorophyll content

Chlorophyll contents in all sample drinks were determined with some modifications of Arnon (1949). One mL of sample and 4 mL of acetone (80% acetone) were well-mixed prior to absorbance (A) measurements at 663 and 645 nm. Total chlorophyll content was reported as µg chlorophyll per 200 mL of product (one serving size) and was calculated by this equation:

$$\text{Total chlorophyll} = (20.2A_{645} + 8.02A_{663})(5/1000)(200)$$

Total phenolic content

The Folin–Ciocalteau micro method of Pinsirodom and Changnoi (2002) was used with some modifications. In brief, 500 µL of sample, 9.5 mL of distilled water and 0.5 mL of Folin–Ciocalteau reagent were thoroughly mixed in a test tube and incubated at room temperature for 5 min. Two mL of 10% (w/v) sodium bicarbonate was added and incubated for 10 min before reading an absorbance at 730 nm. Gallic acid solution (0–150 µg) was used to generate a standard line. Results were reported as mg Gallic equivalent per 200 mL of product (one serving size).

Improved ABTS radical cation decolorization assay

The method based on the ability of antioxidant molecules to quench the long-lived ABTS radical cation ($ABTS^{\cdot+}$) of Re et al. (1999) was modified. The $ABTS^{\cdot+}$ was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12 h before use. The ABTS$^{\cdot+}$ solution was diluted with deionized water and 95% ethanol (1:1) to an absorbance of 0.70 (±0.02) at 734 nm. Twenty µL of sample was mixed with 3 mL of diluted ABTS$^{\cdot+}$ solution. The decrease of absorbance was measured at 1 min after mixing. Vitamin C (0–15 µg) was used as a standard, and results were reported as mg Vitamin C equivalent per 200 mL of product (one serving size).

Ferric reducing antioxidant power (FRAP) assay

The FRAP, a method for measuring total reducing power of electron donating substances, was assessed according to Benzie and Strain (1999) with some modifications. Briefly, 3 mL of working FRAP reagent (300 mM acetate buffer:20 mM $FeCl_3$:10 mM TPTZ = 10:1:1, daily prepared) was mixed with 40 µL of sample and incubated at 37 °C for 30 min before measuring the absorbance at 593 nm. $FeSO_4$ (0–15 µg) was used as a standard and results were reported as mg $FeSO_4$ equivalent per 200 mL of product (one serving size).

Sensory evaluation

One hundred and sixty staffs of Maejo University, Chiang Mai, Thailand were used as a consumer sample group (118 women and 42 men at the age of 23–60). They were instructed to evaluate the aroma intensity of products and express their preference on aroma, color, flavor and overall liking of products by using 10 cm line scale (Fig. 1; Lawless and Heymann 1998).

Statistical analysis

Results of chemical analysis (3 replications) were analyzed by completely randomized design, while sensory results were analyzed by randomized complete block design. Mean comparisons were done by Tukey's HSD (honestly significant difference) test. Bivariate correlation was applied for the correlation test. Internal preference mapping and cluster analysis were used for identifying the preference direction of consumers from overall liking data. All statistical analysis was done by R version 3.2.1 (R Core Team 2015).

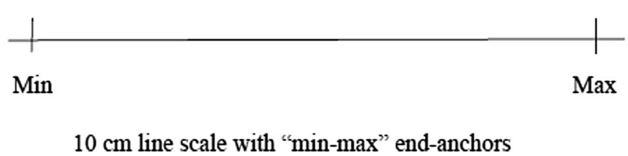

Fig. 1 A 10 cm line scale used for sensory evaluation

Results and discussion

Chemical analysis

Chlorophyll content, total phenolic content and antioxidant activities of five processed grass drinks are shown in Table 1. Their chlorophyll contents were significantly different ($p < 0.05$). Barley grass drink contained more chlorophyll content than grass drinks of Jasmine rice, Sukhothai 2 rice, wheat, and Sukhothai 1 rice, respectively. These chlorophyll contents, 82–958 µg/200 mL (0.06 °Brix) or 0.07–0.8% of solids, were less when compared to those of wheatgrass juice, 70% of solids, which has been used for hemoglobin deficiency treatment (Marwaha et al. 2004; Padalia et al. 2010).

Total phenolic contents were also significantly different ($p < 0.05$). Barley grass drink contained more phenolic compounds than grass drinks of three rice varieties (Sukhothai 2, Sukhothai 1 and Jasmine) and wheat, respectively. Previous studies showed that total phenolic contents of culinary plants were significantly correlated ($p < 0.05$) to their antioxidant activities (Wangcharoen and Morasuk 2007a, b, c), however such correlations were not observed in these five ($p > 0.05$). Grass drinks of Jasmine and Sukhothai 2 rice had significantly higher antioxidant activities, ABTS and FRAP values, ($p < 0.05$) than those of Sukhothai 1 rice, wheat, and barley, respectively. This difference between total phenolic contents and antioxidant activities may be attributed to difference in antioxidant activity in each grass type. For example, apigenin, quercitin and luteolin were reported in wheat grass (Padalia et al. 2010), while ferulic acid was reported in barley grass (Paulíčková et al. 2007), and gallic acid, catechin, rutin, isoquercetin, epicatechin, and epigallocatechin-3-gallate were reported in Sukhothai 1 rice (Phimphilai et al. 2013). The antioxidant potential of phenolic compounds depends on the number and position of hydroxyl groups attached to ring structures of their molecules (Pratt 1992; Rice-Evans et al. 1996).

Antioxidant activities of processed grass drinks, especially of ABTS values was 17.88–35.18 mg Vitamin C equivalent/200 mL (one serving size), it could be considered that these five processed grass drinks had antioxidant potential because one serving size of them had antioxidant activities at least equal to 17.88 mg of Vitamin C or 29.8% of Thai recommended daily intake at 60 mg of Vitamin C (Notification of the Ministry of Publish Health 1998). However, such claim are not permitted.

Table 1 Chlorophyll content, total phenolic content (TPC) and antioxidant activities (ABTS and FRAP) of 5 processed grass drinks (Mean ± SD)

Grass drinks	Chlorophyll (μg/200 mL)	TPC (mg Gallic acid equivalent/200 mL)	Antioxidant activities	
			ABTS (mg Vitamin C equivalent/200 mL)	FRAP (mg $FeSO_4$ equivalent/200 mL)
Rice				
Jasmine	$286^b \pm 8$	$10.50^d \pm 1.06$	$35.18^a \pm 0.74$	$22.62^a \pm 0.62$
Sukhothai 1	$82^e \pm 2$	$18.92^c \pm 0.38$	$25.44^b \pm 0.64$	$16.00^b \pm 0.40$
Sukhothai 2	$128^c \pm 8$	$23.04^b \pm 0.46$	$34.87^a \pm 0.80$	$23.70^a \pm 0.50$
Wheat	$90^d \pm 2$	$5.60^e \pm 0.44$	$22.15^c \pm 0.54$	$10.26^c \pm 0.16$
Barley	$958^a \pm 4$	$26.14^a \pm 0.52$	$17.88^d \pm 0.62$	$5.66^d \pm 0.22$

[a,b,c,d] Means with different letters in the same column are significantly different ($p < 0.05$)

Sensory evaluation

Consumer acceptability results are shown in Table 2. Bivariate analysis showed that overall liking of products was significantly correlated to aroma intensity, preference on aroma and flavor of products ($r = 0.320$, 0.543 and 0.735, respectively, $p < 0.05$). Aroma seemed to be highly influenced to overall liking because any sugar or additives was not used in the drinks. The grass drinks of Jasmine and Sukothai 1 rice were most accepted products ($p < 0.05$), followed by those of Sukhothai 2 rice and wheat, and barley, depending on their aroma intensity.

Internal preference mapping is the construction of a multidimensional product map based on acceptance data. The major source of variation within the preference data was extracted by principal component analysis to create preference dimensions (Greenhoff and MacFie 1994). For overall liking data of five processed grass drinks, 4 principal components (PCs) or preference dimensions were created but the first 2 PCs explained 62.6% variance and were used to create an internal preference map as shown in Fig. 2. Product positions (·) expressed the difference of product acceptance and consumer positions (+) expressed their preference direction.

Cluster analysis is a technique used to identify groups of individuals or objects that are similar to each other but different from individuals in other groups (Norušis 2011). In this study, it could separate all consumer positions on the internal preference map into 3 subgroups, but the member of the third subgroup was too small for further investigation because the minimum number of assessors for acceptance test was recommended to 64 (Wangcharoen et al. 2005) and 70 (Lyon et al. 1992). The first 2 subgroups with 77 and 60 consumers close to the recommended minimum values were examined and two preference directions were found.

The first subgroup (n1 = 77) preferred grass drink of Sukhothai 1 rice than of Jasmine rice, wheat, Sukhothai 2 rice, and barley, while the second subgroup (n2 = 60) preferred grass drinks of Sukhothai 2 and jasmine rice than those of Sukhothai 1 rice, wheat, and barley, as shown in Table 3. The difference of two preference directions showed that there was evidently difference in aroma and flavor between grass drinks of Sukhothai 1 and Sukhothai 2 rice which affected consumer acceptance of both the drinks. Jasmine rice grass drink was accepted by all consumers, while wheat grass drink was neither liked or disliked but barley grass drink was disliked (Tables 2, 3).

Table 2 Sensory evaluation of 5 processed grass drinks (Mean ± SD) by 160 consumers

Grass drinks	Aroma intensity	Acceptance (preference) test			
		Aroma	Color	Flavor	Overall liking
Rice					
Jasmine	$5.63^a \pm 2.28$	$5.79^a \pm 2.18$	$5.61^a \pm 2.31$	$5.51^a \pm 2.30$	$5.90^a \pm 2.08$
Sukhothai 1	$5.53^a \pm 2.36$	$5.86^a \pm 2.26$	$5.61^a \pm 2.36$	$5.63^a \pm 2.35$	$5.87^a \pm 2.31$
Sukhothai 2	$4.58^b \pm 2.20$	$5.04^b \pm 2.38$	$5.89^a \pm 2.22$	$5.18^{ab} \pm 2.51$	$5.25^b \pm 2.34$
Wheat	$4.81^b \pm 2.48$	$5.18^b \pm 2.32$	$5.07^b \pm 2.13$	$4.83^b \pm 2.40$	$4.79^b \pm 2.29$
Barley	$3.89^c \pm 2.56$	$3.08^c \pm 2.24$	$3.08^c \pm 2.24$	$2.84^c \pm 2.15$	$3.00^c \pm 2.20$

0 = Min, 10 = Max

[a,b,c,d] Means with different letters in the same column are significantly different ($p < 0.05$)

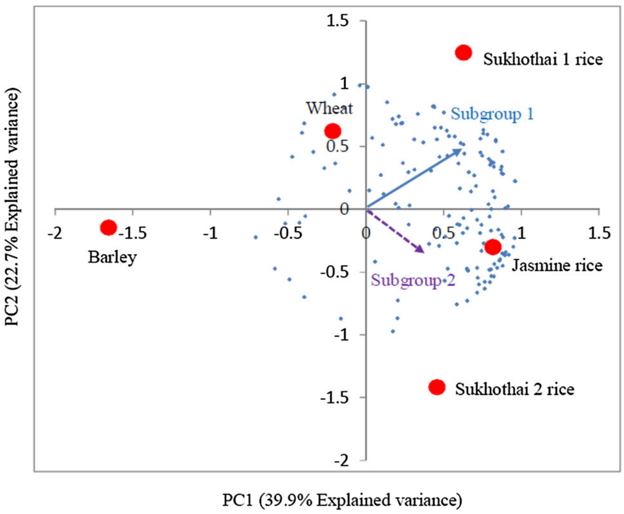

Fig. 2 An internal preference map of 5 processed grass drinks

Table 3 Overall liking of 5 processed grass drinks (Mean ± SD) by 2 subgroups of consumers

	Subgroup 1 (n1 = 77)	Subgroup 2 (n2 = 60)
Rice		
Jasmine	$5.68^{b} \pm 2.03$	$6.58^{a} \pm 1.76$
Sukhothai 1	$7.14^{a} \pm 1.66$	$4.56^{b} \pm 2.03$
Sukhothai 2	$4.07^{c} \pm 1.92$	$7.04^{a} \pm 1.70$
Wheat	$5.35^{b} \pm 2.40$	$4.09^{b} \pm 1.93$
Barley	$2.72^{d} \pm 1.79$	$2.08^{c} \pm 1.43$

0 = Min, 10 = Max

a,b,c,d Means with different letters in the same column are significantly different ($p < 0.05$)

These results explained the similarity of aroma and flavor in jasmine rice and the non similar with wheat, and barley.

Conclusion

This work showed some chemical properties related to health benefits of processed grass drinks. Their chlorophyll contents might be very low when compared to wheatgrass juice but they could be considered as functional drinks with antioxidant potential because of their total phenolic content and other natural antioxidants. Consumer acceptance depended on aroma intensity and preference for aroma and flavor of products. The preference mapping and cluster analysis, the difference in consumer acceptance of grass drinks from Sukhothai 1 and Sukhothai 2 rice was recorded.

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