NFS Journal 33 (2023) 100154

Contents lists available at ScienceDirect

NFS Journal

journal homepage: www.elsevier.com/locate/nfs

The role of herbal teas in reducing the starch digestibility of cooked rice (*Oryza sativa* L.): An *in vitro* co-digestion study

Thiraphong Aumasa [a,c], Yukiharu Ogawa [c], Jaspreet Singh [d,e], Worawan Panpipat [f], Natthawuddhi Donlao [a,b,*]

[a] *Unit of Innovative Food Packaging and Biomaterials, School of Agro-Industry, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[b] *Tea and Coffee Institute, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[c] *Graduate School of Horticulture, Chiba University, 648, Matsudo, Matsudo 271-8510, Japan*
[d] *School of Food and Advanced Technology, Massey University, Palmerston North 4442, New Zealand*
[e] *Riddet Institute, Massey University, Palmerston North 4442, New Zealand*
[f] *Food Technology and Innovation Research Center of Excellence, School of Agricultural Technology and Food Industry, Walailak University, Nakhon Si Thammarat 80160, Thailand*

ARTICLE INFO

Keywords:
Antioxidant activity
Glycemic index
Herbal tea
In vitro digestion
Phytochemicals

ABSTRACT

Herbal teas are well known for their antidiabetic effects due to the abundance of phenolic acids, flavonoids, and tannins. An *in vitro* co-digestion test was conducted to observe influence of herbal teas (HTs) *i.e.*, beal fruit (BA), mulberry leaf (MB), gymnema leaf (GM), and chrysanthemum flower (CS) on starch digestibility of cooked rice and to elucidate correlation analysis of phytochemicals and their antioxidant activities during *in vitro* starch hydrolysis (SH). HTs prepared from GM and MB showed highest reduction of SH, kinetic constant and estimated glycemic index (eGI) of cooked rice, followed by BA and CS. Besides, MB and GM teas decreased the eGI of cooked rice up to 15%, followed by BA and CS teas, respectively. Phytochemicals and their antioxidant activities were positively correlated to SH, with both parameters displaying a higher decrease in intestinal phase when compared to gastric phase during *in vitro* co-digestion. These results indicate a strong interaction among phytochemicals and α-amylase in influencing glycaemic parameters. Thus, HTs has the potential to provide functional health benefits in lowering postprandrial hyperglycemia.

1. Introduction

Rice (*Oryza sativa* L.) is a staple food for people, mostly in Asian countries, with an estimated global consumption of 402 million metric tons in 2016 and 2017, and a global *per capita* food usage of 54.1 kg per person. White rice is the most consumed form of rice. It is usually produced by a physical method to remove the hull and bran layers, thereby exposing the starchy endosperm [1]. This contributes to an overall high starch content of up to 90% (DW). Globally, approximately 382 million people have type 2 diabetes [2], which is strongly associated with the consumption of foods with a high glycemic index (GI). Beside, The estimated national prevalence of diabetes in Thai adults was 2.4 million people. Likewise, the age-adjusted prevalence of diabetes has increased from 7.8% in 2009 to 9.9% in 2014 [3,4]. A cohort study revealed on white rice intake that is linked to an increased likelihood of developing diabetes, with the most notable connection observed in South Asia. In contrast, in other regions, the connection appears to be weaker and not statistically significant [5]. As, South Asian countries, carbohydrates obtained from white rice constitute about 70% to 80% of the calories people consume. Meanwhile, various drugs have been developed as α-glucosidase inhibitors for diabetic treatment, for instance acarbose, voglibose, and miglitol. Those are extensively employed as medications in lowering blood sugar levels in clinical approach. Nevertheless, this group of drugs has a dual nature, as it can lead to undesirable side effects such as flatulence, diarrhea, nausea, and others [6].

Currently, the development of natural and efficient hypoglycemic products is a prominent focus in the realm of diabetes treatment. Numerous studies noticeably reported that plant polyphenols could inhibit starch digestibility through digestive enzymes inhibition [7–9]. The key plant polyphenols are mostly obtained from green tea and herbal tea [10]. Herbal teas, on the other hand, are safer, more effective, and have greater benefits in the prevention and treatment of diabetes

* Corresponding author at: Unit of Innovative Food Packaging and Biomaterials, School of Agro-Industry, Mae Fah Luang University, Chiang Rai 57100, Thailand.
E-mail address: natthawuddhi.don@mfu.ac.th (N. Donlao).

https://doi.org/10.1016/j.nfs.2023.100154
Received 5 July 2023; Received in revised form 10 October 2023; Accepted 11 October 2023
Available online 14 October 2023

than other common tea [11]. Beside, plant phenolics and saponins are positively correlated with antioxidant, anti-inflammatory, anti-glycation, anti-α-glucosidase, and anti-α-amylase activities. Nevertheless, the effectiveness of these bioactivities varies depending on the polarity of the solvent and the method of plant extraction [12].

Currently, hebal teas have been developed into functional beverage from various plant materials including fresh or dried roots, stems, leaves, fruits, flowers, seeds, bark, or whole plants. Numberous investigation have already been conducted on the tea processing methods with the aim of enhancing its bioactive compounds (*e.g.*, carotenoids, phenolic acids, flavonoids, and tannins [13]) and functional activities *i.e.*, fermentation, drying method [14], roasting [15], honey method, salting method, and stewing method [16]. Thereby, popularity of herbal teas has gradually increased among health-conscious consumers and has been tapped into an emerging niche market. Beside, Thailand has several plant species that have been used as herbal teas in traditional medicine by ethnic minority groups and rural Thai communities to treat diabetes [17]. Some plants have been clinically proven to be antidiabetic, including beal fruit, mulberry leaf, Gymnema, and chrysanthemum. These plants are commonly prepared through infusion and decoction, consequently intake with meals. A cohort study revealed people who had a higher intake of green tea with rice showed a reduced risk of diabetes. A high intake of green tea may provide protection against the elevated risk of diabetes associated with a higher intake of rice especially in female [18], ignoring the experimental trials on the influence of tea on it's inhibition capacity against starch digestibility of rice. Also, there is no available data on the correlation analysis of phytochemicals against *in vitro* starch digestibility.

Our group therby undertook investigation regarding co-digestion among herbal teas and cooked rice through static *in vitro* digestion model, in order to elucidate their potential advantage in lowering postprandial hyperglycemie. Hence, this study aimed to evaluate the influence of herbal teas on *in vitro* starch digestibility of cooked rice and to reveal correlation analysis of phytochemicals against *in vitro* starch hydrolysis.

2. Materials and methods

2.1. Samples

Four commercially herbal teas were supplied by the Thai Tea Suwirun Co., Ltd. in Chiang Rai, Thailand. Details are presented in Table 1. The raw materials were subjected to traditional commercial processing including roasting, rolling, and drying. Briefly, BA was roasted at 200 °C for 12 min and dried at 110 °C for 60 min. MB was roasted at 280 °C for 10 min and dried at 100–125 °C for 80 min. GM was spread on bamboo trays, allowed to wither at ambient temperature for 30 min and further roasted at 220 °C for 12 min prior rolling for 10 min using a rolling machine (Yuan Chang Machinery, Taoyuan, Taiwan), and dried at 120 for 30 min. CS was roasted at 220 °C for 12 min and dried at 90 °C for 60 min. All the raw materials were roasted in a drum roaster (Yuan-Chang Machinery, Taoyuan, Taiwan). Drying was performed using an electric convection hot air dryer (Kluay NamThai, Bangkok, Thailand). White Thai Hom mali (*Oryza sativa* L. cv. KDML105) or so-called “Thai jasmine rice” (long grain) was purchased from Khao C.P. Co., Ltd., Ayutthaya, Thailand. The moisture content of the rice sample was 11.41 g/100 g on a wet basis.

2.2. Chemicals

Amyloglucosidase (3260 U/mL) was purchased from Megazyme International Ireland, Ltd. (Wicklow, Ireland). Pepsin (P7000, porcine gastric mucosal >250 U/mg solid), pancreatin (hog pancreas, 4 × USP), invertase (invertase from Baker's yeast, grade VII, 301 U/mg solid), were purchased from Sigma-Aldrich (Missouri, USA). Other chemicals were all analytical grades.

2.3. Preparation of herbal tea infusion

Herbal tea samples were prepared according to the method described by Donloa and Ogawa [15] with slightly modifications. Briefly, herbal tea (2.5 g) was mixed with 250 mL 95 °C hot water for 5 min with continuous stirring. The sample was then filtered through Whatman No. 1 filter paper (GE Healthcare UK, Buckinghamshire, UK). The filtrate was then immediately cooled down to 37 °C in a water bath (Memmort, Schwabach, Germany). The herbal tea infusion was immediately used for *in vitro* digestion testing to prevent any possible changes.

2.4. Rice cooking and sample preparation

Rice samples were cooked as per previously published rice cooking methods by Tamura et al. [19]. Briefly, 30 g of rice grain was placed in a 100 mL beaker and soaked in 45 mL of distilled water**.** Then, the beaker containing rice was placed in a water bath at 30 °C for 30 min**.** The beaker was placed in an electric rice cooker ***(***Shape, Tokyo, Japan***)*** with 250 mL of distilled water and cooked for 45 min**.** The absence of a white core was used to visually indicate that the grains were completely gelatinized. The.

2.5. Simulated in vitro digestion

Co-digestion test was performed according to a simulated *in vitro* digestion model described by Tamura et al. [20]. The total starch in the mixture was initially fixed at 4% *w*/w. To achieve 4% total starch in glass reactor, 8.82 g of cooked rice grains (kernel length was 7.76 ± 1.02 mm) and 161.18 g of each tea infusion were added to a glass reactor prior co-digestion test while the water was replaced as a control sample. The temperature of the reactor was maintained at 37 °C throughout the experiment and the liquid sample in the reactor was continuously stirred using a magnetic stirrer. The pH was adjusted to 2.00 and pepsin solution was added to initiate the gastric phase before adjusting the pH to 1.20. At intervals of 0 and 30 min, liquid samples (0.5 mL) were collected from the reactor and placed in a centrifuge tube containing 3.00 mL 95% (*v*/v) ethanol. The pH of the reactor was adjusted to 6.00 after 30 min of the gastric phase. The small intestine phase was initiated by adding an intestinal enzyme solution containing invertase, pancreatin, and amyloglucosidase. The pH of the solution was adjudted to 6.80. At intervals of 0, 5, 10, 15, 30, 60, 90, 120, 180, 240, 300, and 360 min, a liquid sample (0.5 mL) was collected from the reactor and placed in a centrifuge tube containing 3.00 mL 95% (*v*/v) ethanol. The tubes containing the liquid samples were centrifuged at 1800*g* for 10 min. TPC, TFC, TTC, FRAP, and DPPH were assessed during both the gastric and intestinal phases, whereas eGI was assessed during the intestinal phase of digestion.

Table 1
List of Thai herbal teas in the experiment, with English commom name, Thai common name, scientific name, family, plant parts and moisture content.

English common name	Thai common name	Scientific name	Family	Parts used	Moisture content (g/100 g WB)
Bael fruit (BA)	Ma-Tuum	*Aegle marmelos*	Rutaceae	Fruit	9.58 ± 0.07
Mulberry (MB)	Bai-Mon	*Morus alba*	Moraceae	Leaf	3.46 ± 0.01
Gymnema (GM)	Pak-Chiang-Da	*Gymnema inodorum*	Apocynaceae	Leaf	3.49 ± 0.06
Chrysanthemum (CS)	Dok-Kek-Huay	*Chrysanthemum indicum*	Asteraceae	Flower	3.68 ± 0.02

2.6. Glucose release and kinetics of starch hydrolysis

Glucose release and the kinetics of starch hydrolysis were determined according to the method described by Donlao and Ogawa [21]. The released glucose was measured using a D-glucose test kit (GOPOD Format K-GLUC 04/20) purchased from Megazyme International (Wicklow, Ireland). A 100 mL of supernatant was digested with 0.5 mL and amyloglucosidase in acetate buffer with a pH of 5.20 and incubated for 10 min in a water bath at 37 °C. The mixture was then mixed with 3 mL GOPOD solution and incubated for 20 min in a water bath at 50 °C. The amount of glucose released was determined at 510 nm using a spectrophotometer (Thermo Fisher Scientific, Waltham, United States). The data were expressed as a percentage of starch hydrolysis, as shown in the following equation:

$$\%S_H = S_h/S_i = 0.9 \times G_p/S_i \quad (1)$$

where $\%S_H$ is the percentage of starch hydrolysis, S_h is the quantity of starch hydrolyzed, S_i is the starting amount of starch, and G_p is the amount of glucose generated. A conversion factor of 0.9 was employed, which is commonly calculated as the ratio of the molecular weight of the starch monomer to the molecular weight of glucose (162 / 180 = 0.9).

The first-order equation model described by Goni et al. [22] was used to represent the kinetics of the starch hydrolysis.

$$C = C_\infty \left(1 - exp.^{-k\,t}\right) \quad (2)$$

where k is the kinetic constant, t is the time, C is the proportion of hydrolyzed starch at time t, and C_∞ is the equilibrium starch concentration in the intestinal phase.

The hydrolysis index (HI), which represents starch hydrolysis, was calculated by dividing the area under the curve of starch hydrolysis during the intestinal phase by the area of a reference starch hydrolysis sample or white bread. The estimated glycemic index (eGI), which shows the carbohydrates ingested in various kinds of food based on the postprandial level of blood glucose, was determined according to Eq. (3).

$$eGI = 39.71 + 0.549HI \quad (3)$$

2.7. Determination of phytochemicals

Total phenolic content (TPC) was determined according to the method of Singleton et al. [23]. Briefly, the sample (0.5 mL) was added to 2.5 mL of 10% *v*/v Folin- Ciocalteu reagent. 2.0 mL of Sodium carbonate solution (7.5% *w/v*) was then added. The samples were kept in the dark for 60 min before measuring absorbance at 765 nm using a spectrophotometer. TPC was calculated and expressed as gallic acid equivalents (GAE) per gram of the dried sample.

The aluminum chloride method was used to determine the total flavonoid content (TFC) [24]. In a 10 mL-volumetric flask, 1 mL of the sample was mixed with 4 mL of distilled water. Then, 0.30 mL of 5% sodium nitrite was added and the mixture was allowed to stand at ambient temperature for 5 min. The mixture was then combined with 0.3 mL of 10% aluminum chloride. After 5 min, 2 mL of 1 M sodium hydroxide was added and the final volume was adjusted to 10 mL with distilled water. The absorbance of each sample was measured at 510 nm wavelength using a spectrophotometer. The TFC was calculated and expressed as quercetin equivalents (QUE) per gram of dried sample.

Total tannin content (TTC) was determined using the Folin-Ciocalteu method [25]. In 10 mL-volumetric flask, 0.1 mL of the sample was added to 7.5 mL distilled water and 0.5 mL Folin-Ciocalteu phenol reagent. Then, 1 mL of 35% sodium carbonate solution was added and the final volume was adjusted to 10 mL with distilled water. The mixture was vigorously mixed and allowed to stand at an ambient temperature for 30 min. The absorbance of each sample was measured at 700 nm wavelength using a spectrophotometer. TTC was calculated and expressed as tannic acid equivalents (TAE) per gram of the dried sample.

2.8. Determination of antioxidant activities

The DPPH radical scavenging activity (DPPH assay) was determined using the method described by Roy et al., [26]. Briefly, 50 μL was combined with 2000 μL of 60 μM DPPH reagent. The mixture was allowed to stand at ambient temperature in the dark for 60 min. The absorbance was measured at 517 nm using a spectrophotometer. The percentage inhibition (% inhibition) was calulated by comparing the absorbance of the control and sample. The DPPH activity of the samples was calculated and expressed as micromolar equivalents of trolox (TE) per gram of dried sample.

The ferric reducing antioxidant power activity (FRAP assay) was determined as described by Yen and Chen [27]. Briefly, 200 μL of the sample was combined with 1.3 mL FRAP reagent. After 30 min of incubation at 37 °C, the absorbance at 595 nm of the sample was measured using a spectrophotometer. FRAP was calculated and expressed in micromole equivalents of ferrous sulfate ($FeSO_4$) per gram of the dried sample.

2.9. Statistical analysis

Data are expressed as the mean ± standard deviation. The data were also subjected to analysis of variance (ANOVA) and Duncan's multiple range tests using SPSS 22.0 (IBM Corporation, New York, USA) for Windows. Differences were considered statistically significant at $P < 0.05$. Correlations between TPC, TFC, DPPH, FRAP, and eGI were evaluated using the Minitab 20 (Minitab Pty, Sydney, Australia). The first-order equation from the curve-fitting model was evaluated using SigmaPlot version 12 (Systat Software Inc., California, USA).

3. Results and discussion

3.1. Change of phytochemicals during in vitro co-digestion

The changes in phytochemicals are presented in Fig. 1. The TPC values during the gastric phase decreased slightly in all samples, except for GM (Fig. 1a). The highest TPC reduction was observed for MB (−16.92%), BA (−7.50%), and CS (−0.42%) (Table 2). The slight reduction in TPC during *in vitro* co-digestion resulted from the stability of TPC, which can tolerate acidic conditions such as the gastric phase, rather than alkaline conditions in the intestinal phase [28]. Additionally, Friedman and Jürgens [29] demonstrated that the reduction in TPC could be attributed to the instability of polyphenolic compounds at high pH. Larger molecules may be more stable, whereas smaller molecules such as gallic acid are unstable at high pH, that is, the intestinal phase. However, the increase in TPC in GM (9.80%) resulted from the breaking bond of the oleanane saponin group, that is, gymnemic acids and gymnemasaponins, which are the two major TPC in GM [30]. Breaking the bonds between these compounds results in the fragmentation of polyphenolic compounds under acidic conditions [31]. Fig. 1b shows the changes in TFC during *in vitro* co-digestion; most of the TFC in all samples declined in both the gastric and intestinal phases. However, an increase in the TFC in the BA (13.56%) was observed in the gastric phase (Table 2) as a result of fragmentation of TFC into many fragments. Hazra et al. [32] reported that the major flavonoid present in BA is rutin, a glycoside composed of the disaccharide rutinose and quercetin that can be fragmented under acidic conditions. A reduction in TTC was also observed in all the samples (Fig. 1c) in both the gastric and intestinal phases, except for BA during the gastric phase, which showed an increase in TTC (14.01%) as a result of tannin fragmentation. The percentage changes in phytochemicals during the gastric and intestinal phases and total changes are summarized in Table 2. The percentage total change in TPC, TFC, and TTC was highest in MB (−81.86–58.85, and-63.17%, respectively). Additionally, the percentage change in

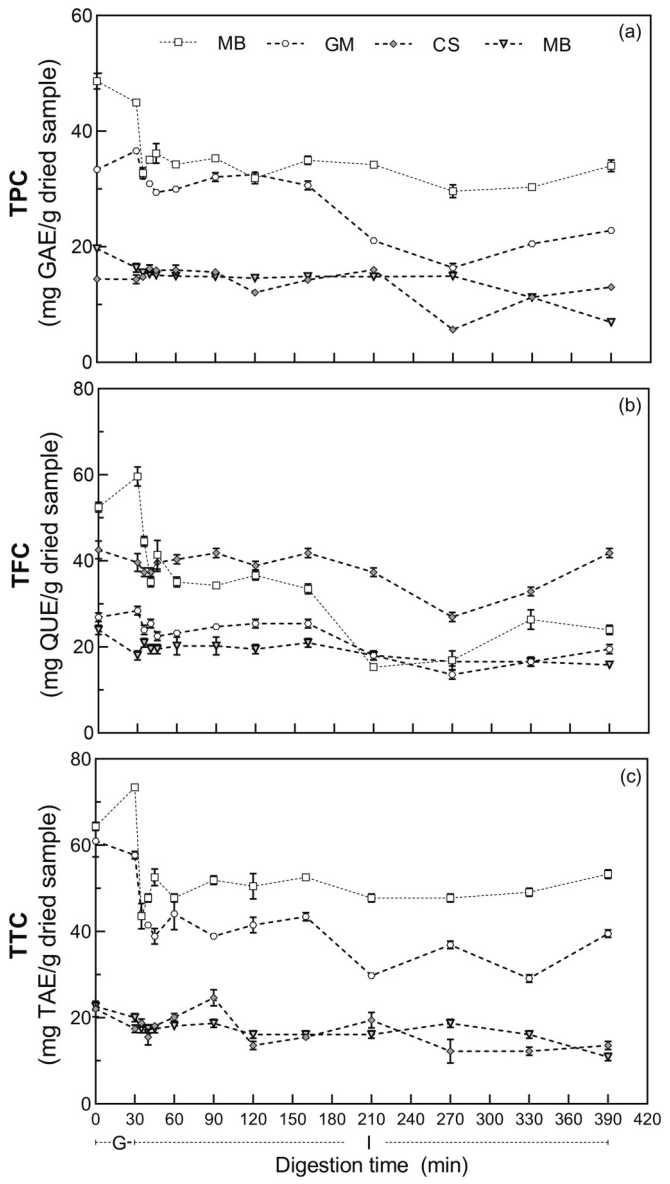

Fig. 1. Changes of TPC (a), TFC (b), and TTC (c) during *in vitro* co-digestion of different type of herbal teas co-digested with intact grain cooked Thai jasmine rice. *Error bars represent standard deviations (n = 3). Abbreviation: gastric phase (G) and intestinal phase (I).

phytochemicals in all samples during the gastric phase was smaller (−24.79 to 14.10%) than that in the intestinal phase (−64.94 to −1.76%). This might be a result of greater sensitivity to phytochemicals during the intestinal phase than during the gastric phase.

3.2. Change of antioxidant activities during in vitro co-digestion

DPPH is one of the chemicals that contains a proton free radical with a distinct absorption that is greatly reduced when exposed to proton radical scavengers. The current investigation demonstrated (Fig. 2a) a reduction in DPPH in both the gastric and intestinal phases in all samples. However, BA showed a slight increase in DPPH during the gastric phase. This result indicated a strong correlation between the increase in TFC and TTC during the gastric phase. Hence, TFC and TTC in BA play important roles in DPPH scavenging. Similarly, a slight increase in DPPH was observed in GM during the gastric phase because of the increase in TPC. In contrast, during the intestinal phase, DPPH gradually decreased in all the samples. A previous study reported that an alkaline pH destroyed antioxidants, resulting in a net reduction in DPPH activity during *in vitro* digestion. Phytochemicals may be subjected to such conditions, and some can be converted into various structural forms with varying chemical characteristics, bioaccessibility, bioavailability, and biological activity [33]. Thus, the variation in DPPH activity may be the result of the presence of specific polyphenol structures. The percentage of total change in DPPH activity is summarized in Table 2. The percentage change in DPPH during the gastric phase was ranged smaller (1.31–16.07%) than that in the intestinal phase (−13.02 to −76.76%) due to lower reduction of TPC, TFC, and TTC during the gastric phase, which could lead to higher DPPH activity. The percentage of total change ranged from −7.31 to - 89.06%. The highest percentage of total change was observed for MB, whereas the lowest percentage was observed for BA. FRAP indicates the reducing power of antioxidants in the samples. Fig. 2b shows that FRAP was slightly decreased in the gastric phase in all samples, except for BA. During the intestinal phase, FRAP gradually decreased with digestion time. Interestingly, it sharply increased after 300 min of digestion. Bouayed et al. [34] demonstrated that numerous factors contribute to the increase in antioxidant activities during *in vitro* digestion, including acid-base reactions and interactions between phytochemicals and other dietary components from cooked rice released during digestion, such as minerals, dietary fiber, and protein. Percentage change in FRAP (Table 2) during the gastric phase ranged smaller (−12.00 to 9.80%) compared to the intestinal phase (−12.00 to −53.75%) and the percentage total change was ranged from −24.00 to −55.90%. The highest total change was observed for MB, in a manner similar to that of DPPH. Hence, the change in FRAP was strongly correlated with the change in DPPH, whereas the lowest change was observed for CS. Additionally, previous studies have indicated that the shift from the gastric to intestinal phase alters the structure of polyphenolic compounds. During intestinal digestion, these molecules can be converted into several structural forms with varying chemical characteristics [35]. Hence, the intestinal phase alters the greater change in antioxidant activities, that is, DPPH and FRAP activities, due to the lower stability of phytochemicals during the intestinal phase compared to the gastric phase.

3.3. Change of starch hydrolysis and estimated glycemic index of cooked rice during in vitro co-digestion

The change in starch hydrolysis (SH) was expressed as a percentage (%) of cooked rice co-digested with different types of HTs during simulated *in vitro* co-digestion, as shown in Fig. 3. During the gastric phase, the SH was close to 0% in all samples because of the absence of α-amylase in gastric juice, which induces no enzymatic hydrolysis of starch. This is in agreement with the results of previous studies [20,21]. During the intestinal phase, SH showed the highest value in the control sample (without the addition of HTs) owing to the presence of pancreatic α-amylases in the intestinal juice. It hydrolyzes starch into maltose and glucose, respectively [36]. Whereas samples with the addition of HTs had lower values of SH. At the equilibrium stage, MB and GM showed lower SH values than BA and CS. This may be a result of the presence of phytochemicals in HTs that can inhibit the action of pancreatic α-amylases during the intestinal phase. Sun, Gidley [37] reported the inhibition of porcine pancreatic α-amylase activity by normal maize starch granules in the presence of tea polyphenols. Tea polyphenols increased the binding rate and decreased the dissociation constant of porcine pancreatic α-amylase. A first-order equation from the curve-fitting model was applied, and the kinetics of starch hydrolysis are presented in Table 3. C∞ showed a significant difference in all samples except for MB and GM, which showed a non-significant difference. Similarly, k, HI, and eGI in the MB and GM were not significantly different and showed the lowest values. A reduction in eGI was observed in both MB and GM by approximately 15%, whereas BA and CS only decreased eGI by approximately 5 and 2%, respectively. These results clearly demonstrated the inhibitory effects of HTs on starch digestibility

Table 2
Phytochemicals and antioxidant activities changes during simulated *in vitro* co-digestion.

Analysis	Sample	Initial value of tea infusion	Gastric phase		Intestinal phase		Total change (%)
			Value	Change (%)	Value	Change (%)	
TPC	BA	50.60 ± 0.81[a]	46.79 ± 0.63[a]	−7.50	33.46 ± 0.45[a]	−28.49	−35.99
(mg GAE/g)	MB	19.68 ± 0.42[c]	16.35 ± 0.76[c]	−16.92	6.90 ± 0.34[d]	−64.94	−81.86
	GM	33.37 ± 0.25[b]	36.64 ± 0.34[b]	9.80	22.84 ± 0.50[b]	−31.56	−21.76
	CS	14.43 ± 0.17[d]	14.37 ± 0.76[d]	−0.42	13.00 ± 0.17[c]	−9.91	−10.33
TFC	BA	52.49 ± 1.12[a]	59.61 ± 2.24[a]	13.56	23.99 ± 1.12[b]	−54.30	−40.73
(mg QUE/g)	MB	23.96 ± 1.05[d]	18.02 ± 1.05[d]	−24.79	15.80 ± 0.00[d]	−34.06	−58.85
	GM	26.93 ± 1.05[b]	28.41 ± 1.05[c]	5.50	19.51 ± 1.05[c]	−27.55	−22.06
	CS	42.59 ± 2.10[b]	39.61 ± 2.10[b]	−7.00	41.84 ± 1.05[a]	−1.76	−8.76
TTC	BA	64.38 ± 0.98[a]	73.40 ± 0.00[a]	14.01	53.29 ± 0.98[a]	−17.23	−3.22
(mg TAE/g)	MB	22.62 ± 0.92[c]	20.02 ± 0.92[c]	−11.49	10.93 ± 0.92[d]	−51.68	−63.17
	GM	60.97 ± 3.68 [a]	57.72 ± 0.92[a]	−5.33	39.53 ± 0.92[b]	−35.15	−40.50
	CS	22.02 ± 1.84[c]	17.46 ± 0.92[d]	−20.71	13.56 ± 0.92[c]	−38.42	−59.13
FRAP	BA	644.31 ± 11.90[b]	707.43 ± 17.85[a]	9.80	384.32 ± 11.90[c]	−40.35	−30.56
(μmol $FeSO_4$/g)	MB	550.03 ± 1.24[d]	538.21 ± 0.62[c]	−2.15	254.41 ± 0.62[d]	−53.75	−55.90
	GM	689.08 ± 3.10[a]	675.50 ± 9.91[b]	−1.97	389.86 ± 9.91[b]	−43.42	−45.39
	CS	600.01 ± 1.86[c]	528.02 ± 4.35[d]	−12.00	528.02 ± 4.35[a]	−12.00	−24.00
DPPH	BA	159.74 ± 0.88[b]	183.54 ± 0.88[a]	14.90	124.26 ± 0.00[b]	−22.21	−7.31
(μmol TE/g)	MB	63.64 ± 3.31[d]	55.81 ± 0.82[d]	−12.30	14.79 ± 0.00[d]	−76.76	−89.06
	GM	178.91 ± 0.83[a]	181.25 ± 0.82[b]	1.31	155.61 ± 0.83[a]	−13.02	−11.72
	CS	76.10 ± 0.83[c]	63.87 ± 0.82[c]	−16.07	56.05 ± 0.83[c]	−26.35	−42.42

TPC, TFC, TTC, FRAP, and DPPH values are presented as the mean ± SD ($n = 3$). Different letters in each analysis in the same column indicate significant differences ($P < 0.05$).

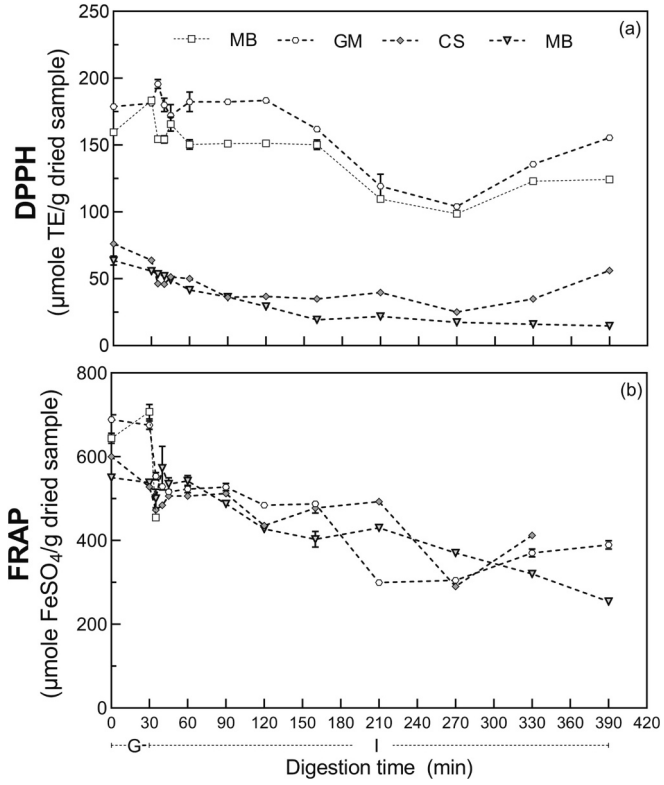

Fig. 2. Changes of DPPH (a) and FRAP (b) activities during *in vitro* co-digestion of different type of herbal teas co-digested with intact grain cooked Thai jasmine rice. *Error bars represent standard deviations ($n = 3$). Abbreviation: gastric phase (G) and intestinal phase (I).

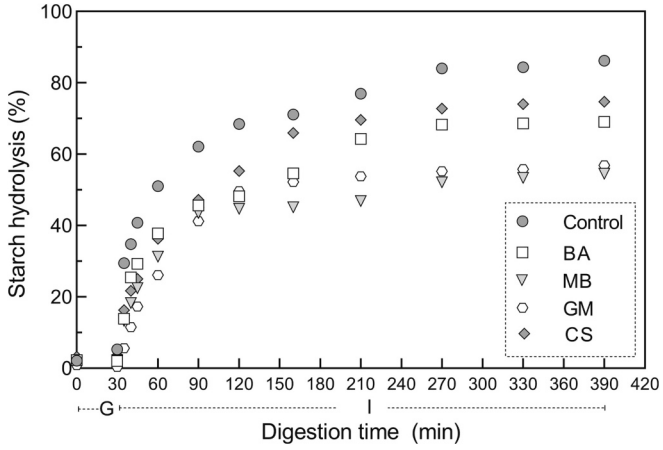

Fig. 3. Changes in starch hydrolysis (%) of intact grain cooked Thai jasmine (Thai Hom Mali 105) rice co-digested with different type of herbal teas during simulated *in vitro* co-digestion. Error bars represent standard deviation ($n = 3$). Abbreviation: Beal fruit (BA), Mulberry (MB), Gymnema (GM), Chrysanthemum (CS), intact grain cooked Thai jasmine rice (HM), Gastric phase (G) and intestinal phase (IG).

through the reduction of SH and kinetics of starch hydrolysis, that is, C∞, k, HI, and eGI. This might be due to the inhibitory effects of the interaction between polyphenols and pancreatic α-amylase during *in vitro* co-digestion. P et al. [38] revealed that the methanol extract of MB inhibited porcine pancreatic α-amylase by approximately 15%. Phytochemical analysis revealed that tannins, cardiac glycosides, flavonoids, and saponins were the main phytochemicals that inhibited porcine pancreatin α-amylase activity. Nirmala et al. [39] also demonstrated the inhibitory potential of GM on α-amylase during *in vitro* digestion. This finding showed that the gymnemic acid fraction significantly decreased the α-amylase activity. At a concentration of 10 mg/mL of the gymnemic acid fraction from GM, 14.25% inhibition was observed. However, CS showed the lowest reduction in eGI, which might indicate slight inhibition of α-amylase activity during *in vitro* co-digestion. This can be attributed to the pH during the intestinal phase (6.80). According to Li et al. [40] revealed the interaction between three flavonoids in CS *i.e.*, buddleoside, acacetin, and luteolin. At different pH values, the binding affinities of the three flavonoids with α-amylase were strongest at pH 6. Additionally, the three flavonoids significantly inhibited α-amylase activity ranging from 6.76 to 21.29%. Overall, the current investigation showed that the inhibition capacity of THs was in the order of MB and GM > BA > CS.

Table 3
Kinetics of starch hydrolysis, equilibrium concentration of hydrolyzed starch (C∞), kinetic constant (k), hydrolysis index (HI) and estimated glycemic index (eGI) for intact grain cooked rice samples co-digested with different type of herbal teas during simulated *in vitro* co-digestion.

Sample	C∞ (%)	k (s^{-1})	R^2	HI	eGI
Control	86.50 ± 4.42[a]	0.0121 ± 0.0017[a]	0.96	69.95 ± 2.63[a]	78.11 ± 1.44[a]
BA	72.58 ± 4.61[b]	0.0094 ± 0.0010[ab]	0.95	62.55 ± 1.71[b]	74.05 ± 0.94[b]
MB	55.23 ± 3.58[c]	0.0112 ± 0.0019[ab]	0.93	50.18 ± 1.33[c]	67.26 ± 0.73[c]
GM	62.27 ± 5.97[c]	0.0087 ± 0.0020[b]	0.91	52.28 ± 2.21[c]	68.41 ± 1.22[c]
CS	80.41 ± 4.61[b]	0.0088 ± 0.0012[b]	0.96	67.77 ± 1.71[a]	76.92 ± 0.94[a]

Values of C∞, k, HI, and eGI are presented as the mean ± SD ($n = 3$). For each sample row, different letters in the same column indicate significant differences ($P < 0.05$).

3.4. Relationship between phytochemicals and antioxidant activites versus starch hydrolysis

The correlation between changes in TPC, TFC, TTC, FRAP, and DPPH (x) *versus* SH (y) at different time intervals during *in vitro* co-digestion was evaluated using the Pearson correlation coefficient analysis (Table 4). Different significance levels ($P < 0.05$, $P < 0.01$, and $P < 0.001$) indicate the confidence levels of the correlation. In particular, a strong positive correlation was observed between TPC and TTC *versus* SH, especially in GM ($P < 0.01$), indicating that the reduction in TPC and TTC decreased SH over time. A strong positive correlation was observed between TFC and SH, particularly in BA ($P < 0.001$). This proves that the reduction in TFC is directly correlated with the reduction in SH. A previous study has demonstrated the interaction of phytochemicals with digestive enzymes and starch. Flavonoids, such as quercetin, luteolin, and diosmetin, in BA have been reported to interact with digestive enzymes through the inhibition of α-amylase activity. α-Amylase-flavonoid complexes are formed through hydrophobic interactions [41]. BA contains quercetin as the main flavonoid and previous results suggest that flavonoids can bind to α-amylase through hydrophobic interactions [42]. A recent investigation showed that quercetin in BA remained the highest during *in vitro* digestion. Thus, it plays an important role in inhibiting α-amylase activity. The correlation between the changes in antioxidant activities, that is, FRAP and DPPH *versus* SH, was positive in all samples. This could be a result of the slight reduction in phytochemicals, which further reduced antioxidant activities. Therefore, phytochemicals were positively correlated with the antioxidant activity. This agrees with a previous study that demonstrated a positive correlation between TPC and DPPH in tea infusions [43].

4. Conclusion

The current study aimed to observe the digestibility characteristics of cooked rice in addition of herbal teas during *in vitro* co-digestion. This result revealed that C∞ and k were significantly decreased resulting in significant decrease in eGI with the addition of herbal teas. The lowest eGI values were observed for MB and GM, followed by BA and CS, respectively. During *in vitro* co-digestion, the percentage change in phytochemical and antioxidant activities was lower in the gastric phase than in the intestinal phase. It has been reported that phytochemicals undergo chemical degradation under basic rather than acidic conditions. The Pearson correlation coefficient revealed a positive correlation between the reduction in phytochemicals and antioxidant activity against the reduction in SH. The reduction in phytochemicals may be due to chemical degradation and the interaction of phytochemicals with α-amylase during *in vitro* digestion. These results revealed that herbal teas prepared from mulberry and gymnema leaves potentially reduce starch digestibility of cooked rice, whereas phytochemicals play an important role in their inhibitory effects. These results further suggest a potential advantage of herbal teas in reducing postprandial hyperglycemia as well as providing antioxidant activities to human health. Yet, our study has not yet illustrated roles of individual phenolic compounds on starch digestibility also their complexation toward digestive enzymes. Molecular docking model and individual phenolic compounds analysis using the HPLC technique are suggested as further investigation.

Table 4
The correlation between the changes of total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC), DPPH radical scavenging activity (DPPH) and ferric reducing antioxidant power activity (FRAP) *versus* starch hydrolysis.

Relationship	Pearson correlation coefficient			
	BA	MB	GM	CS
TPC	0.760*	0.691*	0.718**	0.492^{NS}
TFC	0.925***	0.607*	0.675*	0.374^{NS}
TTC	0.541^{NS}	0.674*	0.745**	0.521^{NS}
FRAP	0.870***	0.823***	0.856***	0.567*
DPPH	0.839***	0.965***	0.673*	0.723**

NS, not significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; *, significant at $P < 0.001$.

Ethical approval

This study did not include any human subjects or animal experiments.

Ethical statement

The authors declare that the present manuscript does not involve any animal and human study.

CRediT authorship contribution statement

Thiraphong Aumasa: Methodology, Validation, Investigation, Formal analysis, Writing – original draft. **Yukiharu Ogawa:** Resources, Supervision, Writing – review & editing. **Jaspreet Singh:** Supervision, Writing – review & editing. **Worawan Panpipat:** Writing – review & editing. **Natthawuddhi Donlao:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest. The foundations supported this study had no role in the study design, data collection, or analysis. The authors alone are responsible for the content and writing of this paper.

Acknowledgements

This work was supported by Mae Fah Luang University; the Outbound Research Exchange for Excellent Research Collaboration Program from the Ministry of Higher Education, Science, Research, and Innovation, Thailand; National Science and Technology Development Agency (NSTDA), Ministry of Science and Technology, Thailand; and Reinventing University has received funding support from The Office of the Permanent Secretary of the Ministry of Higher Education, Science, Research and Innovation, Thailand. The authors would like to express their gratitude to Thai Tea Suwirun Co., Ltd., for providing dried tea leaves for this research.

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