J Appl Biotechnol Rep. 2024 December;11(4):1471-1478

Journal of
Applied Biotechnology Reports

doi 10.30491/jabr.2024.451772.1717

Original Article

Effect of *In Vitro* Gastrointestinal Digestion on Monacolin K and α-Glucosidase and α-Amylase Inhibitory Activities of BRTA and Chemometrics Analysis

Narissara Uthai [1], Putkrong Phanumong [2], Kitisart Kraboun [2*]

[1] Division of Food and Nutrition, Faculty of Home Economics Technology, Rajamangala University of Technology Krungthep, Bangkok 10120, Thailand
[2] Division of Food Safety Management and Technology, Faculty of Science and Technology, Rajamangala University of Technology Krungthep, Bangkok 10120, Thailand

Corresponding Author: Kitisart Kraboun, PhD, Associate Professor, Division of Food Safety Management and Technology, Faculty of Science and Technology, Rajamangala University of Technology Krungthep, Bangkok 10120, Thailand. Tel: +66639515194, E-mail: kitisart.k@mail.rmutk.ac.th

Received April 9, 2024; **Accepted** July 22, 2024; **Online Published** December 30, 2024

Abstract

Introduction: Broken rice tea supplemented with angkak (BRTA) contains monacolin K and Maillard reaction products (MRPs). The dynamic changes in these bioactive compounds and activities of BRTA after *in vitro* gastrointestinal digestion were investigated.

Materials and Methods: Different ratios of roasted broken rice to angkak were supplemented in BRTA as 25:75, 50:50, 75:25, and 100:0. The colors, water activity (a_w), moisture content, monacolin K, inhibition of peroxidation, DPPH radical scavenging assay, chelating ability on Fe^{2+}, and α-glucosidase and α-amylase inhibitory properties of BRTA were investigated after *in vitro* gastrointestinal digestion.

Results: Monacolin K, antioxidant properties, and α-glucosidase and α-amylase inhibitory abilities of BRTA for all ratios of roasted broken rice to angkak increased after *in vitro* gastric digestion. BRTA activities using a ratio of 25:75 (roasted broken rice:angkak) were the highest. After *in vitro* gastrointestinal digestion, monacolin K, antioxidant properties, and α-glucosidase and α-amylase inhibitory abilities of BRTA decreased due to the alkaline condition. Principal component analysis (PCA) and Pearson's correlation coefficient showed that monacolin K was positively correlated with α-amylase inhibition activity.

Conclusions: Changes in monacolin K and α-glucosidase and α-amylase inhibitory abilities after *in vitro* gastrointestinal digestion of BRTA were investigated. Other experiments using angkak as a main ingredient should be conducted to determine dynamic changes in bioaccessibility.

Keywords: Angkak, Antioxidant Properties, PCA, Pearson's Correlation, DPPH

Citation: Uthai N, Phanumong P, Kraboun K. Effect of *In Vitro* Gastrointestinal Digestion on Monacolin K and α-Glucosidase and α-Amylase Inhibitory Activities of BRTA and Chemometrics Analysis. J Appl Biotechnol Rep. 2024;11(4):1471-1478. doi:10.30491/jabr.2024.451772.1717

Introduction

Broken rice is a rice milling by-product that contains the same abundant nutrients as whole grain rice such as 81-86% starch, 5-11% protein, 0.89% lipid, and 9% moisture.[1] Broken rice can be developed into interesting value-added products including germinated purple rice tea and germinated brown rice (Kraboun et al.[2] and Yang et al.[3]) Kraboun et al.[2] found that the manufacturing process of broken rice tea enhanced the levels of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), and ABTS radical scavenging activity. Interestingly, supplementation of nutritional sources possessing antioxidant properties into tea products had a synergistic impact on bioactive compounds. Al-Ghafari et al.[4] noted that Indian tea mixed with ginger, black pepper, and tulsi exhibited higher DPPH free radical scavenging properties and improved organoleptic properties by reducing astringency.[5]

Monascal products, also known as angkak, are prepared from polished rice (*Oryza sativa*) or starchy raw materials fermented by *Monascus* sp. at 30-37 ºC for 2 weeks. The secondary metabolites formed during *Monascus* fermentation are *Monascus* pigments and antioxidants including monacolin K, phenolic compounds, γ-aminobutyric acid (GABA), and dimerumic acid. *Monascus* pigments have long been applied as natural dyes and/or traditional medicines in many countries. Monacolin K blocks the cholesterol synthesis pathway and also inhibits oxidative stress.[6] Yang et al.[3] and Kraboun et al.[7] explained that *Monascus* pigments showed strong antioxidant abilities by inhibiting peroxidation, reducing power, DPPH radical scavenging assay, and chelating ability on Fe^{2+} while Srianta et al.[8] further confirmed that angkak prepared from durian seed substrate possessed α-glucosidase inhibitory activity at > 80%.[9,10]

Different ratios of broken rice tea supplemented with angkak

(BRTA) require detailed study to comprehend and predict the bioavailability and bioaccessibility of monacolin K and phenolic bioactive compounds after *in vitro* gastrointestinal digestion. The development of other products supplemented with angkak should also be investigated.

Determining the relationships among the dependent variables and/or discrimination among treatments by chemometrics analysis is necessary to understand the correlations between and properties of metabolites in the resulting samples. Chemometrics analysis uses multivariate analysis techniques such as principal component analysis (PCA), partial least squares (PLS), and Pearson's correlation coefficient.[11]

This research studied the changes in BRTA properties after *in vitro* gastrointestinal digestion based on L* (lightness), a* (redness), b* (yellowness), water activity (a_w), moisture content, monacolin K, inhibition of peroxidation, DPPH radical scavenging activity, chelating ability on Fe^{2+}, and α-glucosidase and α-amylase inhibitory properties. A chemometrics analysis of these parameters was also conducted using PCA and Pearson's correlation coefficient.

Materials and Methods

Chemicals

Acarbose, monacolin K, pancreatin, bile salt, pepsin, ferrous chloride, linoleic acid, ferrous sulfate monohydrate, ammonium thiocyanate, ferrozine, 2,2-diphenyl-1-picrylhydrazyl (DPPH), α-amylase, α-glucosidase, and *p*-nitrophenyl-α-glucopyranoside (pNPG) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All chemicals were kept at -20 °C before use.

Materials

Broken rice (*Oryza sativa* L.) was purchased from a local market in Sukhothai Province, Thailand and angkak was sourced from Artchit International Pepper and Spice Co., Ltd.

BRTA Preparation

The broken rice was cleaned to remove dirt, roasted in a hot air oven at 220 ºC for 20 min packed in a polyethylene bag and stored in a desiccator at ambient temperature (30 °C) before BRTA preparation.[2] Angkak powder was winnowed through a 700-mesh screen sieve and stored in a polyethylene bag in a desiccator at ambient temperature (30 °C) before use.

To prepare BRTA, the roasted broken rice was blended with angkak powder at ratios of broken rice to angkak 25:75, 50:50, 75:25, and 100:0. The mixtures were stored in polyethylene bags and kept in a desiccator at ambient temperature (30 °C) before analysis.

In Vitro Gastrointestinal Digestion

The *in vitro* digestion simulation procedure followed Kraboun et al.[10] as a 2-step system by 1. imitating gastric digestion, and 2. imitating intestinal digestion. For the *in vitro* gastric digestion, 1 g of sample was mixed with 12 ml of Milli-Q water. The pH was adjusted to 1.5 using HCl 7 N, added with pepsin solution (0.5 g of pepsin dissolved in 4 ml of 0.2 M HCl) at 0.06 g of pepsin per gram of sample, and then reacted at 37 ºC for 3 h. To stop the reaction, the reacted sample was adjusted to pH 7 using 2 M $NaHCO_3$, and the digested BRTA was analyzed. For *in vitro* intestinal digestion, the digested BRTA and 3.60 ml of the reagent (0.2 g of pancreatin and 0.7 g of bile salt dissolved in 30 ml of 0.2 M $NaHCO_3$) were mixed, adjusted to pH 7 using 2 M $NaHCO_3$, and then incubated at 37 °C for 3 h. To inactivate the reaction, the digestive enzyme was soaked in a polyethylene glycol bath at 100 °C for 4 min. After cooling, the mixture was centrifuged at 5000 *g* for 65 min at 2 °C (CS-6R Centrifuge, Beckman Coulter, USA) to obtain soluble and non-soluble fractions which were later analyzed.

Color, Moisture Content and Water Activity (a_w)

The L*, a*, and b* values (indicating lightness, redness, and yellowness, respectively) were analyzed using a color meter (WS Series; FRU, China), with calibration using a reference plate (X = 99.68, Y = 87.91, and Z = 91.87). A moisture analyzer was used to investigate moisture content. Water activity (a_w) was determined using a water activity meter (AquaLab Series 3, Decagon Devices, USA).

Monacolin K

A 1 g sample (from raw BRTA or BRTA after *in vitro* gastrointestinal digestion) was extracted with 30 ml of 80% ethanol at 60 °C for 5 h and then filtrated through a 0.45 µm membrane. The filtrate was analyzed by high performance liquid chromatography (HPLC) using a Shimadzu LC-10AT VP Liquid Chromatograph, a FCV-10AL VP pump, a LDC Analytical SpectroMonitor 3100 detector set at 240 nm, and an LDC Analytical CI-4100 integrator. An Ascentis C18 (5 µm, 250×4.6 mm) chromatography column was connected to a 20 µl loop injector. The mobile phase was a ratio of 65:35, v:v (acetonitrile:water), with flow rate and temperature 2 ml/min and 30 °C, respectively.[10] Monacolin K was dissolved in 80% ethanol, which was used as a standard at 2-10 mg/ml. The monacolin K content was expressed as mg/kg BRTA or BRTA extract.

Sample Extraction for Analysis of Antioxidant Abilities and Inhibitory Properties of α-Glucosidase and α-Amylase

The sample extraction followed the protocol of Kraboun et al.[2] with slight modifications. A 100 g sample was extracted with 200 ml of methanol and then oscillated by a shaker at 170 rpm for 24 h. The mixture was filtered through Whatman No. 4 filter paper and the precipitate was again extracted using 200 ml of methanol following the above condition. The solvent was evaporated using an evaporator

under 50 °C, and the extract was kept in an amber glass bottle at -20 °C before analysis.

Inhibition of Peroxidation

The inhibition of lipid peroxidation based on the ferric thiocyanate method was performed following the procedure of Kuo et al.[12] Briefly, a 0.03 mM linoleic acid emulsion was prepared from 0.28 g of linoleic acid and 0.30 g of Tween 20 in 60 ml of 0.03 M phosphate-buffered saline (PBS). A 3 ml aliquot of linoleic acid emulsion was mixed with 3 ml of 0.3 M PBS and the mixture was placed into tubes consisting of 5 ml of BRTA extract (from raw BRTA or BRTA after *in vitro* gastrointestinal digestion) at different concentrations (2-12 mg/ml). The mixtures were incubated at 60 °C for 80 h in a dark room. Before analysis, the samples were mixed with 5 ml of 80% ethanol, 0.2 ml of 35% (w/v) ammonium thiocyanate, and 0.2 ml of 30 mM ferrous chloride (in 4% HCl), with 80% ethanol used as a blank. After reacting for 3 min the absorbance was read at 500 nm (GENESYS 20, Thermo Fisher Scientific, USA). The IC_{50} values of peroxidation inhibition were expressed as mg/ml BRTA extract with interpolation to 50% inhibition from the linear regression analysis.

DPPH Radical Scavenging Ability

The DPPH radical scavenging ability was investigated following the protocol of Kraboun et al.[10] Briefly, 40 µl of 2-12 mg/ml BRTA extract *(*from raw BRTA or BRTA after *in vitro* gastrointestinal digestion*)*, 400 µl of 0.03 mM DPPH solution, and methanol 4 ml were placed in a test tube. The mixture was reacted at ambient temperature for 15 min and then analyzed for absorbance at 517 nm (GENESYS 20, Thermo Fisher Scientific, USA). The IC_{50} of DPPH radical scavenging ability was calculated as $mg.ml^{-1}$ BRTA extract with interpolation to 50% inhibition from the linear regression analysis.

Chelating Ability on Fe^{2+}

The chelating ability on Fe^{2+} was assessed following the method of Kraboun et al.[2] A 500 µl aliquot of 3 mM ferrous sulfate monohydrate was transferred into 700 µl of BRTA extract from raw BRTA or BRTA after *in vitro* gastrointestinal digestion (2-12 $mg.ml^{-1}$) + 600 µl of 5 mM ferrozine. After incubation at 30 °C for 20 min, the absorbance of the reacted mixture added with 10 ml of ethanol was read at 570 nm (GENESYS 20, Thermo Fisher Scientific, USA). The IC_{50} of chelating ability on Fe^{2+} was reported as mg/ml BRTA extract with interpolation to 50% inhibition from the linear regression analysis.

α-Amylase Inhibitory Activity

The analysis of α-amylase inhibition followed the method of Liu et al.[13] A 50 µl aliquot of α-amylase (1.6 unit/ml) was placed in a test tube containing 1 ml of sodium phosphate buffer (0.03 M, pH 7.0 with 7 mM NaCl) and 1 ml of 2-12 mg/ml BRTA extract *(*from raw BRTA or BRTA after *in vitro* gastrointestinal digestion*)* or acarbose with incubation at 37 °C for 20 min. The mixture was added with 500 µl of 1% (w/v) starch solution in sodium phosphate buffer (0.03 M, pH 7.0 with 7 mM NaCl) and then incubated for 30 min. The resulting sample was mixed with 0.2 ml of dinitrosalicylic acid and then boiled for 5 min. After cooling, the sample was diluted by adding 10 ml of distilled water, and the absorbance was read at 540 nm (GENESYS 20, Thermo Fisher Scientific, USA). Acarbose was used as a positive control. The IC_{50} of α-amylase inhibitory activity was calculated as mg/ml BRTA extract with interpolation to 50% inhibition from the linear regression analysis.

α-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity was determined following the method of Zhang et al.[14] A 15 µl aliquot of α-glucosidase ($1.5\ unit.ml^{-1}$) was dissolved in 70 µl of phosphate buffer (0.2 mM, pH 7) and 200 µl of 2-12 mg/ml BRTA extract (from raw BRTA or BRTA after *in vitro* gastrointestinal digestion) or acarbose. The mixture was reacted in a 96-well plate reader (BioTek 800 TS Absorbance Reader, Agilent Technologies, USA) with incubation at 37 °C for 20 min. The reacted sample and 60 µl of pNPG solution (3 mM of pNPG in 0.5 mM phosphate buffer) were mixed to start the enzyme reaction and the absorbance was read at 405 nm. Acarbose was used as a positive control. The IC_{50} of α-glucosidase inhibitory activity was calculated as $mg.ml^{-1}$ BRTA extract with interpolation to 50% inhibition from the linear regression analysis.

Statistical Analysis

All experiments were conducted in triplicate with results expressed as mean ± SD. SPSS 26.0 (SPSS Inc., USA) was used for the analysis of variance (ANOVA) with different separations using Duncan's new multiple range test (DMRT) ($p \leq 0.05$) and principal component analysis (PCA).

Results and Discussion

Color, Moisture Content, Water Activity (a_w), Monacolin K, Inhibition of Peroxidation, DPPH Radical Scavenging Ability, Chelating Ability on Fe^{2+} and α-Amylase and α-Glucosidase Inhibitory Activities of Raw BRTA (Without In Vitro Gastrointestinal Digestion)

The color (L*, a*, b*), moisture content, and a_w of raw BRTA at ratios 25:75, 50:50, 75:25, and 100:0 (roasted broken rice:angkak) are presented in Table 1. Higher levels of angkak directly affected the values of L*, a*, and b* of raw BRTA ($p \leq 0.05$). The lightness of raw BRTA decreased at higher levels of angkak but not the a* and b* values. This indicated that higher increments of angkak enhanced the

intensity of *Monascus* pigments and redness in raw BRTA.[6] The moisture content and a_w of raw BRTA also increased with higher angkak content because the moisture content (15%) and a_w (0.876) of angkak (data not shown) were higher than roasted broken rice (5.39% and 0.761, respectively) (Table 1). Moisture from angkak rapidly moved into the structure of roasted rice.[2,15]

The levels of monacolin K, inhibition of peroxidation, DPPH radical scavenging ability, chelating ability on Fe^{2+}, and α-amylase and α-glucosidase inhibitory activities in raw BRTA for ratios of 25:75, 50:50, 75:25, and 100:0 (roasted broken rice: angkak) are shown in Table 2. The amount of monacolin K in raw BRTA increased at higher angkak content. The monacolin K contents in BRTA treatments > 25% angkak ranged between 15.23 and 25.35 $mg.kg^{-1}$ dry weight but monacolin K was not found in the control **(**without angkak addition**)**. Similarly, the IC_{50} values of peroxidation inhibition, DPPH radical scavenging ability, chelating ability on Fe^{2+}, and α-amylase and α-glucosidase inhibitory activities in raw BRTA at ratio 25:75 (roasted broken rice: angkak) were lower than for other treatments of raw BRTA. Higher contents of angkak directly impacted enhanced monacolin K content and also decreased the IC_{50} values in raw BRTA (Table 2).

Table 1. L*, a*, b*, Moisture Content, and Water Activity (a_w) of BRTA for Ratios of 25:75, 50:50, 75:25, and 100:0 Roasted Broken Rice:Angkak

Roasted broken rice:angkak	L*	a*	b*	Moisture content (%)	a_w
25 : 75	15.35 ± 0.12^{a}	12.32 ± 0.05^{d}	7.23 ± 0.12^{c}	12.67 ± 0.11^{d}	0.899 ± 0.002^{b}
50 : 50	20.39 ± 0.23^{b}	10.11 ± 0.08^{c}	6.45 ± 0.08^{b}	9.78 ± 0.13^{c}	0.835 ± 0.003^{b}
75 : 25	25.23 ± 0.24^{c}	7.32 ± 0.01^{b}	6.56 ± 0.03^{b}	7.14 ± 0.03^{b}	0.862 ± 0.001^{b}
100 : 0	30.23 ± 0.11^{d}	3.54 ± 0.05^{a}	5.39 ± 0.05^{a}	5.34 ± 0.01^{a}	0.761 ± 0.004^{a}

ND = not detecte; Different letters in a same column define as significantly different ($p\leq0.05$).

Table 2. Levels of Monacolin K, Inhibition of Peroxidation, DPPH Radical Scavenging Ability, Chelating Ability on Fe^{2+}, and α-Glucosidase and α-Amylase Inhibitory Activities of BRTA for Ratios of 25:75, 50:50, 75:25, and 100:0 Roasted Broken Rice:Angkak

Roasted broken rice:angkak	Monacolin K ($mg.kg^{-1}$ BRTA)	Inhibition of peroxidation (IC_{50} $mg.mL^{-1}$ BRTA extract)	DPPH free radical scavenging ability (IC_{50} $mg.mL^{-1}$ BRTA extract)	Chelating ability on Fe^{2+} (IC_{50} $mg.mL^{-1}$ BRTA extract)	α-amylase inhibitory activity (IC_{50} $mg.mL^{-1}$ BRTA extract)	α-glucosidase inhibitory activity (IC_{50} $mg.mL^{-1}$ BRTA extract)
25 : 75	25.35 ± 0.12^{c}	3.22 ± 0.01^{a}	1.02 ± 0.03^{a}	$1.07+0.01^{a}$	1.52 ± 0.04^{ab}	2.08 ± 0.01^{a}
50 : 50	20.39 ± 0.23^{b}	4.26 ± 0.02^{b}	2.55 ± 0.06^{b}	$2.45+0.02^{b}$	2.14 ± 0.03^{b}	4.76 ± 0.01^{b}
75 : 25	15.23 ± 0.24^{a}	4.17 ± 0.04^{b}	2.47 ± 0.01^{b}	$2.43+0.03^{b}$	2.15 ± 0.01^{b}	4.81 ± 0.07^{b}
100 : 0	ND	4.11 ± 0.02^{b}	2.63 ± 0.04^{b}	$2.61+0.02^{b}$	2.12 ± 0.01^{b}	4.82 ± 0.06^{b}

ND = not detecte; Different letters in a same column define as significantly different ($p\leq0.05$).

Effect of In Vitro Gastrointestinal Digestion on Monacolin K, Inhibition of Peroxidation, DPPH Radical Scavenging Ability and Chelating Ability on Fe^{2+} of BRTA

The levels of monacolin K, inhibition of peroxidation, DPPH radical scavenging ability, and chelating ability on Fe^{2+} in BRTA for ratios of 25:75, 50:50, 75:25, and 100:0 (roasted broken rice: angkak) after *in vitro* gastrointestinal digestion are presented in Table 3.

The BRTA treatments > 25% angkak after gastric digestion led to higher amounts of monacolin K but the content decreased after intestinal digestion. After gastric digestion, monacolin K contents in BRTA treatments > 25% angkak enhanced from 40.55 to 67.90 mg/kg BRTA, equal to 123.98 to 167.85% compared to raw BRTA. After intestinal digestion, total monacolin K contents obtained from both soluble and insoluble fractions were 60.90, 42.06, and 31.18 mg/kg BRTA for 25:75, 50:50, and 75:25 **(**roasted broken rice: angkak**)**, respectively and reduced by 7.90 and 23.10% compared to after gastric digestion. This result concurred with Kraboun et al.[10] who noted that monacolin K level in monascal waxy corn (MWC) after *in vitro* gastric digestion increased by 1.35% and decreased by 14% after *in vitro* pancreatic digestion. Imitating this stomach condition increased the release of monacolin K extracted from angkak and altered the structure of monacolin K and phenolic compounds in angkak, resulting in an improvement of antioxidant effectiveness.[16] After intestinal digestion, the contents of monacolin K in the soluble fraction of BRTA treatments 75:25, 50:50, and 25:75 (roasted broken rice: angkak) were 27.97, 36.25, and 50.00 mg/kg BRTA extract, respectively and higher than the insoluble fraction (3.21, 5.81, and 10.90 mg/kg BRTA extract, respectively). Results confirmed that monacolin K dissolved well in water because of its higher water-soluble capacity. The reduction of monacolin K after imitating intestinal digestion was due to the mild alkaline condition and O_2 concentration affecting its structure and oxidative stability.[17]

Lipid peroxidation is formed by the autoxidation of unsaturated fatty acids. Lipid peroxides directly affect changes in the properties of cell membranes containing phospholipid bilayers leading to various diseases. Lipid hydroperoxides (LOOH) from this reaction should be scavenged before they develop into aldehydes or ketones **(**rancid odor**)**, malondialdehyde (MDA), and 4-hydroxynonenal (4-HNE).[18] The DPPH free radical scavenging indicates the ability of DPPH free radical inhibition, with hydrogen donation from antioxidants into the odd electrons of nitrogen atoms.[19] The chelating ability on Fe^{2+} relates to the potential for disrupting the complex between ferrozine and Fe^{2+} and manifests as a decrease in red color.[20]

The IC_{50} values of peroxidation inhibition, DPPH free radical scavenging ability, and chelating ability on Fe^{2+} in BRTA treatments > 25% angkak after *in vitro* gastrointestinal digestion were similar to the results of monacolin K. The IC_{50} values of DPPH free radical scavenging ability of BRTA after gastric digestion were 0.07, 0.84, 1.23, and 2.13 mg/ml BRTA extract at 25:75, 50:50, 75:25, and 100:0 (roasted broken rice:angkak), respectively. The IC_{50} values for peroxidation inhibition and chelating ability on Fe^{2+} in all BRTA treatments after gastric digestion ranged from 2.17-4.56 and 1.07-6.03 mg/ml BRTA extract, respectively. After intestinal digestion, the IC_{50} values of DPPH free radical scavenging ability and inhibition of peroxidation and chelating ability on Fe^{2+} of both soluble and insoluble fractions in all BRTA treatments increased. Compared to the inhibition of peroxidation and chelating ability on Fe^{2+}, the DPPH free radical scavenging ability in BRTA was higher in both *in vitro* gastric and intestinal digestion. This implied that BRTA had superior properties of hydrogen atom donation due to the synergistic effect between the Maillard reaction products (MRPs) in roasted broken rice and monacolin K in angkak. Monacolin K available in angkak is well known to have strong antioxidant activity. The MRPs found in roasted broken rice such as melanoidins (high molecular weight (HMW) products) and acrylamides (formed by α-hydroxycarbonyl compounds (reducing sugars) reacting with asparagine) indicated the high potential of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), and ABTS radical scavenging activity.[2,21]

Table 3. Monacolin K, Inhibition of Peroxidation, DPPH Radical Scavenging Ability, and Chelating Ability on Fe^{2+} of BRTA for ratios of 25:75, 50:50, 75:25, and 100:0 Roasted Broken Rice:Angkak after *in vitro* Gastrointestinal Digestion

Mimic digestion processes		Roasted broken rice:angkak	Monacolin K (mg.kg^{-1} BRTA extract)	Inhibition of peroxidation (IC_{50} mg/mL^{-1} BRTA extract)	DPPH free radical scavenging ability (IC_{50} mg/mL^{-1} BRTA extract)	Chelating ability on Fe^{2+} (IC_{50} mg/mL^{-1} BRTA extract)
Gastric digestion		25 : 75	67.90±3.45^{g}	2.17±0.05^{a}	0.07±0.01^{a}	1.07±0.01^{a}
		50 : 50	45.67±2.33^{ef}	3.54±0.05^{b}	0.84±0.05^{a}	3.84±0.01^{b}
		75 : 25	40.55±1.66^{ef}	3.45±0.03^{b}	1.23±0.02^{ab}	4.23±0.03^{bc}
		100 : 0	ND	4.56±0.05^{cd}	2.13±0.03^{b}	6.03±0.03^{cd}
Intestinal digestion	Soluble fraction	25 : 75	50.00+1.05^{f}	4.11±0.05^{cd}	2.11±0.02^{b}	5.89±0.02^{cd}
		50 : 50	36.25±0.93^{e}	5.05±0.01^{d}	5.67±0.01^{c}	5.17±0.02^{c}
		75 : 25	27.97±0.76^{d}	5.78±0.03^{d}	8.08±0.01^{d}	8.34±0.01^{d}
		100 : 0	ND	10.45±0.02^{fg}	9.67±0.05^{e}	8.11±0.05^{d}
	Insoluble fraction	25 : 75	10.90±0.12^{c}	8.45±0.01^{e}	5.29±0.01^{c}	9.29±0.06^{de}
		50 : 50	5.81±0.03^{b}	9.12±0.11^{f}	7.42±0.01^{d}	10.12±0.03^{e}
		75 : 25	3.21±0.18^{a}	10.23±0.15^{fg}	9.11±0.01^{e}	10.11±0.02^{e}
		100 : 0	ND	11.21±0.02^{g}	10.44±0.02^{f}	10.24±0.05^{e}

ND = not detecte; Different letters in a same column define as significantly different ($p \leq 0.05$).

Table 4. IC_{50} Values of α-Glucosidase and α-Amylase Inhibitory Activities of BRTA for Ratios of 25:75, 50:50, 75:25, and 100:0 Roasted Broken Rice:Angkak and Acarbose (Positive Control) after *In Vitro* Gastrointestinal Digestion

Mimic digestion processes		Roasted broken rice:angkak	α-amylase inhibitory activity (IC_{50} mg/mL^{-1} BRTA extract)	α-glucosidase inhibitory activity (IC_{50} mg/mL^{-1} BRTA extract)
Acarbose (without digestion)		-	6.43±0.01^{e}	8.02±0.04^{e}
Gastric digestion		25 : 75	1.25±0.02^{a}	1.78±0.25^{a}
		50 : 50	2.07±0.08^{b}	2.58±0.01^{ab}
		75 : 25	2.02±0.05^{b}	2.67±0.07^{ab}
		100 : 0	3.53±0.09^{c}	4.13±0.01^{b}
Acarbose (through *in vitro* gastric digestion)		-	6.12±0.02^{e}	8.02±0.05^{e}
Intestinal digestion	Soluble fraction	25 : 75	3.25±0.05^{c}	5.78±0.05^{c}
		50 : 50	4.67±0.06^{d}	6.58±0.07^{c}
		75 : 25	4.16±0.01^{cd}	6.67±0.02^{c}
		100 : 0	5.21±0.04^{d}	7.56±0.01^{d}
	Insoluble fraction	25 : 75	5.89±0.12^{f}	5.07±0.06^{f}
		50 : 50	6.67±0.08^{e}	6.89±0.02^{cd}
		75 : 25	6.36±0.05^{e}	6.54±0.07^{c}
		100 : 0	7.53±0.09^{e}	7.01±0.05^{d}
Acarbose (through *in vitro* gastrointestinal digestion)		-	9.16±0.12^{f}	12.18±0.14^{f}

Different letters in a same column define as significantly different ($p \leq 0.05$).

Effect of In Vitro Gastrointestinal Digestion on α-Amylase and α-Glucosidase Inhibitory Activities of BRTA

In patients with type 2 diabetes, insulin rapidly stimulates glucose uptake from the blood into the cells.[22] Hence, an effective therapy is required to control the amount of glucose in the blood immediately after consuming carbohydrate foods.[23] Both α-amylase and α-glucosidase are involved with carbohydrate digestion. α-Amylase hydrolyzes long chain carbohydrate polymers while α-glucosidase hydrolyzes disaccharides into reducing sugars. To break down these enzymes, patients with type 2 diabetes take acarbose to suppress α-glucosidase activity in the small intestine epithelium resulting in a delay in glucose absorption.[24]

Table 4 shows the IC_{50} values of α-amylase and α-

glucosidase inhibitory activities of BRTA for ratios of 25:75, 50:50, 75:25, and 100:0 (roasted broken rice:angkak) and acarbose after *in vitro* gastrointestinal digestion. The IC_{50} values of α-amylase and α-glucosidase inhibitory activities of BRTA after gastric digestion were lower than raw BRTA. The lowest IC_{50} value of α-amylase inhibitory activity of BRTA after gastric digestion was 1.25, followed by 2.07, 2.02, and 3.53 mg/ml for BRTA extract at 25:75, 50:50, 75:25, and 100:0 (roasted broken rice:angkak), respectively. The IC_{50} values of α-glucosidase inhibitory activity of BRTA after gastric digestion were 1.78, 2.58, 2.67, and 4.13 mg/ml BRTA extract for 25:75, 50:50, 75:25, and 100:0 (roasted broken rice:angkak), respectively and higher than α-amylase inhibitory activity. The IC_{50} values of α-amylase and α-glucosidase inhibitory activities from acarbose (an antidiabetic drug for treating type 2 diabetic patients) before and after *in vitro* gastric digestion were 6.12-6.43 and 8.02, respectively (p>0.05) and lower than BRTA after gastric digestion. Compared to acarbose, BRTA was more satisfactory for retarding carbohydrate polymers transformed into disaccharides by α-amylase, confirming that monacolin K in angkak plays a direct role as an α-amylase and α-glucosidase inhibitor. This experimental condition under gastric digestion caused higher release of significant compounds from BRTA[10] as monacolin K and MRPs, promoting α-amylase and α-glucosidase inhibitory activities.[8,25] *M. purpureus* produced monacolins and also phenolic compounds γ-aminobutyric acid, and dimerumic acid which have strong antioxidant activities and also α-amylase and α-glucosidase inhibitory activities.[6] This result concurred with Kraboun et al.,[10] who noted that increased levels of monacolin K in angkak after *in vitro* gastric digestion resulted in higher α-amylase and α-glucosidase inhibitory activities.

After intestinal digestion, the IC_{50} values of the soluble fraction of α-amylase inhibitory activity, ranging 3.25-5.21 $mg.ml^{-1}$ BRTA extract, were lower than in the insoluble fraction (5.89-7.53 $mg.ml^{-1}$ BRTA extract). The highest α-amylase and α-glucosidase inhibitory activities in both the soluble and insoluble fractions were recorded in BRTA ratio 25:75 (roasted broken rice: angkak). The α-amylase and α-glucosidase inhibitory activities in both fractions in all BRTA treatments decreased after *in vitro* intestinal digestion. This confirmed that the α-amylase and α-glucosidase inhibitors (monacolin K and MRPs) in BRTA were destroyed by the mild alkaline condition within the imitation intestinal system and the structures and/or activities of these inhibitors were altered due to oxidative degradation and polymerization reactions.[17,26] This result concurred with Ohta et al.[27] who suggested that significant soluble antioxidants in food constituents mostly occurred in water-based systems.

The Correlation among the Dependent Variables of BRTA before and after In Vitro Gastrointestinal Digestion

The loading plots of principal component analysis (PCA) and Pearson's correlation coefficient from data of the dependent variables of BRTA in all treatments before and after *in vitro* gastrointestinal digestion are presented in Figure 1 and Table 5, respectively. PCA was performed to classify the correlation among the dependent variables, whereas Pearson's correlation coefficient represents the correlation direction between two dependent variables and their significance.[10] For raw BRTA, the eigenvalues of the two principal components (PCs) accounted for 87.681% of the total variance. PC1 explained 71.814% of the total variance containing a_w, moisture content, a*, and b*. In PC1, moisture content correlated positively with a* (r = 0.976, $p\leq$0.05) and a_w correlated positively with b* (r = 0.991, $p\leq$0.01) while PC2 only explained 16.867% of the total variance as monacolin K, α-amylase and α-glucosidase inhibition activities, and L*. No significant correlation of dependent variables was found in PC2. For the dependent variable relationships between PC1 and PC2, significantly

Table 5. Pearson's Correlation Coefficient among Dependent Variables of BRTA for Ratios of 25:75, 50:50, 75:25, and 100:0 Roasted Broken Rice:Angkak before (A) and after (B) *In Vitro* Gastrointestinal Digestion

A	L*	a*	b*	Moisture content	a_w	Monacolin K	α-amylase inhibitory activity
a*	0.250						
b*	0.577	0.934					
Moisture content	0.163	0.976*	0.890				
a_w	0.682	0.878	0.991**	0.822			
Monacolin K	0.066	0.178	0.134	-0.029	0.143		
α-amylase inhibitory activity	-0.539	-0.947	-0.992**	-0.881	-0.979*	-0.253	
α-glucosidase inhibitory activity	0.059	-0.930	-0.770	-0.975*	-0.678	0.049	0.770

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

B	Monacolin K	Inhibition of peroxidation	DPPH free radical scavenging ability	Chelating ability on Fe^{2+}	α-amylase inhibitory activity
Inhibition of peroxidation	-0.418				
DPPH free radical scavenging ability	-0.432	0.910**			
Chelating ability on Fe^{2+}	-0.623*	0.894**	0.862**		
α-amylase inhibitory activity	-0.188	-0.319	-0.213	-0.284	
α-glucosidase inhibitory activity	-0.554	0.789**	0.898**	0.817**	-0.073

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

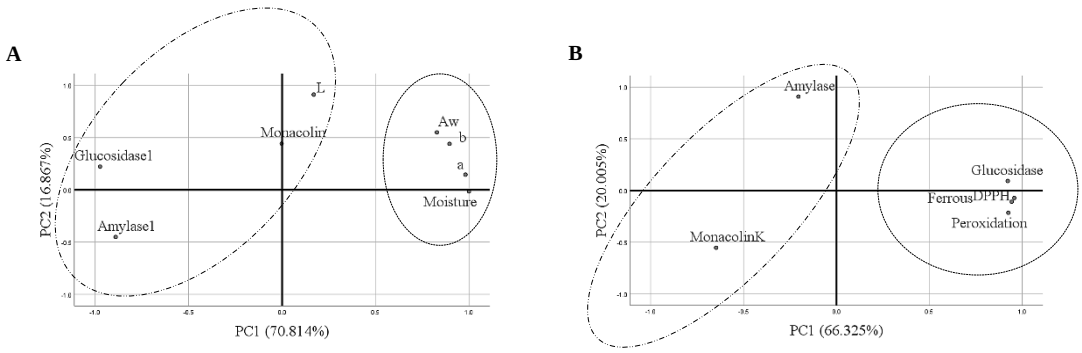

Figure 1. Loading Plots of Principal Component Analysis (PCA) from the Dependent Variables of BRTA for Ratios of 25:75, 50:50, 75:25, and 100:0 Roasted Broken Rice:Angkak before (A) and after (B) *In Vitro* Gastrointestinal Digestion.

negative correlations were found between b* and α-amylase inhibitory activity (r = -0.992, $p \leq 0.01$), a_w and α-amylase inhibitory activity (r = -0.979, $p \leq 0.05$), and moisture content and α-glucosidase inhibition activity (r = -0.975, $p \leq 0.05$).

After *in vitro* gastrointestinal digestion, the eigenvalues of the two principal components explained 86.33% of the total variance. PC1 contained α-glucosidase inhibition activity, inhibition of peroxidation, DPPH free radical scavenging ability, and chelating ability on Fe^{2+}, accounting for 66.325% of the total variance. Positive correlations shown in PC1 were (1) α-glucosidase inhibitory activity and inhibition of peroxidation (r = 0.789, $p \leq 0.01$), (2) α-glucosidase inhibitory activity and DPPH free radical scavenging ability (r = 0.898, $p \leq 0.01$), (3) α-glucosidase inhibitory activity and chelating ability on Fe^{2+} (r = 0.817, $p \leq 0.01$), (4) inhibition of peroxidation and DPPH free radical scavenging ability (r = 0.910, $p \leq 0.01$), (5) inhibition of peroxidation and chelating ability on Fe^{2+} (r = 0.894, $p \leq 0.01$), and (6) DPPH free radical scavenging ability and chelating ability on Fe^{2+} (r = 0.862, $p \leq 0.01$). PC2 accounted for only 20.005% of the total variance, representing monacolin K and α-amylase inhibition activity. This result indicated a negative correlation between monacolin K and chelating ability on Fe^{2+} (r = -0.623, $p \leq 0.05$).

Raw BRTA, α-amylase and α-glucosidase inhibition activities could be used as indices for b* and a_w, whereas after *in vitro* gastrointestinal digestion, α-glucosidase inhibitory activity could be used as an index for all antioxidant activities.

Conclusion

The addition of angkak produced a darker BRTA sample and enhanced monacolin K, DPPH free radical scavenging ability, inhibition of peroxidation and chelating ability on Fe^{2+}, and α-amylase and α-glucosidase inhibitory activities. After *in vitro* gastric digestion, the monacolin K content in BRTA at ratio 25:75 (roasted broken rice:angkak) was enhanced by 167.85% compared to raw BRTA. The lowest IC_{50} values of DPPH free radical scavenging ability, inhibition of peroxidation and chelating ability on Fe^{2+}, and α-amylase and α-glucosidase inhibitory activities found in this treatment were 0.07, 2.17, 1.07, 1.25, and 1.78 mg/ml BRTA extract, respectively. After *in vitro* gastrointestinal digestion, monacolin K, DPPH free radical scavenging ability, chelating ability on Fe^{2+}, and α-glucosidase and α-amylase inhibitory activities in all BRTA treatments decreased, with levels in the soluble fraction higher than in the insoluble fraction. Using PCA and Pearson's correlation coefficient to consider the correlation of dependent variables in BRTA before and after *in vitro* gastric digestion showed that monacolin K could be used as an index for α-amylase inhibition activity.

Authors' Contributions

PP and NU provided resources, designed the experiments and wrote the original manuscript. KK supervised the research, interpreted the data and edited the manuscript. All authors reviewed and approved the articles.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

References

1. Balindong JL, Ward RM, Liu L, Rose TJ, Pallas LA, Ovenden BW, et al. Rice grain protein composition influences instrumental measures of rice cooking and eating quality. J Cereal Sci. 2018;79:35-42. doi:10.1016/j.jcs.2017.09.008
2. Kraboun K, Phanumong P, Nudang S, Klinpikul N. Optimization of roasting treatment to reduce acrylamide content and increase melanoidins content and diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), copper chelating activity and ABTS radical scavenging activity in roasted polished-rice tea. JFHB. 2022;15:10-18.
3. Yang JH, Tseng YH, Lee YL, Mau JL. Antioxidant properties of methanolic extracts from monascal rice. LWT-Food Sci Technol. 2006;39:740-7. doi:10.1016/j.lwt.2005.06.002
4. Al-Ghafari AB, Shorbaji AM, AL-Sarori LA, Baduwailan EO, Basaar AA, Al Doghaither HA, et al. Phenolic contents and antioxidant activities of green tea with and without lemon. Nat Sci. 2016;8:247-55. doi:10.4236/ns.2016.86029

5. Gupta RK, Chawla PR, Tripathi M, Shukla AK, Pandey AR. Synergistic antioxidant activity of tea with ginger, black pepper and tulsi. Int J Pharm Pharm Sci. 2014;6(5):477-9.
6. Kraboun K, Kongbangkerd T, Rojsuntornkitti K, Phanumong P. Factors and advances on fermentation of *Monascus* sp. for pigments and monacolin K production: a review. Int Food Res J. 2019;26:751-61.
7. Kraboun K, Tochampa W, Chatdamrong W, Kongbangkerd T. Effect of MSG and peptone on antioxidant activity of monascal waxy corn. Int Food Res J. 2013;20:623-31.
8. Srianta I, Kusumawati N, Nugerahani I, Artanti N, Xu GR. *In vitro* α-glucosidase inhibitory activity of *Monascus*-fermented durian seed extracts. Int Food Res J. 2013; 20:533-6.
9. Miltenburg J, Bastiaan-Net S, Hoppenbrouwers T, Wichers H, Hettinga K. Gastric clot formation and digestion of milk proteins in static *in vitro* infant gastric digestion models representing different ages. Food Chem. 2024;432:137209. doi:10.1016/j.foodchem.2023.137209
10. Kraboun K, Phanumong P, Tochampa W, Jittrepotch N, Rojsuntornkitti K, Chatdamrong W, et al. Impact of *in vitro* digestion phases on antioxidant properties of monascal waxy corn from 2-step fermentation. J Microbiol Biotechnol Food Sci. 2018;7:454-6. doi:10.15414/jmbfs.2018.7.5.454-456
11. Aleixandre-Tudo JL, Castello-Cogollos L, Aleixandre JL, Aleixandre-Benavent R. Chemometrics in food science and technology: A bibliometric study. Chemom Intell Lab Syst. 2022;222:104514. doi:10.1016/j.chemolab.2022.104514
12. Kuo CF, Hou MH, Wang TS, Chyau CC, Chen YT. Enhanced antioxidant activity of *Monascus pilosus* fermented products by addition of ginger to the medium. Food Chem. 2009;116:915-22. doi:10.1016/j.foodchem.2009.03.047
13. Liu S, Li D, Huang B, Chen Y, Lu X, Wang Y. Inhibition of pancreatic lipase, α-glucosidase, α-amylase, and hypolipidemic effects of the total flavonoids from *Nelumbo nucifera* leaves. J Ethnopharmacol. 2013;149: 263-9. doi:10.1016/j.jep.2013.06.034
14. Zhang J, Zhao S, Yin P, Yan L, Han J, Shi L, et al. α-Glucosidase inhibitory activity of polyphenols from the burs of *Castanea mollissima* Blume. Molecules. 2014;19:8373-86. doi:10.3390/molecules19068373
15. Hajjaj H, Niederberger P, Duboc P. Lovastatin biosynthesis by *Aspergillus terreus* in a chemistry defined medium. Appl Environ Microbiol. 2001;67:2596-2602. doi:10.1128/AEM.67.6.2596-2602.2001
16. Baublis AJ, Lu C, Clydesdale FM, Decker EA. Potential of wheat-based breakfast cereals as a source of dietary antioxidants. J Am Coll Nutr. 2000;19:308S-311S. doi:10.1080/07315724.2000.10718965
17. Rufian HJA, Delgado AC. Effect of digestive process on Maillard reaction indexes and antioxidant properties of breakfast cereals. Int Food Res. 2009;42:394-400. doi:10.1016/j.foodres.2009.01.011
18. Gil MI, Thomas Barberan FA, Hess-Pierce B, Hplcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relation ship with phenolic composition and processing. J Agric Food Chem. 2000;48:4581-9. doi:10.1021/jf000404a
19. Contreras-Guzman ES, Strong FC. Determination of tocopherols (vitamin E) by reduction of cupric ion. J AOAC Int. 1982;65:1215-21. doi:10.1093/jaoac/65.5.1215
20. Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR. Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. Afr J Biotechnol. 2008;7: 3188-92.
21. Gniechwitz D, Reichardt N, Meiss E, Ralph J, Steinhart H, Blaut M, et al. Characterization and fermentability of an ethanol soluble high molecular weight coffee fraction. J Agric Food Chem. 2008;56:5960-9. doi:10.1021/jf800231q
22. Orme CM, Bogan JS. Sorting out diabetes. Science. 2009;324:1155-6. doi:10.1126/science.1174841
23. Yu Z, Yin Y, Zhao W, Liu J, Chen F. Anti-diabetic activity peptides from albumin against α-glucosidase and α-amylase. Food Chem. 2012;135:2078-85. doi:10.1016/j.foodchem.2012.06.088
24. Rosak C, Mertes G. Critical evaluation of the role of acarbose in the treatment of diabetes: patient considerations. Diabetes Metab Syndr Obes. 2012;5:357-67. doi:10.2147/DMSO.S28340
25. Hwanga IG, Kim HY, Woo KS, Hong JT, Hwang BY, Jung JK, et al. Isolation and characterisation of an α-glucosidase inhibitory substance from fructose–tyrosine Maillard reaction products. Food Chem. 2011;127:122-6. doi:10.1016/j.foodchem.2010.12.099
26. Talcott ST, Howard LR. Phenolic autoxidation is responsible for color degradation in processed carrot puree. J Agric Food Chem. 1999;47:2109-15. doi:10.1021/jf981134n
27. Ohta T, Yamasaki S, Egashira Y, Sanada H. Antioxidative activity of corn bran hemicellulose fragments. J Agric Food Chem. 1994;42:653-6. doi:10.1021/jf00039a010