

Some physical characteristics and bioactive compounds of young flattened rice (Khao-Mao)

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Abstract

Young flattened rice (Khao-Mao) is made from young glutinous rice. Khao Mao is consumed as an indigenous rice dessert in several Southeast Asian countries such as Thailand, Laos, and Vietnam. Khao Mao samples obtained from Trakan Phuet Phon district (KM1) and Phibun Mangsahang district (KM2) in Ubonratchathani province were investigated to determine their physicochemical properties and bioactive compounds. No difference was found in the physicochemical properties of KM1 and KM2. γ -Oryzanol content was higher in KM2 (cycloartenylferulate 13.01 mg/100 g, 24-methylenecycloartanylferulate 21.50 mg/100 g, campesterylferulate 33.11 mg/100 g, sitosterylferulate 45.27 mg/100 g and total γ -Oryzanol 112.80 mg/100 g) than those of KM1 (cycloartenylferulate 6.11 mg/100 g, 24-methylenecycloartanylferulate 9.99 mg/100 g, campesterylferulate 15.86 mg/100 g, sitosterylferulate 22.95 mg/100 g and total γ -Oryzanol 55.65 mg/100 g). KM1 had higher γ aminobutyric acid (GABA) content (7.71 mg/100 g) than that of KM2 (4.07 mg/100 g). The results could serve as baseline information for the improvement of the preparation method and for further product development of Khao Mao.

Keywords

Rice

bioactive compound

γ -oryzanol

GABA

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Introduction

Rice (*Oryza sativa*) is a semi-aquatic, annual grass which can be grown under tropical or subtropical condition. It is one of the leading food crops and is the major staple food for 60% of the world's population (Marshall and Wadsworth, 1994). In 2010, FAO reported that the approximate world rice paddy production and area harvested were 672 million tons and 153 million hectare, respectively (FAOSTAT, 2010). In the same year, the Thai Office of Agricultural Economics estimated the quantity and value of rice exports as 8,939,630 metric tons and 168,193 million baths, respectively (Economics, 2012).

One popular rice product is Khao Mao made from young flattened glutinous rice. In Thailand, "Khao" means rice, and "Mao" means immature grains, which are formed between 13th and 19th day after anthesis (DAA), depending on the variety. To produce Khao Mao the young grains are soaked in water for several hours, steamed for about 25 min in bamboo pot (Huat) then roasted in medium heat in a large wok while being continuously stirred for approximately one h and 20 min. The roasted grains are left to cool down then pounded in a manually operated or motorized

mortar. The flattened grains are then separated from broken husk and fine bran by sieving and winnowing. Pounding, sieving and winnowing may be repeated a few more times to obtain clean Khao Mao. Khao Mao mixed with sugar, salt and shredded coconut is a traditional rice dessert in some Southeast Asian countries such as Thailand, Laos and Vietnam. RD6 cultivar of glutinous rice, widely grown in Northern and Northeastern Thailand, is the main cultivar used for making Khao Mao. Physical characteristics of rice, attributed mainly to chemical components of the grains, are important in the processing, cooking and eating qualities of the product (Hamaker, 1994). Rapid Visco Analyser (RVA) can measure viscosity versus temperature and time, indicating thickening ability and gelatinization of rice starch, which are important physicochemical properties when cooking and eating quality of rice is evaluated (Wang 2010). In addition, Differential Scanning Calorimeter (DSC) can determine gelatinization temperature, the amount of energy to gelatinize starch and the extent to which it gelatinizes. There are many factors that influence the physicochemical properties of rice starch, such as cultivars (Wang *et al.*, 2002; Singh, 2005; Yoon *et al.*, 2009), starch composition and structure (Ibáñez *et al.*, 2007), processing method and storage condition

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(Yu *et al.*, 2012).

Rice is a rich source of bioactive compounds, which include γ -oryzanol (steryl ferulates) and γ -Aminobutyric acid (GABA). γ -oryzanol, which esterifies tran-ferulic acid and sterols or triterpenols, is well known as the main bioactive compound in rice. The four major γ -oryzanol constituents in rice are cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, campesterol ferulate and sitosterol ferulate (Rogers *et al.*, 1993). Several studies have reported health benefits of γ -oryzanol, such as its antioxidant activity, improvement of plasma lipid pattern, reduction of total plasma cholesterol and increase of HDL cholesterol levels, and inhibition of the platelet aggregation (Cicero and Gaddi, 2001). Gamma aminobutyric acid (GABA) is a non-protein amino acid compound that is synthesized from glutamic acid by glutamate decarboxylase (GAD). GABA is a neurotransmitter in the brain and spinal cord of mammals (Banchuen *et al.*, 2009). It can regulate blood pressure, heart rate, sensations of pain and anxiety, lipid levels in serum and assist in insulin secretion to prevent diabetes. In addition to rice grain, several studies on bioactive compounds of germinated rice have also been reported (Varayanond *et al.*, 2005; Lin and Lai, 2011).

Many studies have been done on the physicochemical characteristics and bioactive compounds of glutinous rice and germinated rice, but there have not been any report on those of Khao Mao. Therefore, the objective of this research is to investigate some of these physicochemical and chemical properties of the product. Khao Mao samples from Phibun Mangsahang district and Trakan Phuet Phon district, Ubonratchathani province, Thailand, were used in the studies.

Materials and Methods

Young glutinous rice and young flattened rice (Khao Mao) samples

Young glutinous rice (*Oryza sativa* L.) cv. RD6 and Khao Mao samples made from the same rice were purchased from Phibun Mangsahang district and Trakan Phuet Phon district, Ubonratchathani province, Thailand. The preparation method for Khao Mao is shown in Figure 1. The young RD6 grains were soaked in tap water for several hours. After soaking, the grains were first steamed in a bamboo basket (*Huat*) for Khao Mao from Trakan Phuet Phon district (KM1), or were roasted right away for Khao Mao from Phibun Mangsahang district (KM2). The grains were roasted in medium heat for 2 hr, left to cool then pounded in a manual or motorized

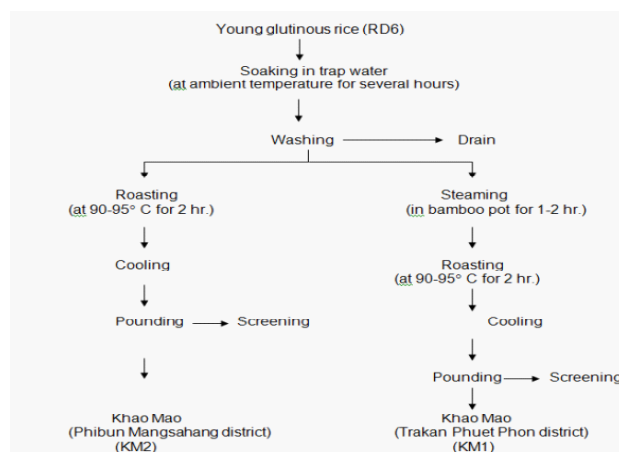


Figure 1. Schematic diagram of the preparation of Khao Mao samples from Trakan Phuet district (KM1) and Phibun Mangsahang district

mortar. After pounding, the mixture is sifted through a bamboo sieve and winnowed with a bamboo tray to separate Khao Mao from the husk and fine bran. Young glutinous rice grain and Khao Mao samples were packed in plastic bags, sealed, and kept at 4°C or ambient temperature until analysis.

Chemicals

All chemicals used in the analyses and the determination of chemical composition and bioactive compounds of young glutinous rice and Khao-Mao samples were of analytical grade.

Determination of physicochemical properties

Moisture and Protein: Moisture and protein contents of Khao Mao samples were determined by AOAC standard method (A.O.A.C, 1999). The factor of 6.25 was used for the conversion of nitrogen to protein.

Colour: Colour of Khao Mao samples was determined using CIE colour system (Hunter, Colour Flex, USA). Colour values: L^* (brightness), a^* (redness-greenness) and b^* (yellowness-blueness) were measured using white and black standards to calibrate the colourimeter before measurements. The measurements were performed in triplicate for each sample.

Water activity (a_w): Water activity of the samples was determined using the Novasina water activity meter (Thermoconstanter Novasina, Switzerland). The samples, placed into three individual containers for the three separate chambers were allowed to equilibrate for 25–30 min before reading. Readings were taken after the values stabilized.

Thermal denaturation behavior: Pulverized grain and Khao Mao samples were heated from 30 to 120°C at 10°C/min heating rate in differential scanning calorimeter (DSC) (Mettler Toledo, Model DSC1,

USA). Samples (4 mg) were placed in aluminum pans, and 10 μ l of distilled water was added. An aluminum pan filled with 10 μ l of distilled water was used as reference. Denaturation temperature (T_d) and heat of transition or denaturation enthalpy (ΔH) were calculated using STAR^e SW 9.10 program.

Pasting properties: The changes in the apparent viscosity behavior of the Khao Mao and young glutinous rice samples were determined using Rapid Visco Analyzer (RVA) (model:RVA-4S/N 2001102, Newport Scientific, Australia). The samples (3 g) was added to distilled water (25 mL) and placed in the RVA. The suspension was kept at 50°C (the speed rate was 920 rpm for 10 s. and then 160 rpm for 50 s), heated to 95°C and kept at 95°C for 2.7 min, then cooled to 50°C at 12°C/min and kept at 50°C for 2 min. The RVA experiments were performed in triplicates. The pasting profiles were analyzed using ThermoLine for Window for RVA4. Paste viscosity, plotted in arbitrary RVA units (RVU) versus time, was used to determine the peak viscosity (PV), trough viscosity, final viscosity (FV), breakdown viscosity (BKD = PV- trough), and setback viscosity (SB = FV- trough).

Determination of bioactive compounds

γ -Oryzanol: γ -Oryzanol content was determined using the method of Chen and Bergman (2005) with slight modification (Chen and Bergman, 2005). Khao Mao samples (0.05-0.1 g) were extracted in 3 mL of methanol HPLC grade. The mixtures were shaken using a vortex for 1 min. After the extraction, the samples were centrifuged for 10 min at 825xg. The supernatants were collected by filtering and the residues were extracted two more times and 50 μ L of the combined samples were injected into the Agilent HPLC (1200 Series, Japan), with Agilent Eclipse XDB-C18 column, 4.6x150 mm, 5 μ m. The HPLC was equipped with a UV-Vis photodiode array detector set at 330 nm wavelength. The mobile phases were methanol: acetonitrile: dichloromethane: acetic acid (50:44:3:3) operated at ambient temperature, and the flow rate of 1.5 mL/min. γ -Oryzanol was used as standard for calibration. The 4 components of γ -oryzanol were identified as cycloartenylferulate, 24-methylenecycloartanylferulate, campesterylferulate and sitosterylferulate.

Gamma-aminobutyric acid (GABA): GABA content was determined with the method of Varayanond *et al.* (2005) with slight modifications (Varayanond *et al.*, 2005). One-fifth to one-half gram (0.2-0.5 g) of ground Khao Mao samples were weighed in plastic tubes and 1.8 mL of deionized water was added and the slurries were shaken at room

temperature for 1.5 hours. 200 μ L of 3% (by volume) sulfosalicylic acid was added and the mixtures were centrifuged at 4500xg for 10 min. To 50 μ L of the supernatants were added 50 μ L of 100 mM NaHCO₃ and 50 μ L of 4 mM 4-dimethylaminoazobenzene-4-sulfonyl chloride acetonitrile solutions. The mixtures were heated to 70°C for 10 min to effect derivatization. After the derivatization, the samples were added 250 μ L of absolute ethanol and 250 μ L of 25 mM phosphate buffer (pH 6.8). The samples were then filtered and 5 μ L of the filtrate were injected into Agilent HPLC (1200 Series, Japan), with Supelcosil LC-DABS column, 4.6x150 mm, 3 μ m (Supelco, Bellefonte, PA). The HPLC was equipped with a UV-Vis photodiode array detector set at 465 nm wavelength. The mobile phases were 25 mM acetate buffer and acetonitrile (65:35) operated at the flow rate of 0.5 mL/min, and 55°C. Pure GABA was used as standard for calibration.

Statistical analysis

All analyses and measurements were performed in at least triplicates and the results are expressed as mean and standard deviation. Mean values of physicochemical properties (except thermal properties) and bioactive compounds were compared, using t-test. For thermal properties, a completely randomized design was used for the experiments. The data were analyzed using analysis of variance (ANOVA) and the differences between means were determined using Duncan's new multiple range test. Statistical analysis of the data was performed, using SPSS version 11.5 program.

Results and Discussion

Physicochemical properties of Khao Mao

Moisture and protein: Moisture and protein contents of Khao Mao samples from both locations (KM1 and KM2) were in the range of 22.11-22.92% and 6.24-6.64% wb, respectively (Table 1). This range of moisture may cause microbial problems during storage. Protein content of Khao Mao was similar to that of in glutinous rice (*Oryza sativa* L.) cv. RD6 (6.90%) (Kongseree, 1979).

Colour: Colour of KM2 was darker, more red and more yellow than those of KM1 as indicated by lower L*, a* and b* values (Table 1). Colour formation in Khao Mao was influenced by various processing steps, especially roasting. The longer the roasting time and the higher the roasting temperature the more browning substances developed, from Maillard reactions between reducing sugar and amide.

Water activity (a_w): Khao Mao samples had a_w of 0.97 (Table 1), which indicates high amount of

Table 1. Physicochemical properties of Khao Mao from Trakan Phuet Phon district (KM1) and Phibun Mangsahan district (KM2)

Physicochemical properties	KM1	KM2
Moisture (%)	22.11 ± 0.13	22.92 ± 0.91
Protein (%)	6.64 ± 0.17	6.24 ± 0.06
L*	55.19 ± 0.24	50.27 ± 0.06
a*	0.84 ± 0.08	0.41 ± 0.14
b*	32.31 ± 0.19	27.22 ± 0.41
Water Activity (a _w)	0.97 ± 0.01	0.97 ± 0.05

moisture being available for microbial growth and enzyme activity. In general, almost all microbial activities are inhibited below a_w 0.6, most fungi below 0.7, most yeast below 0.8 and most bacteria below 0.9 (Fellow, 2000). At a_w of 0.97, Khao Mao would support all microbial growth if stored at room temperature. Thus, the moisture content and a_w can serve as useful guides in designing the packaging and storage methods for the product.

Thermal properties: Thermal properties of KM1 and KM2, in comparison to young glutinous rice grains (RD6) are shown in Table 2. There were no significant differences in gelatinization temperatures, which consist of onset temperature (To) (65.58-67.79°C), peak temperature (Tp) (87.25-87.80°C) and end temperature (Te) (102.13-103.92°C), between KM1 and KM2. Compared to those of young glutinous rice, Khao Mao had significantly higher gelatinization temperatures. This suggested that Khao Mao requires high temperature for gelatinization (from 65.58 to 103.92°C), indicating that the products are more heat stable than the grains. These may be due to partial melting of the product followed by recrystallization during cooling, thus delaying the onset of gelatinization (and hence the Tp) of all the Khao Mao samples. There was also significant difference (p < 0.05) between the two Khao Mao samples and the young grains in their enthalpy (ΔH). This was most likely due to the heat treatments during Khao Mao preparation. KM2 was prepared by roasting only, while KM1 was prepared by both steaming and roasting. Normally, gelatinization is the process that takes place when starch containing water is heated, resulting in the irreversible disruption of molecular order within the starch granule. The loss of order results in irreversible granule swelling and of birefringence, and loss of crystallinity. For gelatinization to occur the regions of amorphous starch must melt and undergo glass transition (Bao and Bergman, 2004). Therefore, some physicochemical characteristics of Khao Mao can be attributed to these changes which take place during its processing.

Pasting properties: Pasting properties of Khao

Table 2. Thermal properties of Khao Mao from Khao Mao from Trakan Phuet Phon district (KM1), Phibun Mangsahan district (KM2) in comparison with young glutinous rice (RD6)

Samples	Onset Temp. (°C)	Peak Temp. (°C)	End Temp. (°C)	Enthalpy (ΔH) (J/g)
Young glutinous rice (RD6)	61.01b ± 0.20	71.35b ± 0.34	78.07b ± 0.41	4.13b ± 0.50
KM1	67.79a ± 2.56	87.25a ± 4.45	102.13a ± 0.42	1.27c ± 0.15
KM2	65.58a ± 4.25	87.80a ± 0.11	103.92a ± 1.73	6.57a ± 0.06

Mean values followed by the same letter in the same column are not significantly different at 5% significance level by Duncan's multiple range tests.

Mao samples are shown in Table 3. KM2 had higher peak viscosity (1638.33 cp), trough viscosity (1382.66 cp), break down (255.66 cp), final viscosity (1671.66 cp) and setback viscosity (289.00 cp) than KM1. The samples from the two sources showed no significant difference in pasting time (5.13-5.73 min) and pasting temperature (51.62-55.13°C). Difference in pasting properties of Khao Mao from the two sources could probably be due to different rice starch composition such as amylose, protein and lipid in the young grains. This difference suggests that protein and lipid may influence the pasting properties through the interaction between lipid, protein and starch during processing. Several studies have reported the formation of a complex between protein, lipid and amylose (Dautant *et al.*, 2007; Kang *et al.*, 2011; Kaur and Singh, 2000).

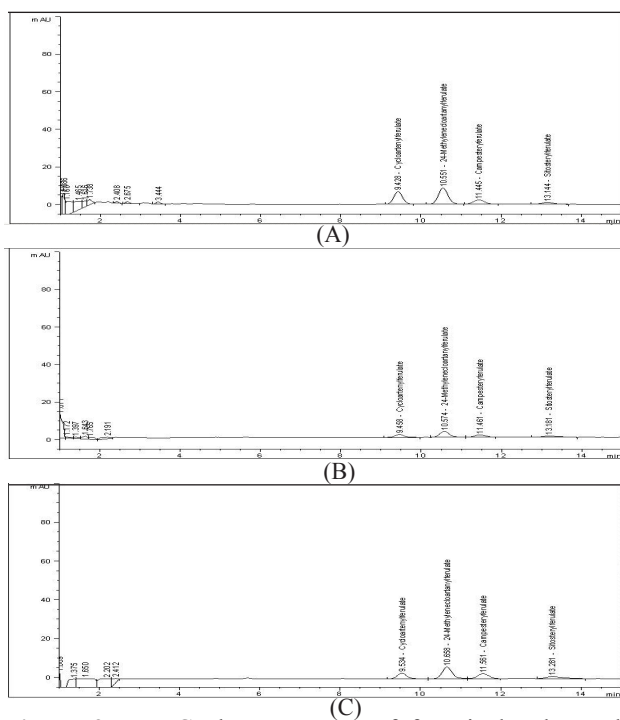
Table 3. Pasting properties of Khao Mao from Trakan Phuet Phon district (KM1) and Phibun Mangsahan district (KM2)

Pasting properties	KM1	KM2
Peak Viscosity (cp)	1440.66 ± 110.56	1638.33 ± 53.89
Trough viscosity (cp)	1316.00 ± 85.55	1382.66 ± 45.96
Break down (cp)	124.66 ± 42.45	255.66 ± 50.14
Final viscosity (cp)	1509.33 ± 90.71	1671.66 ± 39.55
Set Back (cp)	193.33 ± 4.16	289.00 ± 12.12
Peak Time (min)	5.73 ± 0.29	5.13 ± 0.23
Pasting Temp (°C)	55.13 ± 3.22	51.61 ± 1.80

Bioactive compounds of Khao Mao

γ-Oryzanol: The γ-oryzanol fractions of Khao Mao samples were determined using HPLC and four isolated steryl ferulates were used as standards. The four peaks of Khao Mao samples on HPLC chromatogram were identified as cycloartenylferulate, 24-methylenecycloartenylferulate, campesterylferulate and sitosterolferulate (Figure 2). The external calibration curve of each compound at the concentration range of 1-40 μg produced a good linear correlation (r² = 0.999). The individual γ-oryzanol contents in Khao Mao samples were calculated by comparing the slopes of the standards. The results are shown in Table 4. The contents (mg/100 g) of the four components and total γ-oryzanol in KM2 were

higher than those of KM1. The contents (mg/100 g) of the four components in KM2 were as follows: sitosterylferulate (45.27) > campesterylferulate (33.11) > 24-methylenecycloartanylferulate (21.50) > cycloartenylferulate (13.01). Those in KM1 were as follows: sitosterylferulate (22.95) > campesterylferulate (15.86) > 24-methylenecycloartanylferulate (9.99) > cycloartenylferulate (6.11). Total γ -oryzanol contents of KM2 and KM1 were 112.80 mg/100 g and 55.65 mg/100 g, respectively. The results indicate that KM2 had higher total γ -oryzanols than KM1. Factors such as cultivar, growing location, and environment can affect these components, but in this case it is most likely that heat treatment of the grains during processing was the major factor. KM1 had two heating steps, i.e. steaming and roasting, while KM2 only went through roasting. Thus, KM2 retained greater amount of these bioactive compounds.



cereal. It appears to be an indigenous product that has excellent commercial potential.

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