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Proximate Composition and Antioxidant Activity of Young Flattened Rice (Khao-Mao)

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Abstract

Khao-Mao producer group in Thailand's Nakhon Phanom province's Phon Sawan district, Na Hua Bo sub-district is a community that has produced Khao-Mao according to conventional wisdom, for a very long time. Khao-Mao has an emerald, green color, fragrant, delicate texture, and delicious taste, however the essential nutritional facts are not provided. Consequently, this study's goal was to evaluate nutritional values by proximate analysis. All 6 types of young flattened rice-: Laos Wan, Kor Khor 6(RD6), Ai Lueng, San Pa Tong, Yee Pun, and Hom Nang Nuan have phenolic compounds by Folin-Ciocalteu method and antioxidant properties by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. According to the findings, rice's primary constituents are carbohydrates, which range from 69.76 to 73.07 %, protein, which ranges from 4.79 to 6.11 %, crude fat, which ranges from 1.68 to 2.22 %, fiber, which ranges from 0.00 to 0.03 % moisture, which ranges from 17.96 to 21.31 %, and ash, which ranges from 1.13 to 1.70 %. The San Pa Tong Khao-Mao has the highest 6.11% of protein content. Ai Lueng Khao-Mao has the greatest levels of total phenolic compounds, 0.79 mg/g dry weight. The DPPH radical scavenging assay test was used to measure the antioxidant activity. Their half-maximal inhibitory concentration (IC_{50}) value falls between 1.18 and 2.00 mg/mL. The Hom Nang Nuan Khao-Mao has the lowest 1.18 mg/mL of IC_{50}. According to the study's findings, this community's Khao-Mao has good nutritional and antioxidant properties.

Keywords: Khao-Mao, Young flattened rice, Antioxidant, Phenolic, Proximate composition

Introduction

Rice is a staple food for many people, and has been a primary part of the diet for Thai people for a long time. Rice is a crucial grain for human nutrition and a source of energy. Importantly, many research have shown that rice is a good source of vitamins, minerals, dietary fiber, proteins, fat, and carbohydrate. Most of the land is used for agriculture in Thailand's Na Hua Bo sub-district, Phon Sawan district, and Nakhon Phanom province. Most of the population works in agriculture. Rice, which is produced from the sale of paddy by the community's yearly farming, is the main product that brings in money for the area. The investment in paddy is not cost-effective, but young flattened rice, or "Khao-Mao," is sold at a high price of 150 Baht/kg. This leads to its production and sales as a source of additional revenue in the neighborhood. Khao is Thai slang for rice, and Mao refers to immature grains that form between 13th and 19th day after anthesis, depending on the rice genotype and climate [1]. Khao-Mao is also referred to as beaten rice, rice flakes, pounded rice or young rice and pressed rice.

Khao-Mao is made of flat, smooth, fragrant and green-coloured grains. It is produced during pre-harvest season between September and October, using 75 - 90 days old pre-harvest rice by separating grains and awn. For 1 to 2 h, the grains were submerged in water, after which it is roasted over high heat, the rice husks are pounded off, and the product is screened to ensure it is pure Khao-Mao. This Khao-Mao is unpolished rice. **Figure 1** shows the production process of Khao-Mao. Research has been done on the

chemical make-up, bioactive substances, and biological activity of rice. Numerous rice antioxidants, including phenolic compounds, anthocyanins, flavonoids, proanthocyanins, γ-oryzanol, tocotrienols, tocopherols and phytic acid have been reported [2]. Many studies have been reported on pigment rice [3-6], brown rice [7,8], germinated rice [9-12] and rice bran oil [13]. Carbohydrates, which make up the majority of rice composition, account for 80 % of its dry weight. They are essential for human life as an energy source. Most people believe that rice protein is hypoallergenic [14]. It is used as an alternative protein [15]. There are other important substances as well as antioxidants. Vitamins C and E, as well as phenolic chemicals, are well-known antioxidants found in plants and cereal [16]. Phenolic compounds, prevalent in plants and cereals, have antioxidant properties making them important in human diet and of interest. Numerous studies have shown a close connection between these substances and antioxidant properties [17]. Few studies have been conducted on the chemical makeup and biological traits of Khao-Mao. However, some physiochemical properties and bioactive compounds such as γ-oryzanol and γ-aminobutiric acid from Khao-Mao from Trakan Phuet Phon and Phibun Mangsahang districts in Ubonratchathani province, Thailand have been studied [1]. Thai people believe that eating Khao-Mao promotes good health and helps the brain function normally, restoring physical and mental development. Studies have found that unpolished rice contains bioactive chemicals that have positive health effects. For instance, cancer prevention [18] and the avoidance of diabetic problems [19]. Farmers in Thailand's northeast focus on producing Khao-Mao using pre-harvest rice to increase its value. Khao-Mao samples obtained from Phon Sawan district, Nakhon Phanom province, Thailand which have not been reported. In terms of general qualities including color, fragrance, taste, and foreign matter, this community's Khao-Mao is consistent with the Khao-Mao standard [20].

Therefore, the goals of this study are to investigate the nutritional, total phenolic contents, and antioxidant activity of Khao-Mao. They were made from 6 varieties of sticky rice: Laos Wan, Kor Khor 6(RD6), Ai Lueng, San Pa Tong, Yee Pun, and Hom Nang Nuan. It is anticipated that the knowledge gained from this research will support nutritional advice and benefit the Khao-Mao of this community.

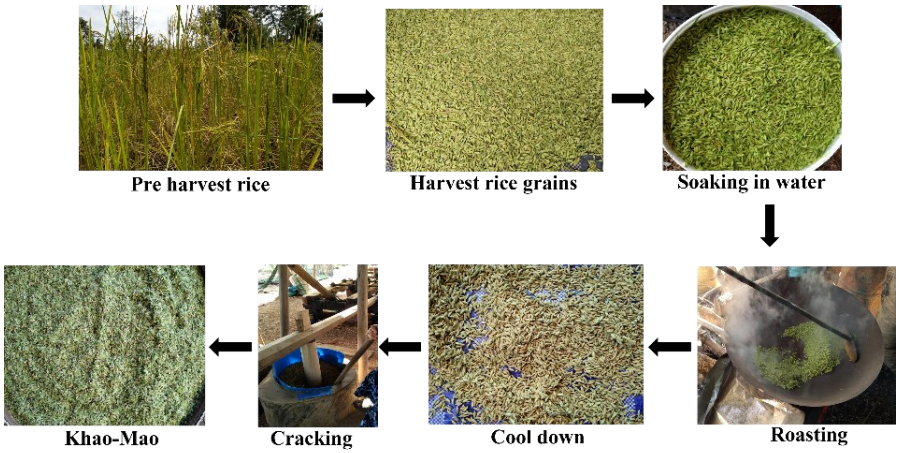

Figure 1 Production flow chart for Khao-Mao.

Materials and methods

Reagents and standards

2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteu phenol reagent were purchased from Sigma-Aldrich Chemical Co. USA. All the other chemicals used in the proximate analysis and antioxidant activity were analytical grade.

Khao-Mao samples

Khao-Mao is made from 6 rice accessions (*oryza sativa* L.) which are Laos Wan, Kor Khor 6(RD6), Ai Lueng, San Pa Tong, Yee Pun and Hom Nang Nuan. Khao-Mao was produced in Phon Sawan district in Nakhon Phanom province, Thailand. There are 3 main steps in preparing Khao-Mao: Soaking, roasting and cracking. The soaking: Preharvest rice grains were soaked in tap water for 1 - 2 h. Roasting, the grains were roasted right away with high heat (90 °C) for 10 min. After that, the rice grains were left to cool. Last step: the cracking, the roasted rice was pounded in a motorized mortar. The mixture of rice and husk was separated by a sieve (**Figure 1**). Khao-Mao samples were packed in plastic bags, sealed, and kept at −40 °C until analysis.

Khao-Mao extraction

One hundred grams of each type of Khao-Mao were mashed and dissolved with 80 % ethanol in an Erlenmeyer flask. These samples were shaken at 150 rpm for 24 h. The supernatant was separated by centrifugation at 3,500 rpm for 10 min. The extracts were evaporated under a vacuum by a rotary evaporator. The crude extracted was kept at −40 °C. These crudes extracted were used for total phenolic compounds and antioxidant activity analysis.

Proximate analysis

The proximate composition of Khao-Mao was determined using the AOAC method [21]. The moisture content was determined in a dry oven at 110 °C until a constant weight was obtained. All organic material was burned at a temperature of 550 °C to determine the amount of ash. The crude fiber was measured by initial digestion of 5 g of a dried sample under reflux in equal concentration of acid and base followed by sequential washing with hot water, acetone, and hydro ethanol. Crude fat content was estimated by acid digestion prior to continuous extraction using hexane in the Soxhlet system, at 60 °C for 6 h following drying in hot air oven at 120 °C. Total nitrogen was estimated using the Kjeldahl method and nitrogen content (%N) was multiplied with a factor of 6.25 to obtain protein content. Carbohydrate content was calculated by 100 – Σ (%moisture + %protein + %crude fat + %ash + %fiber).

Energy value

The energy was calculated by the Atwater factor (4 for %protein and %carbohydrate and 9 for %crude fat).

Determination of total phenolic compounds

The determination of total phenolic compounds was measured by a Folin–Ciocalteu method [22]. Crude extracted from each sample was taken with 50 µL applied per well (96 well plates) followed by 80 µL of 10 % Folin–Ciocalteu phenol reagent and 150 µL of 7 % sodium carbonate. The mixture was incubated for 2 h in the dark at room temperature. Gallic acid solution was used as the standard. The absorbance was determined at 765 nm. Methanol was applied as a control. The total phenolic contents of the sample were computed as the gallic acid equivalents (mg/g dry weight).

Determination of antioxidant activity by DPPH scavenging assay

The antioxidant activity of Khao-Mao extracts was measured by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity [22]. Briefly, aliquots of rice extract 50 µL were mixed with 0.1 mM DPPH methanolic solution in a 96-well plate. The mixture was mixed and then discard in the dark at room temperature for 20 min. The absorbance of the solution was measured using a microplate reader at 515 nm. The DPPH radical solution in the respective extraction solvent was a negative control. The percent of DPPH scavenging activity was calculated using the equation below.

$$\text{DPPH scavenging activity (\%)} = ((A_{control} - A_{sample}) / A_{control}))\times 100$$

$A_{control}$ is the absorbance of the DPPH radical solution in the respective extraction solvent, A_{sample} is the absorbance of each Khao-Mao extract in DPPH radical solution. The results were expressed as half maximal inhibitory concentration (IC_{50}).

Statistical analysis

The data was operated as mean value ± standard deviation (SD). The comparison was executed by Tukey's further test and $p \leq 0.05$ was applied for statistically significant.

Results and discussion

The 6 Khao-Mao rice varieties displayed different ratios of proximate components, as shown in **Table 1**. The tested types differ significantly from one another ($p \leq 0.05$).

The percentage of carbohydrate is between 69.62 and 73.07 %. The San Pa Tong Khao-Mao contains the highest carbohydrate overall, at 73.07 %. Carbohydrate serve as the body's main source of energy. Because they aid in the proper operation of the heart, brain, immune system and digestive system. Additionally, they serves as the precursor for the synthesis of several biomolecules [23].

The Kjeldahl technique was used to determine the protein content. The highest content is found in the San Pa Tong Khao-Mao (6.11 %), followed by Ai Lueng (6.00 %) and RD6 (5.50 %). San Pa Tong Khao-

Mao protein content was close to that of KM2 Khao-Mao (6.24 %, (*Oryza sativa* L.) cv. RD6) [1]. Proteins are essential because they assist the immune system and act as precursors to the creation of hormones and enzymes. Additionally, proteins support regular bodily function by regulating the actions of numerous organs, balancing blood sugar levels, repairing different cell types, keeping cell water levels at the proper level, and preserving the pH balance.

Khao-Mao has crude fat level that ranges from 1.68 to 2.22 %. The highest amount of crude fat is seen in RD6 Khao-Mao. Khao-Mao contains relatively less fat than other types of rice, such as Tung Samrit jasmine rice, which has 4.02 % fat [24] and Chiang Phatthalung rice, whose bran has 16.32 % fat [13]. Unsaturated fats, which make up most vegetable fats, are healthier than saturated fatty acids found in animal fats. The RD6 Khao-Mao has similar fat content as Kpurukpuru Afikpo rice (2.23 %) and Awilo Ikwo rice (2.23%) [25]. As a source of energy, fat is a vital nutrient for the body, aids in dissolving and absorption of a variety of vitamins, the generation of hormones, brain function, and the regular operation of the metabolic system.

The RD6 cultivar has the highest fiber, with the Khao-Mao samples' fiber content ranging from 0.00 to 0.03 %. Dietary fiber aids in the absorption of trace nutrients in the gastrointestinal tract and encourages healthy excretion, colon cancer prevention and cholesterol reduction.

The range of the moisture content is 17.96 to 21.31 %. The RD6 and Yee Pun Khao-Mao have the maximum moisture content (21.31 %), which is less than the glutinous rice (*Oryza sativa* L.) cv. RD6 Khao-Mao from Trakan Phuet Phon district (KM1, 22.11 %) and Phibun Mangsahang district (KM2, 22.92 %) in Ubonratchathani province, Thailand [1]. Khao-Mao has a delicate, palatable texture because of its moisture content. Khao-Mao may be stored for a longer period because of its low moisture content, which reduces microbial contamination and enzyme activity.

The ash content is between 1.13 and 1.70 %. With 1.70 %, the Ai Lueng Khao-Mao has the highest ash. All dietary components, especially minerals essential to the body's operation, are present in ash. Additionally, the inclusion of ash in the diet aids in creating and maintaining the blood's acid-base balance and controlling blood sugar levels [26].

Energy values were calculated for all the species using their nutritional composition. The overall calculated energy values of the analyzed Khao-Mao were as given in **Figure 2**. The San Pa Tong Khao-Mao have the highest energy value (332 kcal). This is because San Pa Tong Khao-Mao has the highest level carbohydrate. It is indicated that San Pa Tong Khao-Mao is a rich source of energy for the body. The caloric value was the highest compared to Khao-Mao of Yee Pun (318 kcal), Hom Nang Nuan (319 kcal), RD 6 (320 kcal), Laos Wan (322 kcal) and Ai Lueng (322 kcal).

Table 1 Percentage of proximate content of Khao-Mao with 6 rice cultivars.

Khao-Mao types	Proximate content (%)					
	Carbohydrate	Protein	Crude fat	fiber	Moisture	Ash
Laos Wan	71.35^{d}±0.100	5.39^{b}±0.039	1.68^{a}±0.000	0.01^{b}±0.008	20.22^{b}±0.140	1.35^{b}±0.038
RD6	69.62^{a}±0.020	5.50^{b}±0.039	2.22^{e}±0.002	0.03^{d}±0.010	21.31^{c}±0.094	1.32^{b}±0.026
Ai Lueng	69.76^{a}±0.030	6.00^{c}±0.078	2.16^{d}±0.003	0.02^{c}±0.010	20.37^{b}±0.493	1.70^{c}±0.046
San Pa Tong	73.07^{e}±0.010	6.11^{c}±0.067	1.72^{b}±0.002	0.02^{c}±0.020	17.96^{a}±0.329	1.13^{a}±0.003
Yee Pun	70.23^{b}±0.010	4.90^{a}±0.039	1.95^{c}±0.000	0.00^{a}±0.010	21.31^{c}±0.069	1.61^{c}±0.074
Hom Nang Nuan	70.63^{c}±0.020	4.79^{a}±0.067	1.94^{c}±0.001	0.01^{b}±0.010	21.03^{b}±0.227	1.61^{c}±0.045

Values are presented as Mean ± SD. Values with the different letters in superscript along the same column are significantly different by Tukey's test ($p \leq 0.05$).

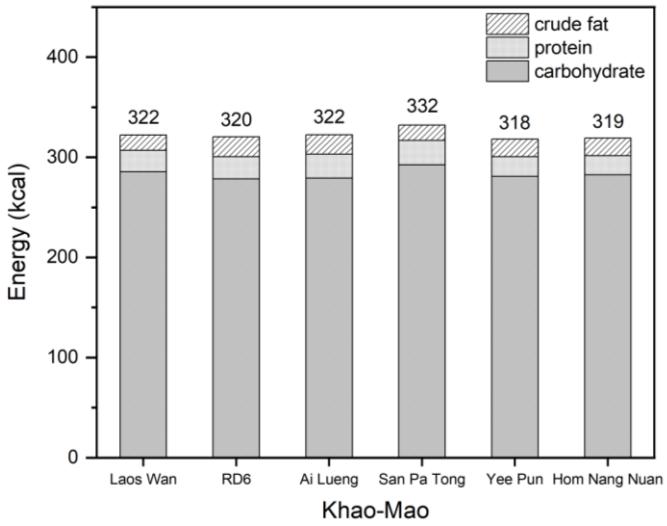

Figure 2 Energy values of Khao-Mao with 6 rice cultivars.

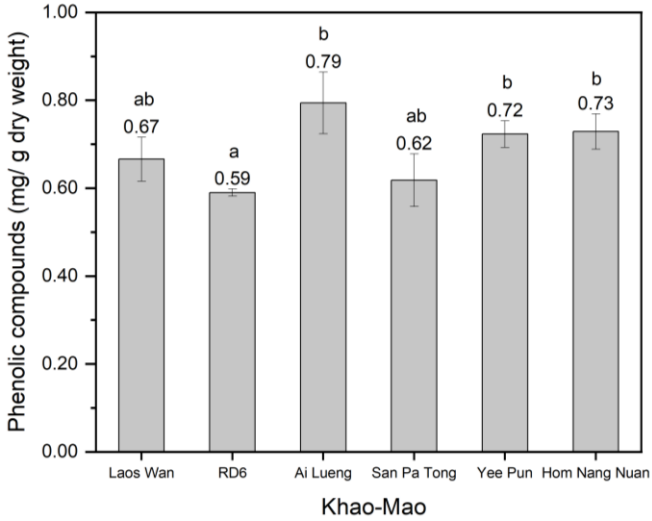

Figure 3 Total phenolic compound contents of 6 types of Khao-Mao. Data reported are the mean ± SD of triplicate determinations. Different letters above the bars of each phenolic sample type indicate significant differences by Tukey's test ($p \leq 0.05$)

Total phenolic concentration in Khao-Mao ranged from 0.59 to 0.79 mg/g of dry weight (**Figure 3**). The Ai Lueng Khao-Mao contained the highest amount of total phenolic followed by Hom Nang Nuan and Yee Pun with 0.79, 0.73 and 0.72 mg/g dry weight, respectively. The results of this investigation showed that phenolic compounds in Khao-Mao were 1.9 times higher than in white rice. White rice (*Oryza sativa* L. 9311), exhibited a total phenolic content (soluble-free, soluble-conjugated, and insoluble-bound) of 0.40 mg gallic acid equivalent/g rice grain at completely mature stages [27]. The phenolic acids, primarily ferulic acid and coumaric acid, make up most of the phenols in rice kernels with a light brown husk [28]. These phenolic compounds contain a variety of crucial biological properties that are advantageous to human health, including antioxidant, anti-cancer and anti-inflammatory [28].

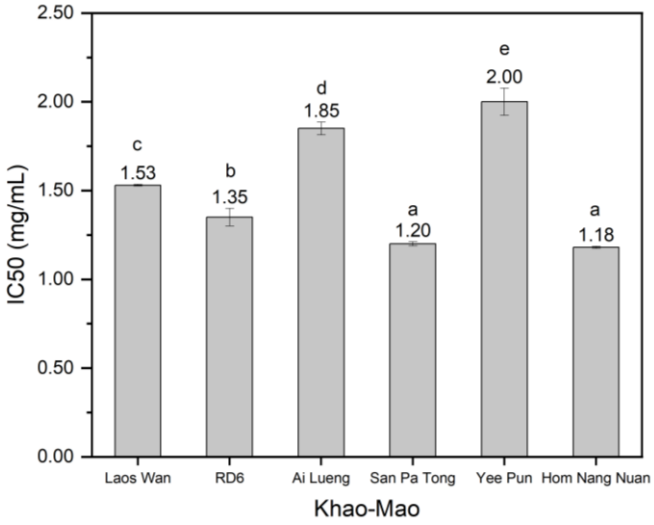

Figure 4 Antioxidant activities of 6 types of Khao-Mao. Data are presented as means SD of determinations made in triplicate. Different letters above the bars of each IC_{50} sample type indicate significant differences by Tukey's test ($p \leq 0.05$).

The IC_{50} value was found to be between 1.18 and 2.00 mg/mL in the extracts of all 6 cultivars of Khao-Mao. The extract from Hom Nang Nuan rice varieties had the lowest IC_{50} value is 1.18 mg/mL. According to figure 4, the IC_{50} values for the rice varieties San Pa Tong, RD6, Laos Wan, Ai-Lueng and Yee Pun were 1.20, 1.35, 1.53, 1.85, and 2.00 mg/mL, respectively. All these samples of Khao-Mao had IC_{50} values that were lower than those of brown rice (7.40 mg/mL) and germinated brown rice (5.31 mg/mL) of Hom Mali 105 rice [8].

It's possible that proteins and phenolic chemicals are responsible for Khao-Mao's capacity to suppress free radicals. For instance, Hom Nang Nuan Khao-Mao has better antioxidant ability than other Khao-Mao because of its greater phenolic compound concentration (0.73 mg/g dry weight) and San Pa-Tong Khao-Mao has 0.62 mg/g dry weight phenolic content which high protein content (6.11 %). Rice has proteins with a molecular weight range of 14 to over 97 kDa which may be prolamin, globulin, albumin and glutelin [29,30]. In other words, proteins are formed from long chains of amino acids joined together by peptide bonds. In amino acids there are carboxylic groups (-COOH) and amino groups ($-NH_2$) that can donate hydrogen atoms to free radicals, resulting in non-oxidative action. In addition, some amino acids have side chains that can provide hydrogen atoms to free radicals, such as positively charged amino acids (histidine, lysine and arginine) [31]. Furthermore, proteins and phenolic compounds are substances with good antioxidant capacity which are commonly found in plants and grains. Phenolic compounds can give hydrogen atoms in the OH group binds to free radicals to stabilize free radicals [32].

Conclusions

The investigation of the nutritional components and antioxidant potential of Khao-Mao from Thailand's Nakhon Phanom province's Phon Sawan district. This homemade product has not before been researched. According to the findings, Khao-Mao is primarily composed of carbohydrates and has a comparatively high protein content. This demonstrates that Khao-Mao can be a staple food that gives the body enough energy. Additionally, it possesses beneficial antioxidant effects that may have therapeutic potential. Khao-Mao is typically eaten with savory and sweet meals which is an interesting product. It can be consumed separately or added as a component of breakfast cereals. Additionally, fragrant green bran that is left over after making Khao-Mao can be used in other ways. For instance, it could be added to the feed of both terrestrial and aquatic animals. Khao-Mao is a native product with significant commercial potential. As a result, the findings of this study may be used to reinforce nutritional information about Khao-Mao and enhance its value in this community.

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