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Microbiological quality and some bioactive compounds in relation to sensory profiles during germination of brown-purple-pigmented rice

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Abstract

Brown rice contains many nutrients such as carbohydrates, proteins and bioactive compounds such as GABA and antioxidant compounds (e.g. anthocyanins and phenolic compounds). These may increase during grain germination which in turn could affect the sensory attributes. Therefore, the present work was aimed to study the effect of different germination times (24, 36 and 48 h) on changes of the bioactive compounds, the physical parameters in colour, texture properties and sensory attributes of germinated glutinous purple brown rice, Niew Dam (ND-GBR) and germinated non-glutinous purple brown rice, Hom Nil (HN-GBR). The germination of the two cultivars led to the differences in GABA, polyphenolic compound content, and DPPH radical scavenging activity. The ND-GBR yielded higher GABA and polyphenolic compounds than those found in HN-GBR. Moreover, the aerobic mesophilic bacteria and yeasts and mould were found in the early phase of germination, but the lactic acid bacteria (LAB) were found in the late phase of the course. The physical properties including colour, textural properties and sensory characteristics were examined. The cooked HN-GBR was more polished than the cooked ND-GBR with longer time of germination. The samples had positive a^* values and negative b^* values. The cooked ND-GBR was harder but had higher chewiness than the cooked HN-GBR. The result indicated that the germination of rice could improve the softness of the GBR texture. These interactions provided the unique characteristics in sensory profiles. It was found that HN-GBR provided high endurance rice bran and fermented odour. Meanwhile, the deeply purple-coloured, rubbery and gluey-like attributes, oiliness and brittleness are unique attributes for ND-GBR. Furthermore, the stickiness, the steamed banana leaf-like odour and the glossiness and less sweetness were dominantly in longer germinating time of ND-GBR.

Introduction

All along, rice (*Oryza sativa* L.) has been an important staple food for many countries. More than 90% of the world's rice is grown and consumed in Asia, especially in South East Asia (Roohinejad *et al*., 2009; Patil and Khan, 2011). In general, there are two types of rice commonly consumed and commercialised, which are classified by the degree of milling; white rice and brown rice (Lin *et al*., 2015). White rice is a form of rice with complete removal of testa and bran. Therefore, white rice only consists of the starchy endosperm, thus giving them chewiness and easy swelling. Black rice or pigmented-rice is another genotype of rice which has long been known for its health benefit (Ahuja *et al*., 2007; Ujjawal, 2016). However, the hard texture of black rice after cooking leads to its poorer preference as compared to white rice. The testa layer of the rice grain exists in various visible colours after dehulling or outer husk removal. These contain a flavonoid group called anthocyanin pigments and some bioactive compounds (Oki *et al*., 2002; Sutharut and Sudarat, 2012). Moreover, rice bran is a nutritional source of protein, dietary fibre, fat, minerals and vitamins (B_1, B_2, E, C and D) (Ohtsubo *et al*., 2005; Patil and Khan, 2011), as well as the phytochemicals such as tocopherol, tocotrienol, γ-oryzanol, γ-aminobutyric acid (GABA) and ferulic acid (Tian *et al*., 2004; Miller and Engel, 2006; Lai *et al*., 2009).

Brown rice is the rice grain with partial removal of testa and bran. Adversely, brown rice has several

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disadvantages, especially it is not tasty as white rice, which makes them inferior in preference as compared to white rice (Champagne *et al*., 2004). However, brown rice has become a popular alternative among health-conscious individuals. In addition, brown rice can be value added product by the process of germination (Patil and Khan, 2011). Therefore, many researchers have studied brown rice germination and their variety in cultivars, germinating conditions, nutrients and bioactive compounds, and their impacts on health (Komatsuzaki *et al*., 2007; Charoenthaikij *et al*., 2010; Watchararparpaiboon *et al*., 2010). Germination is introduced by steeping the rice in water to hydrate the inactive tissue (Maisont and Narkrugsa, 2010). After that, endogenous metabolism occurs and the storage macronutrients are broken down by different enzymes such as α-amylase and proteases in order to be used in sprouting (Ohtsubo *et al*., 2005; Komatsuzaki *et al*., 2007; Saman *et al*., 2008; Parnsakhorn and Langkapin, 2013).

One compound that enhances the nutritional value of rice is γ-aminobutyric acid (GABA), which is naturally biosynthesised during rice germination. GABA, an amino acid derivative-neurotransmitter, plays important roles in various physiological functions and capability of human to manage stress, including postponement of intelligence degradation, relief of nervous tension, reduction of hypertension, regulation of hepatic cholesterol metabolism, and postponement of the development of cancer cells. GABA is endogenously synthesised from L-glutamate by glutamate decarboxylase (GAD) in our body (Mayer *et al*., 1990; Shiahs and Yatham, 1998; Okada *et al*., 2000; Patil and Khan, 2011). However, GAD activity gradually declines with aging. Therefore, the supplementation of GABA may be necessary to the elderlies. Moreover, other beneficial bioactive compounds have also been discovered in germinated brown rice (GBR), including dietary fibres, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium, zinc, γ-oryzanol, and prolyl-endopeptidase inhibitor (Shoichi, 2004). GBR has several health benefits such as prevention of headache, colon cancer, heart disease, Alzheimer's diseases and Parkinson's like disease, and regulation of blood pressure and blood sugar (Kayahara *et al*., 2000; Chompoopong *et al*., 2016).

The germination of brown rice grain usually occurs in a humid and warm condition, thus undesirable microorganisms would readily proliferate on the outer surface of the brown rice and after the immersion of water (Toyoshima *et al*., 2004; Lu *et al*., 2010). In previous reports, the microorganisms including total mesophilic aerobic bacteria, lactic acid bacteria and *Enterobacteriaceae* have been found during seedling or germination (Simon-Sarkadi and Holzapfel, 1995; Gabriel, 2005; Kim *et al*., 2012). Lactic acid bacteria (LAB) such *as Weissella confusa*, *Pediococcus pentosaceus* and *Lactobacillus fermentum* were predominant during brown rice germination (Kim *et al*., 2012). The microbial contamination may lead to the fermented odour or other unpleasant odour in GBR that could be perceived by consumers and remain in cooked GBR (Bandara *et al*., 1991; Ohtsubo *et al*., 2005). Moreover, this could also threaten the safety level of GBR. Therefore, the microbial contamination during germination on the sensory characteristics of GBR, especially flavour should be elucidated.

Unfortunately however, the scientific data on Thai purple brown rice are less available, especially on rice germination and odour characteristics in relation to microbial changes. Therefore, in the present work, two popular Thai purple brown rice; glutinous purple brown rice (local name Kao Niew Dam, ND), and non-glutinous purple brown rice (local name Kao Hom Nil, HN), were investigated on the effect of germination on their GABA and antioxidant activity as well as microbial contamination. The odour characteristic was analysed by sensory analysis. The data derived from chemical, microbial and sensory analysis were interpreted by a multivariate statistical method, viz principal component analysis (PCA) (Massart *et al*., 1988; Naes *et al*., 1996), along with descriptive analysis for the variations of sensory characteristics of GBR during germination. PCA operation makes it possible to distinguish the samples and also to identify the most important variables in a multivariate data matrix (Gómez-Díaz and Navaza, 2003).

Materials and methods

Rice samples and preparation

The glutinous purple brown rice (local name Kao Niew Dam, ND) (*Oryza sativa* var. *glutinosa*) was obtained from a local market in Borrabeu district, Maha Sarakham, Thailand. The non-glutinous purple brown rice (local name Kao Hom Nil, HN) (*Oryza sativa* L.) was obtained from Chumpea district, Khon Kean, Thailand. The quality of the rice samples was also inspected according to Thai Agricultural Standard (2012).

Chemicals and reagents

Gallic acids, γ-butyric acid (GABA), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (USA).

Acetonitrile, ethanol (HPLC grade), Folin-Ciocalteau's phenol reagents, and sodium carbonate were purchased from Fluka Analytical Co. Ltd. (Germany). Hexane, di-sodium hydrogen phosphate, and phenyl isothiocyanate were purchased from Merck Co. Ltd. (Germany).

Microbiological media

Nutrient broth (NB), plate count agar (PCA), de Man Rogosa and Sharpe (MRS) agar and potato dextrose agar (PDA) were purchased from Himedia (India).

Preparation of germinated brown rice

Germination process was modified from the previous study by Bourneow and Santimalai (2014). Briefly, a batch of brown rice sample (100 g) with 80% germination rate was immersed in 300 mL tap water at ambient temperature (25.0 ± 2.0°C) for 12 h. Every 4 h, the used water was replaced to minimise the fermented odour. After that, the sample was drained off and covered with three layers of humid cloth sheets (Lee *et al*., 2007; Bourneow and Santimalai, 2014). The sample was collected at 24, 36 and 48 h of incubating time in the dark. Finally, the samples were washed with distilled water, blotted on dried cotton cloth sheets, and kept in a plastic box at 4°C until use.

Chemical analysis

Determination of total phenolic content

The determination of total phenolic content was modified from Umnajkitikorn *et al*. (2013). Briefly, 4 g of germinated seeds were ground using mortar and pestle with excessive dry ice to maintain tissues and various sensitive nutrients. After that, 10 mL chilled 80% ethanol was added and mixed before transferring to centrifuge tubes. The supernatant was collected after centrifugation at 10,000 g at 4°C for 20 min. Next, 400 µL supernatant were mixed with 2 mL 10% Folin-Ciocalteu reagent (v/v) for 8 min. Then 1.6 mL 7.5% sodium carbonate (w/v) was added and then incubated for 2 h. The absorbance was measured at 765 nm with a visible spectrophotometer (Model Thermo Spectronic, USA).

Determination of total anthocyanin content

The total anthocyanin content of the samples was determined using the modified pH differential methods of Giusti and Wrolstad (2001). Briefly, 100 µL sample extract was mixed with 5 mL KCl buffer (pH 1.0) and sodium acetate buffer (pH 4.5), separately. The mixture was vigorously vortexed and incubated for 15 min before centrifugation at 10,000 g at 4°C for 20 min. The clear solution was then measured at 515 and 700 nm against distilled water in a UV-visible spectrophotometer. The total anthocyanidin content was then calculated according to Konwatchara *et al*. (2014) by using the molar absorptivity coefficient (ε) of cyaniding-3-glucoside of 26,900.

Determination of γ-amino butyric acid

The extraction and determination of γ-amino butyric acid (GABA) were conducted following the modified method of Ohtsubo *et al*. (2005) and Lee *et al*. (2007). The ground rice sample (2.5 g) was mixed with 25 mL 70% ethanol, and agitated in a vortex mixer for 10 min. The mixture was then centrifuged for 20 min at 12,000 g (4°C). Next, the supernatant was derivatised, and analysed by HPLC. The sample (10 µL) and a gradient mobile phase of 0.05% tri-fluoroacetic acid (TFA), 100% acetonitrile and absolute methanol (1.0 mL/min) was injected into a water symmetry reversed-phase analytical column (C18, 150 × 3.9 mm, 5 µm) at 40°C. The accumulation rate of GABA was also calculated by GABA content (g/Kg) to the germination time (h).

Determination of DPPH radical scavenging activity

The antioxidant activity of the samples was determined by DPPH radical scavenging and hydrogen donating activity following the method of Mohd Esa *et al*. (2013). An aliquot of samples was mixed and serially diluted with 100 mM Tris-HCl buffer (pH 7.4). Then, an equivalent volume of 500 µM DPPH in ethanol was added to the mixture and shaken vigorously. After incubation for 20 min in the dark, the absorbance was measured at 518 nm with a microplate reader. The DPPH radical scavenging activity was then calculated in term of IC_{50} (concentration providing 50% inhibition/scavenging).

Microbiological determination

All the microbial enumeration for GBR samples was conducted following the standard plate count method. Briefly, 0.1 mL aliquot of each diluted sample was spread on plate count agar (PCA). The total viable counts (TVC) were determined after 24 h of incubation at 37°C. The proliferation of lactic acid bacteria (LAB) during the rice germination was also analysed using MRS agar. For yeasts and moulds counts, 0.1 mL of each decimal diluted sample was spread on the potato dextrose agar (PDA) with 10% of tartaric acid. The growth of yeasts and moulds was also observed after the plates were incubated at 37°C

for 3-5 d (Andrews *et al*., 2015).

Analysis of colour values

The colour values of ND-GBR and HN-GBR were determined after steamed cooking in triplicate by a Colour Difference Meter (Model JC801, Tokyo, Japan). The colour meter was calibrated with a standard white plate with L*, *a** and *b** values following the method of Parnsakhorn and Langkapin (2013).

Analysis of textural properties

The textural properties of the cooked rice samples were evaluated by a texture analyser LLOYD (Model LRX plus) using the compression rate with load cell of 500 N and 50 mm/min. A cylinder stainless steel probe of 1.55 cm2 pressure area accompanied by a spherical-shaped stainless-steel plunger of 6.2 mm radius was used to analyse the cooked samples following the method of Reyes Jr and Jindal (1990). Next, 4 g cooked sample was prepared and placed into the sample cell. The running test was performed with the plunger set 1 mm above the cell base, and the distance of moving up and down was measured.

Sensory analysis

The samples were cooked by using 250 g sample with 500 mL drinking water. After that, the cooked rice was presented to 10 trained panellists (18 to 35 years). The panellists were screened and recruited from MSU panellist database, based on their sensory sensitivity and ability to describe sensations perceived from food products. They first participated in discussion sessions for orientation and development of terms. A generic descriptive analysis method (Lawless and Heymann, 1999) was applied. Panellists undertook a 24-hour training programme. A 10-cm unstructured line scale was used in the training sessions to evaluate the sensory intensity.

The panellists were asked to describe the perceived sensations in terms of quality, intensity and time. The development of terms for taste, oral and odour perception was done separately. For the development of organoleptic terms for their perception, the samples were served by using clean plastic cups. Whereas, the development of terms for odour, the samples were presented in covered glass flasks to mask interfering colours and to control the transfer of odorants. The panel generated terms that described sensory attributes as they perceived. The initial list of attributes was revised to clarify and remove subjective, duplicate, or ambiguous terms. The final attributes and terms were agreed and defined by panellists.

After the training, the panellists' performance was tested to determine reliability and validity in all attributes. During the sample evaluation sessions, the panellists individually worked in separate booths and were independently instructed to rate samples by placing marks on the scale to indicate the perceived intensity of the sensory attributes. The temperature in the sensory evaluation room was controlled at 25°C, and the room was free from distracting noises and odours. In taste/odour-related intensity evaluation, 2.5 g sample was served in a glass bottle. Each sample was presented in an aluminium foil-covered glass bottle to mask any influences of colour and to control the transferring of any odorant. The panellists were asked to sniff and hold their breath for 3-5 s. They were required to clear their nasal cavities with soft tissue papers between samples. To evaluate the oral perceived intensity, 2.5 g sample was served in a plastic cup. All samples were presented in the booths with red light to mask any colour interference. The panellists were asked to rate the hotness intensities perceived via mouth. After testing each sample, the panellists were required to rinse their mouths once with water and then wait for 5 min before evaluating the next sample.

Statistical analysis

The analysis of variances was tested for the significant difference at $p < 0.05$ by the Duncan's multiple range tests of the triplicate determinations (n = 3) using SPSS packaging program (version 10.5). The completely randomised design was used to study the chemical and microbiological quality of the samples. The study on the sensory analysis of the samples was designed as randomised complete block. The data obtained from the panel was subjected to analysis of variance (ANOVA). Principal Component Analysis (PCA) was applied to observe the sensory profiling of the ND-GBR and HN-GBR samples. The PCA was applied to present sensory attributes of samples by using SPSS software.

Results and discussion

Changes of GABA and antioxidant activity of GBR during germination

In the present work, the chemical qualities of GBR were represented by GABA, total phenolic compound, total anthocyanins content and DPPH radical activity of the whole rice grains after 24, 48 and 36 h of germination in both cultivars (Table 1).

After soaking in distilled water for 12 h (0 h), GABA content of ND-GBR were significantly higher than those of HN-GBR ($p < 0.05$), which was

Table 1. Effect of germination times on the γamino butyric acid (GABA) content, total phenolic compounds content and the IC_{50} of the DPPH radical scavenging activity of ND-GBR and HN-GBR at various geminating times at ambient temperature.

Sample	Germination time (h)	GABA content (g/Kg)	Total phenolic content (mg GE/100 g)	Total anthocyanin contents (mg /100 g)ns	DPPH, IC_{50} (mg GE/100 g)
ND-GBR	0	0.27 ± 0.05^{ab}	0.57 ± 0.43^{d}	0.20 ± 0.01	0.28 ± 0.01^{c}
	24	1.13 ± 0.23^{d}	0.56 ± 0.19^{cd}	0.22 ± 0.02	0.26 ± 0.02^{c}
	36	1.79 ± 0.15^{e}	0.35 ± 0.04^{abc}	0.19 ± 0.01	0.35 ± 0.03^{d}
	48	2.35 ± 0.07^{f}	0.09 ± 0.01^{a}	0.21 ± 0.02	0.22 ± 0.08^{bc}
HN-GBR	0	0.12 ± 0.01^{a}	0.55 ± 0.04^{cd}	0.20 ± 0.01	0.25 ± 0.15^{bc}
	24	0.29 ± 0.11^{ab}	0.51 ± 0.17^{cd}	0.21 ± 0.02	0.11 ± 0.02^{a}
	36	0.38 ± 0.21^{b}	0.22 ± 0.09^{abc}	0.18 ± 0.02	0.18 ± 0.02^{ab}
	48	0.80 ± 0.11^{c}	0.09 ± 0.02^{a}	0.19 ± 0.01	0.18 ± 0.04^{ab}

ND-GBR: germinated glutinous purple brown rice (Niew Dam). HN-GBR: germinated non-glutinous purple brown rice (Hom Nil). Data are means ± SD. Means with different letters in the same column indicate significant difference at $p < 0.05$. **ns: not significant.

observed throughout the germination (24, 36 and 48 h). GABA accumulation rate was also 3.34 times faster in ND-GBR (0.0435 g/Kg/h) as compared to HN-GBR (0.0128 g/Kg/h). GABA content in both reached maximum at 48 h of germination which was at 2.35 and 0.80 g/Kg for ND-GBR and HN-GBR, respectively. From the results, there was a significant difference in the production and accumulation of GABA by cultivars and germination time ($p < 0.05$). This agrees with a study by Karladee and Suriyong (2012), in which the GABA content in the purple rice was 4-5 times higher in the range of 1.44 – 2.36 g/kg dry matter within 24 h of germination.

After being soaked in distilled water for 12 h (0 h), the total phenolic content of ND-GBR and HN-GBR was 0.57 and 0.55 mg GE/100 g. Subsequently, the phenolic content of both decreased to 0.09 mg/100 g. There was a loss of about 80% of total phenolic content throughout germination. Among those, the total anthocyanin content did not significantly differ and was in the range of 0.18-0.22 mg/100 g in both cultivars. Setyaningsih *et al*. (2015) reported that the level of melatonin and total phenolic of polished black glutinous rice was not as high as that in black glutinous rice. In the present work, the repeated cycle of soaking might affect the total phenolic compounds and anthocyanins by elution effect on the bioactive deposited on the rice bran. The GABA content in rice grains is synthesised from glutamic acid by GAD and the activity of GAD has been shown to have high correlation with the germination ratio that corresponds to germination time. GABA could be increased substantially by soaking rice germ in water during which hydrolytic enzymes are activated. During germination, storage protein is decomposed, changed into transportable amide and conveyed to the growing parts of the rice seedling. Furthermore, the concentration of GABA rises remarkably by several times in response to many diverse stimuli, including, hypoxia, cold shock and darkness.

As shown in Table 1, DPPH radical scavenging assay was used to evaluate the antioxidant activity of both cultivars and was represented by the inhibitory concentration (IC_{50}). ND-GBR had significantly higher IC_{50} of DPPH (0.28-0.22 mg GAE) than those of HN-GBR (0.25 – 0.11 mg GAE) throughout germination, indicating that ND-GBR had lower antioxidant activity as compared to HN-GBR ($p < 0.05$).

The result also showed a decreasing trend on the total phenolic content during germination due to the effect of hydration of the rice during soaking. In addition, the four times of soaking might affect to release and elute some of bioactive compounds from the rice testa and bran. Thus, the phenolic content in the rice grain also decreased. Moreover, the water absorption of the rice grain may also influence on the total phenolic content. In contrast, the antioxidant activity had increased as indicated by the DPPH radical assay. This might be caused by the endogenous synthesis of phenolic compounds and bioactive compounds in the rice germ cells that accumulated for the differentiation of apical and roots (Jannoey *et al*., 2010; Umnajkitikorn *et al*., 2013; Zhang *et al*., 2014; Ding *et al*., 2016; Jirapa *et al*., 2016).

Microbiological changes during rice germination

The microbiological profiles of both samples are shown as total viable counts (TVC) and the content of yeasts and moulds in Table 2. The TVC of both was in the range of 1.44 – 2.76 log CFU/g, which was lower than the total mesophilic aerobic bacteria content in GBR (7.47 log CFU/g) reported by Kim *et al*. (2012). Whereas, the contents of yeast and

moulds were in the range of 1.97-2.87 log CFU/g, which did not significantly differ. The mesophilic aerobic bacteria were in a range of 3.91-6.38 log CFU/g (Lu *et al*., 2010; Kim *et al*., 2012; Park *et al*., 2012). After sprouting at 36 h of mung bean the total bacterial counts had increased from 5.4 to 7.8 log CFU/g (Simon-Sarkadi and Holzapfel, 1995). In addition, LAB did not predominantly grow as in the early phase of germination. The LAB content in ND-GBR was high during the 36 – 48 h of germination with 1.65 – 1.80 log CFU/g. Meanwhile, the LAB in HN-GBR was 1.84 log CFU/g at 48 h of germination. Kim *et al*. (2012) reported that LAB such *as Weissella confusa*, *Pediococcus pentosaceus* and *Lactobacillus fermentum* increased markedly and might play an important role during germination of GBR. Additionally, Anwachkul and Jiamyangyuen (2009) reported that supplement of yogurt with LAB may enhance GABA development in GBR (Munpu rice). Subhasree *et al*. (2013) reported that GBR can be used as an alternative substrate for probiotic food formulation using Lactobacillus spp. The lower microbial content might be due to the germinating condition in the present work. As previously mentioned, the present work was aimed to elucidate and improve the sensory characteristics by the process of germination, especially fermented smell. Therefore, the annealing of hydro-priming was conducted by immersion of the whole rice grain and changed to a new one in every 4 h and soaking for 5 min, thus some contaminated microorganism might be reduced during germination (Bourneow and Santimalai, 2014).

Table 2. The microbiological profiles of ND-GBR and HN-GBR at various germination times at ambient temperature.

Sample	Geminating time (h)	TVC (log CFU/g)	LAB (log CFU/g)^{ns}	Yeasts and Moulds (log CFU/g)^{ns***}
ND-GBR	0	2.16 ± 0.69^{ab}*	ND**	2.12 ± 0.43
	24	2.43 ± 0.60^{ab}	ND	1.97 ± 1.30
	36	2.60 ± 0.32^{ab}	1.80 ± 0.22	2.84 ± 0.08
	48	2.34 ± 0.40^{ab}	1.65 ± 0.10	2.87 ± 0.15
HN-GBR	0	2.76 ± 0.06^{b}	ND	2.78 ± 0.00
	24	1.86 ± 0.74^{ab}	ND	2.00 ± 0.48
	36	2.14 ± 0.64^{ab}	ND	2.23 ± 0.48
	48	1.44 ± 0.37^{a}	1.84 ± 0.28	2.78 ± 0.00

ND-GBR: germinated glutinous purple brown rice (Niew Dam). HN-GBR: germinated non-glutinous purple brown rice (Hom Nil). Data are means ± SD. Means with different letters in the same column indicate significant difference at $p < 0.05$. **ND: not detected, including less than the detection limit (<30 CFU/g). ***ns: not significant.

Table 3. Physical characteristics of the cooked germinated brown rice of the purple rice and black sticky rice at various germinating times.

Physical characteristic	ND-GBR Germination time			HN-GBR Germination time		
	24 h	36 h	48 h	24 h	36 h	48 h
Colour						
L*	15.19 ± 0.52 ^{a}	16.32 ± 0.81^{a}	18.02 ± 0.59 ^{b}	16.46 ± 0.60 ^{a}	18.31 ± 0.27 ^{b}	19.11 ± 0.53 ^{c}
a^{*ns}	5.26 ± 0.87	5.33 ± 0.91	4.59 ± 0.65	6.84 ± 1.15	6.21 ± 0.40	5.12 ± 0.55
b^{*ns}	-2.01 ± 1.22	-1.80 ± 1.54	-1.21 ± 1.01	-1.02 ± 1.24	-1.04 ± 1.23	-0.98 ± 1.04
Texture						
Hardness (N)	24.29 ± 0.98 ^{a}	20.53 ± 0.35^{b}	20.17 ± 0.26 ^{b}	19.21 ± 0.83^{bc}	18.08 ± 0.22 ^{c}	18.17 ± 0.71^{c}
Adhesiveness (g”sec) ^{ns}	382.27 ± 187.55	421.52 ± 392.42	302.42 ± 221.60	204.67 ± 187.61	212.54 ± 190.88	281.92 ± 202.52
Chewiness	3.41 ± 0.70 ^{a}	3.22 ± 0.11^{a}	3.01 ± 1.12^{a}	2.20 ± 0.6 ^{ab}	1.51 ± 0.56 ^{b}	1.87 ± 0.38 ^{b}
Gumminess ^{ns}	5.18 ± 2.54	5.20 ± 1.18	5.01 ± 2.24	3.26 ± 1.28	3.33 ± 2.27	3.98 ± 1.61
Springiness ^{ns}	0.16 ± 0.42	0.18 ± 0.32	0.08 ± 0.58	0.15 ± 0.33	0.13 ± 0.41	0.06 ± 0.20
Cohesiveness ^{ns}	0.07 ± 1.15	0.06 ± 1.81	0.02 ± 0.95	0.08 ± 0.92	0.12 ± 0.53	0.07 ± 0.12

ND-GBR: germinated glutinous purple brown rice (Niew Dam). HN-GBR: germinated non-glutinous purple brown rice (Hom Nil). Data are means ± SD. Means with different letters in the same row indicate significant difference at $p < 0.05$. ns = not significant.

Physical characteristics

Colour values

The colour parameters (L*, *a**, *b**) of the cooked rice samples are shown in Table 3. The whiteness (L*) values of the cooked HN-GBR was higher than those observed in the cooked ND-GBR with time of germination. The samples were positive in *a** values (4.59 to 6.84) and negative in *b** values (-0.98 to -2.01) which indicated their purple-pigmented rice colour. The polish appearance of the cooked rice after cooked by steaming might provide different colour due to the difference in variety of rice cultivar. Indigenous pigments such anthocyanins, carotene, and derivatives make them unique in colour. Moreover, the amylose and amylopectin ratio may also be a major cause of some characteristics such as opacity, glossiness, and chroma. During germination, it was found that the L* values increased with time. This might be caused by the partial loosing of pigments on the grain surface and the enzymatic hydrolysis inside the grains.

Textural properties

The cooked samples of ND-GBR and HN-GBR were examined after equilibration at room temperature for 30 min. The cooked ND-GBR had the hardness in a range of 20.17-24.29 N that was significantly higher than those found in the cooked HN-GBR (18.17-19.21 N). However, the germination process showed improving on softening to the both as shown in Table 3. The chewiness values of cooked ND-GBR (3.01-3.41) were higher than those of cooked HN-GBR (1.51-2.20). Whereas, the other physical characteristics including adhesiveness, gumminess, cohesiveness and springiness did not significantly differ between the cooked GBR of two cultivars. Generally, the cooked white rice was softer than cooked brown rice, however, the germination or malting process of rice could mprove their textural properties. Sirirombon *et al*. (2017) reported that the germination causes a decrease in the amount of amylose content, as a result of amylolytic activity that alters their textural properties. Moongngarm (2010) reported that the germination process could change the packed starch granules to rough and eroded shapes. In addition, the endogenous nutrients and active compounds such as fatty acids, amino acids, proteins, anthocyanin, amylose and amylopectin in the purple-pigmented rice may also affect to the rice texture.

Sensory characteristics

The sensory lexicon was developed by 10 panellists including attribute descriptive and defined specific terms (Table 4). The sensory rating scores of individual samples for deep purple-colour, glossiness, rice bran odour, fermented odour, rubbery and gluey, brittleness, stickiness, oiliness, sweetness and steamed banana leaf-like odour were defined and elucidated in the present work. A one-way ANOVA test was performed to determine whether any significant difference occurred in the rice verities and germination time (Table 5). There was no significant difference in all samples for sweetness. PCA illustrates an overview of sensory characteristics of all samples.

Table 4. The specific terms of the consensus perception on the characteristics of the cooked geminated brown rice defined by trained panellists (n = 10).

Characteristic	Description
Colour	
Purple colour	The visible deep purple-magenta colour of the cooked rice
Attributes	
Glossiness	The visible glossy of the reflect light on the surface of the cooked rice
Odour	
Fermented odour	The sour smell of the cook rice by smelling
Rice bran odour	The odour of the cooked rice with the rice bran odour
Steamed banana leaf-like odour	The odour of the cooked rice with the steamed banana leaves like odour by smelling
Texture	
Rubbery and gluey	The cooked rice with rubber like texture with adhesion like teeth pull force
Brittleness	Brittle of the cooked rice after crushing
Stickiness	Adhesion of the cooked rice to the teeth
Perception and taste	
Oiliness	The residual of the cooked rice taste after crushing detected on the tongue
Sweetness	Oral perception of the cooked rice by the sweetness

Table 5. The sensory intensity of the consensus perception on the cooked germinated brown rice of the purple rice and black sticky rice at various germinating times.

Sensory characteristic	ND-GBR Germination time			HN-GBR Germination time		
	24 h	36 h	48 h	24 h	36 h	48 h
Deep purpled colour	5.57 ± 2.50^{b}	6.84 ± 1.98^{c}	8.14 ± 0.91^{d}	2.60 ± 1.85^{a}	3.31 ± 2.41^{a}	3.53 ± 2.50^{a}
Glossiness	6.72 ± 1.99^{b}	5.53 ± 2.65^{b}	3.45 ± 2.46^{a}	4.06 ± 1.19^{a}	5.64 ± 1.56^{b}	6.14 ± 2.29^{b}
Rice bran odour	4.40 ± 2.72^{abc}	3.36 ± 2.13^{a}	3.39 ± 1.81^{a}	5.21 ± 2.36^{c}	4.88 ± 2.38^{bc}	3.66 ± 2.63^{ab}
Fermented odour	3.31 ± 2.30^{ab}	2.82 ± 1.67^{a}	5.00 ± 3.03^{cd}	4.24 ± 1.86^{bc}	5.57 ± 2.88^{de}	6.36 ± 2.32^{e}
Oiliness	4.43 ± 2.06^{b}	5.84 ± 2.69^{c}	5.80 ± 1.82^{c}	3.50 ± 1.54^{ab}	3.34 ± 2.52^{ab}	2.64 ± 1.49^{a}
Sweetness^{ns}	2.95 ± 1.69	2.90 ± 2.47	4.30 ± 2.00	3.10 ± 2.18	3.54 ± 2.56	3.32 ± 2.39
Rubbery	4.21 ± 1.52^{bc}	5.18 ± 1.55^{cd}	5.76 ± 2.10^{d}	3.85 ± 2.01^{ab}	2.97 ± 2.15^{a}	3.30 ± 2.57^{ab}
Brittleness	4.38 ± 1.71^{b}	5.80 ± 1.95^{c}	6.07 ± 2.50^{c}	3.83 ± 2.24^{ab}	3.03 ± 2.01^{a}	2.77 ± 2.59^{a}
Stickiness	5.67 ± 2.07^{b}	6.07 ± 2.50^{b}	3.50 ± 2.78^{a}	5.48 ± 1.90^{b}	5.51 ± 2.62^{b}	6.31 ± 2.33^{b}
Steamed banana leaf-like odour	4.22 ± 2.87^{c}	4.83 ± 2.73^{c}	2.43 ± 1.16^{a}	3.86 ± 2.60^{bc}	2.77 ± 2.00^{ab}	4.07 ± 2.60^{c}

ND-GBR: germinated glutinous purple brown rice (Niew Dam). HN-GBR: germinated non-glutinous purple brown rice (Hom Nil). Data are means ± SD. Means with different letters in the same row indicate significant difference at $p < 0.05$. ns = not significant.

The bi-plots of quality values-products PCA are shown in Figures 1a-d. The first two components of physical properties (Figures 1a-b) showed 79.94% variance with 50.80% of PCs1-1 and 29.15% of PCs2-1. The results suggested that the stickiness was highly correlated to the gumminess and springiness with linear correlation (r) of 0.81 and 0.77 ($p < 0.05$), respectively. Moreover, the deep purple colour was positively correlated to the b^* value ($r = 0.73$, $p < 0.05$), whereas, the cohesiveness was negatively correlated to the oiliness ($r = -0.86$, $p < 0.05$). Another two components (PCs1-2) explained 84.55% of the variance in the data set, which means the sensory characteristics can be mostly viewed by looking at the plots of PCs1-2. PC1 shows the deep purple colour, rubbery and gluey, oily and brittle attributes

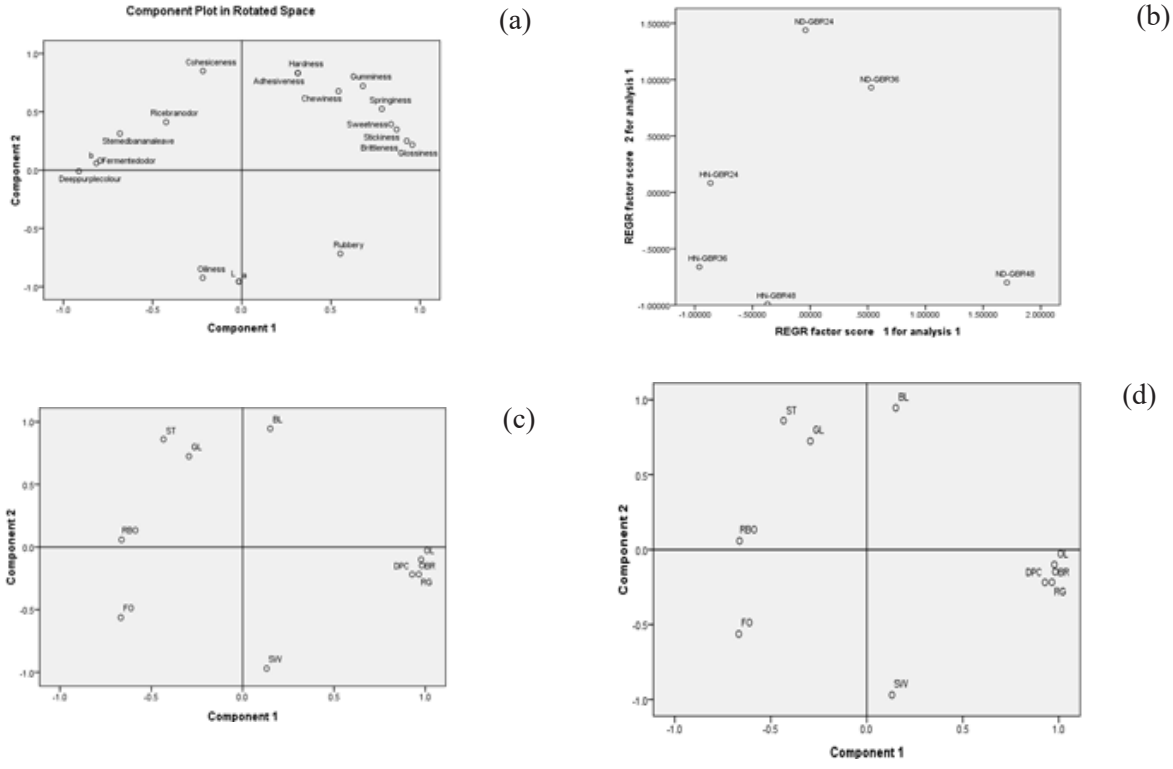

Figure 1. PCA bi-plots on physical properties (a-b) and sensory characteristics (c-d) of brown-purple-pigmented rice samples during germination. DPC = purple-coloured, GL = glossiness, RBO = rice bran odour, FO = fermented odour, RG = rubbery and gluey, BR = brittleness, ST = stickiness, OL = oiliness, SW = sweetness

in positive side, and rice bran odour and fermented odour in negative side with accounts for 53.46% of the variation. Whereas, PC2 shows the steamed banana leaf-like odour, stickiness and glossiness in positive side, and sweetness in negative side with accounts for 31.09% of the variation. The interpretation of each PC gives an overview of sensory profiles of the samples. The PCA characterises the cooked HN-GBR samples and gave high rice bran and fermented odour and less deep purple colour, rubbery and gluiness, oiliness and brittleness. On the other hand, the deep purple coloured, rubbery and gluey-like attributes, oiliness and brittleness are unique attributes for ND-GBR samples. The cooked ND-GBR obtained from 24 and 36 h of germination was described to have high stickiness, steamed banana leaf-like odour and glossiness and less sweetness.

There is lack of reported information on the effect of germination on sensory profiles of GBR. Konwatchara and Ahromrit (2014) reported that the thermal treatment leads to the losses of antioxidant capacity of the GBR. Cooking by pressure might enhance the GABA and γ-oryzanol stability. Nevertheless, germination might improve pasting property of some purple pigmented rice by starch hydrolysis activity (Panchan and Naivikul, 2009). Therefore, the germination process could improve the physicochemical properties and microbial profiles, thus also impact on the sensory characteristics.

Conclusion

In the present work, it was demonstrated that the germination of the two cultivars (ND-GBR and HN-GBR) yielded differences in GABA, polyphenolic compound content, and DPPH radical scavenging activity. The ND-GBR yielded higher GABA and polyphenolic compounds than those found in HN-GBR. But, the HN-GBR yielded higher DPPH radical scavenging activity. Aerobic mesophilic bacteria and yeasts and moulds were found throughout germination. Meanwhile, LAB was found after 36 h of germination. The whiteness (L*) values of the cooked HN-GBR was higher than those observed in the cooked ND-GBR with longer time of germination. The samples had positive *a** values and negative *b** values which indicated their unique characteristics of the purple-pigmented rice. The cooked ND-GBR was harder than the cooked HN-GBR. The chewiness values of cooked ND-GBR were higher than those of cooked HN-GBR. The results indicated that the germination process of rice could improve their textural properties. These interactions provided the unique characteristics in sensory profiles that could be characterised in odour as steamed banana leaf-like odour, texture as rubbery and gluey state, brittleness, stickiness, oiliness and sweetness. Through the sensory profiles by PCA, it was found that HN-GBR provided high endurance of rice bran and fermented odour. On the other hand, the deeply purple-coloured, rubbery and gluey-like attributes, oiliness and brittleness were unique attributes for ND-GBR. Furthermore, the stickiness, the steamed banana leaf-like odour and the glossiness and less sweetness were dominantly in longer germinating time of ND-GBR. These were the result of microbial activity and the endogenous biological changes during rice germination.

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