



Original article

White mulberry leaf (*Morus alba* L.) infusion as a strategy to reduce starch digestibility: The influence of particle size of leaf powder

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ABSTRACT

Mulberry leaf (*Morus Alba* L.) has been found in clinical trials to be effective in reducing diabetes in Asia. The powdered tea market is expanding in popularity due to its functional properties. This study aimed to examine the influence of different particle sizes of mulberry leaf powder (MLP) infusion on the digestibility of starch in cooked *Japonica* rice (cv. Koshihikari) and the bioaccessibility of phytochemicals. Dried mulberry leaf was pulverized and sieved into several particle sizes: 160 μm (MLP160), 250 μm (MLP250), 404 μm (MLP404), and 774 μm (MLP774). Through simulated *in vitro* digestion, we assessed starch hydrolysis (%S_H), the kinetics of starch hydrolysis, estimated glycemic index (eGI), as well as total phenolic content (TPC) and total flavonoid content (TFC). The smaller particle size of MLP showed a greater reduction of eGI. Specifically, infusions prepared from MLP160 resulted in a reduction of 15 % in eGI for cooked grains and 3 % for slurries, respectively. The reduction in eGI was attributed to the interaction among flavonoids and digestive enzymes, demonstrating a concentration-dependent manner on enzyme inhibition effect. Pulverization significantly influenced the concentration of phytochemicals and their bioaccessibility in infusions. This study offers valuable insights into determining optimal particle sizes for MLP, considering both physical and functional characteristics as well as implications for the food industry. The results further suggest that MLP infusion holds promise as a functional beverage, potentially providing benefits in reducing postprandial hyperglycemia.

1. Introduction

Type II diabetes (T2D) is the most common disease, accounting for an estimated 90 % of diabetes patients, with type I diabetes accounting for the remaining 10 %. T2D is primarily caused by decreased insulin production and secretion by β cells, as well as insulin resistance [1]. For

centuries, T2D has been a major public health concern, and it is expected to be the sixth-largest cause of death by 2030 [2]. According to the most recent International Diabetes Federation (IDF) data, the global prevalence of T2D in adults was 536.6 million (10.5 %) in 2021, with 783.2 million (12.2 %) living with diabetes by 2045 [3].

Carbohydrates are the primary dietary constituents that significantly

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affect blood glucose levels. The concept of glycemic index (GI) emerged in the early 1980s to address the varying abilities of carbohydrates to raise blood glucose levels. GI categorizes carbohydrates based on their impact on postprandial glycemia [4]. Bhupathiraju et al. found a relationship between GI and the risk of T2D in three large groups of US men and women [5]. Individuals who ate meals high in GI and low in cereal fiber had a roughly 40 % greater risk of T2D than those who ate diets high in cereal fiber and low in GI [5]. Interestingly, rice is considered a stable meal as a main source of carbohydrates, which accounts for 20 % of the world's calorie intake [6]. Rice is most often consumed as white rice, a refined grain produced by milling that eliminates the bran and germ while leaving the starchy endosperm, which contains starch up to 90 % dry weight [7,8]. As a result, higher white rice intake was closely associated with an increased incidence of type 2 diabetes (T2D) in prospective cohort studies conducted in Asian countries, as well as in other regions.

Several studies on the regulation of starch digestibility have revealed that plant polyphenols are promising digestive enzyme inhibitors, specifically α -amylase and α -glucosidase. For example, cocoa, pomegranates, cranberries, grapes [9], mulberry leaves [10], green tea [11], and black tea [12]. In general, polyphenols inhibit digestive enzymes through the formation of both competitive and non-competitive complexes against digestive enzymes. This complexation arises from hydrophobic interactions between polyphenols and enzymes [13]. Herbal tea prepared from mulberry leaf is popular in Asian countries as a functional beverage [14]. White mulberry leaf (*Morus alba*) is well recognized as an antidiabetic activity plant attributed to the presence of numerous bioactive compounds, including flavonoids, soluble dietary fiber, and imino sugars, which reduce starch digestion and absorption in the human intestine [10,12,15]. Tea is typically prepared through infusion and decoction with hot water, which allows for the extraction of certain phytochemicals. In contrast, tea powder offers a higher surface area, resulting in greater extractable phytochemicals. Studies demonstrated that superfine grinding of tea leaves decreased particle size, improved specific surface area [16], increased solubility [17] and increased bioavailability of phytochemicals in teas [18]. Yet, there exists a gap in the published research that focuses on the potential of mulberry leaf powder to impede starch digestion during *in vitro* digestion, as well as the most effective particle size of the powder for achieving these inhibitory effects. This study investigates the influence of different particle sizes of mulberry leaf powder (MLP) infusion on starch digestion and the bioavailability of phytochemicals during simulated *in vitro* digestion. The results of this study could provide significant knowledge for establishing the most suitable particle sizes for powder, considering both physical and functional attributes. These findings could have implications for the food industry and potentially provide health benefits for consumers.

2. Materials and methods

2.1. Samples

Dried white mulberry leaf (*Morus alba* L.) was commercially produced by Thai Tea Suwirun Co., Ltd. in Chiang Rai, Thailand (moisture content was 3.69 % w.b.). Mulberry leaves underwent traditional commercial processing involving withering, roasting, rolling, and drying. The fresh leaves were spread on bamboo trays and allowed to wither at 25 °C for 30 min. Leaves were then roasted in a drum roaster (Yuan Chang Machinery, Taoyuan, Taiwan) at 300 °C for 15 min. The roasted leaves were rolled using a rolling machine (Yuan Chang Machinery, Taoyuan, Taiwan) for 10 min. Leaves were cut and twisted during this step. After the rolling process, leaves were dried in an electric convection hot-air dryer (Kluay Nam Thai, Bangkok, Thailand) at 200 °C until the moisture content was reduced to <10 g/100 g (w.b.). This sample is referred to as dried mulberry leaves or ML. It was further ground with a laboratory-scale grinder (Sansho Industry Co., Ltd., Osaka, Japan) and

sieved with different sieve opening sizes, *i.e.*, 125, 250, 355, and 500 μ m. The grinding time was fixed at 2 min at 6000 rpm to obtain mulberry leaf powder (MLP) [19]. Polished rice grain (cv. Koshihikari) was collected from Niigata Prefecture and distributed by Iris Ohyama Inc., Sendai, Japan. To assess particle size diameter, each MLP was sputter-coated with gold using an ion sputter (JFC-1100, Jeol, Tokyo, Japan) and examined using a scanning electron microscope (SEM) (SU1510, Hitachi, Tokyo, Japan) with an accelerating voltage of 5 kV. The particle size diameter (μ m) was determined by calculating the mean values from one hundred replicates obtained from each sieve opening, particularly 125, 250, 355, and 500 μ m. The measurements yielded particle sizes of 160 μ m (MLP160), 250 μ m (MLP250), 404 μ m (MLP404), and 774 μ m (MLP774), respectively. Particle size distribution was determined prior to sieving the MLP; one hundred replicates were counted, and their diameters were measured. Subsequently, each sample (100–1000 μ m) was expressed as a fraction.

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 \times USP), and 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 MLP infusions

MLP infusions were prepared according to the method described by Donlao & Ogawa [20] with slight modifications. Briefly, each MLP (2.5 g) was mixed with 250 mL 95 °C hot water, then stirred continuously for 5 min before filtering 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 (Memmert, Schwabach, Germany).

2.4. Preparation of cooked rice grain and slurry

A modified experimental cooking method has been described by Tamura et al. [21]. For the grain sample, 30 g rice grain was placed into a 100 mL beaker and soaked in 45 mL of distilled water. Then, beakers containing Koshihikari rice were placed in a water bath at 30 °C for 30 min. The beakers were placed in an electric rice cooker (Sharpe, Tokyo, Japan) with 250 mL of distilled water and heat for 45 min. Cooked rice grains (RG) were transferred into a beaker and then added with distilled water (1:2 of water to cooked rice grain) prior to homogenization using a hand blender (TESCOM, Tokyo, Japan) for 2 min to obtain cooked rice slurry (RS).

2.5. Simulated *in vitro* digestion

A simulated *in vitro* digestion model was applied according to Aumasa et al. [22], with slight modifications. Briefly, each cooked rice sample (RG and RS) was added to the reactor, followed by 170 mL of each MLP infusion. The amount of total starch in the mixture was fixed at 4 % w/v. 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 at 200 rpm. The pH was adjusted to 2.00, and 19 mL of pepsin solution containing 91.20 mg of pepsin in gastric fluid buffer (pH 1.20) 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 25 mL of intestinal enzyme solution containing invertase (15 mg), pancreatin (200 mg), and amyloglucosidase (4 mL) in intestinal fluid buffer (pH 6.8). The pH of the

solution was adjusted 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 and then stored in a refrigerator before further analysis.

2.6. Glucose released and kinetics of starch hydrolysis

Glucose release and the kinetics of starch hydrolysis were determined according to Donlao & Ogawa [23]. 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 of 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 (%S_H), 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 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 Goñi et al. [24] was used to represent the kinetics of the starch hydrolysis.

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

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 calculated according to Eq. (3) that described by Goñi et al. [24].

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

2.7. Determination of phytochemicals

Total phenolic content (TPC) was determined according to the Folin-Ciocalteu's method [25]. Briefly, the sample (0.5 mL) was added to 2.5 mL of 10 % v/v Folin-Ciocalteu reagent, and then 2.0 mL of sodium carbonate solution (7.5 % w/v) was added. The samples were kept in the dark for 60 min before measuring the absorbance at 765 nm using a spectrophotometer (Thermo Fisher Scientific, USA). TPC was calculated and expressed as gallic acid equivalents (GAE) (mgGAE/g DW).

Total flavonoid content (TFC) was determined according to the aluminum chloride method [26]. 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 kept 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 (Thermo Fisher Scientific, USA). The TFC was calculated and expressed as quercetin equivalents (QUE) (mgQUE/g DW).

2.8. Bioaccessibility of phytochemicals

Bioaccessibility refers to the amount of a certain nutrient that is released into the gastrointestinal tract during digestion, and it is the maximum amount of a nutrient that can be absorbed through the intestinal epithelium [27]. The bioaccessibility of TPC and TFC was calculated as the following equation:

$$\text{Bioaccessibility (\%)} = \frac{\text{TPC or TFC after in vitro digestion}}{\text{TPC or TFC before in vitro digestion}} \times 100$$

2.9. Statistical analysis

Data are expressed as the mean ± standard deviation. The data were also subjected to analysis of variance (ANOVA) and Turkey's test-mean comparison test using SPSS 22.0 (IBM Corporation, New York, USA) for Microsoft Windows. Differences were considered statistically significant at *P* < 0.05. 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. Physical properties of MLP

A previous study reported that the maximum concentration of TPC in green tea was in between the particle size of 100 to 180 μm, while particle size of 20–50 μm has low functional properties due to the heat generated during superfine grinding and powder agglomeration [19]. During grinding, heating promoted biochemical alterations of samples and might result in changes to their physicochemical qualities, such as color change, which reduced their organoleptic properties and might result in bioactivity losses. In the present investigation, sieves opening sizes ranging from 150 to 500 μm were selected and pulverization at 6000 rpm for 2 min to minimize the alteration of functional properties, e.g., phenolic compound, a flavonoid compound, etc. The particle size distribution indicates the proportion of particles of a particular size. It is one of the most crucial properties of particulate and powder samples. The size of particles had a direct impact on the qualities of the sample, such as dissolution rate, stability, flowability, solubility, etc. Fig. 2. shows the particle size distribution curve, smaller particle sizes, i.e., 200 to 100 μm, showed a greater percentage compared to those with larger particle sizes, i.e., 400 to 1000 μm. This demonstrated that pulverization was an effective method in increasing the percentage of small particle sizes of MLP. While the mean particle size diameter was 366.39 μm.

3.2. Change in bioaccessibility of phytochemicals during in vitro digestion as affected by infusions prepared from different particle size of MLP

A previous study termed bioaccessibility as the amount of a molecule or nutrient that is liberated from its matrix and accessible for absorption in the gastrointestinal system as well as accessible through the bloodstream. In the present investigation, bioaccessibility and concentration of TPC and TFC were evaluated after simulated *in vitro* gastrointestinal digestion. The bioaccessibility of TPC and TFC are shown in Table 1. The result showed that the concentration of TPC in different particle sizes of MLP infusions was not significantly different in those samples except MLP774 infusion (16.5 ± 0.5 and 13.9 ± 0.3 mg GAE/g in RG and RS, respectively). This observation may be attributed to the high cellulosic fractions in MLP774, which primarily includes petiole, veins, and midrib rather than leaf material (Fig. 1d). The petiole, veins, and midrib contain a higher cellulose content (10 % to 15 %) compared to the leaves (7.2 % to 14 %), making them less powdery and more difficult to grind to a smaller size [28]. Besides, larger particle sizes are associated with lower dissolution and solubility of phytochemicals from their matrix [17]. Incorporating these fractions in the pulverization of dried leaves is

Table 1

The bioaccessibility of phytochemicals for different particle size of MLP infusion co-digested with intact grain (RG) and slurry (RS) cooked rice after simulated *in vitro* digestion.*

Sample	TPC		TFC	
	Concentration (mg GAE/g)	Bioaccessibility (%)	Concentration (mg QUE/g)	Bioaccessibility (%)
ML-RG	21.7 ± 0.1 ^a	143 ± 0.1 ^c	12.7 ± 4.5 ^c	35.5 ± 7.6 ^d
MLP774-RG	16.5 ± 0.5 ^b	191 ± 1.2 ^a	4.3 ± 2.9 ^d	66.9 ± 5.3 ^a
MLP404-RG	21.9 ± 0.3 ^a	145 ± 0.8 ^b	11.2 ± 0.8 ^c	40.6 ± 1.5 ^c
MLP250-RG	21.9 ± 1.1 ^a	141 ± 0.3 ^c	14.3 ± 0.1 ^b	43.1 ± 1.5 ^b
MLP160-RG	21.1 ± 0.1 ^a	131 ± 0.3 ^d	21.7 ± 0.8 ^a	66.5 ± 1.5 ^a
ML-RS	16.1 ± 0.2 ^a	101 ± 0.5 ^c	16.6 ± 1.1 ^c	69.1 ± 2.1 ^a
MLP774-RS	13.9 ± 0.3 ^b	123 ± 0.6 ^a	6.2 ± 1.1 ^d	38.9 ± 2.1 ^c
MLP404-RS	18.3 ± 0.5 ^a	113 ± 0.9 ^b	22.5 ± 1.1 ^c	56.9 ± 2.1 ^b
MLP250-RS	18.1 ± 0.2 ^a	99.7 ± 0.5 ^c	30.7 ± 2.1 ^b	67.4 ± 3.9 ^a
MLP160-RS	17.3 ± 0.5 ^a	84.1 ± 1.0 ^d	32.9 ± 1.1 ^a	35.4 ± 2.0 ^d

(P < 0.05). Values are presented as the mean ± SD (n = 3).

ML, dried mulberry leaf; MLP774, particle size of 774 µm; MLP404, particle size of 404 µm; MLP250, particle size of 250 µm; MLP160, particle size of 160 µm.

* Different letters in the same column mean the values are significantly different.

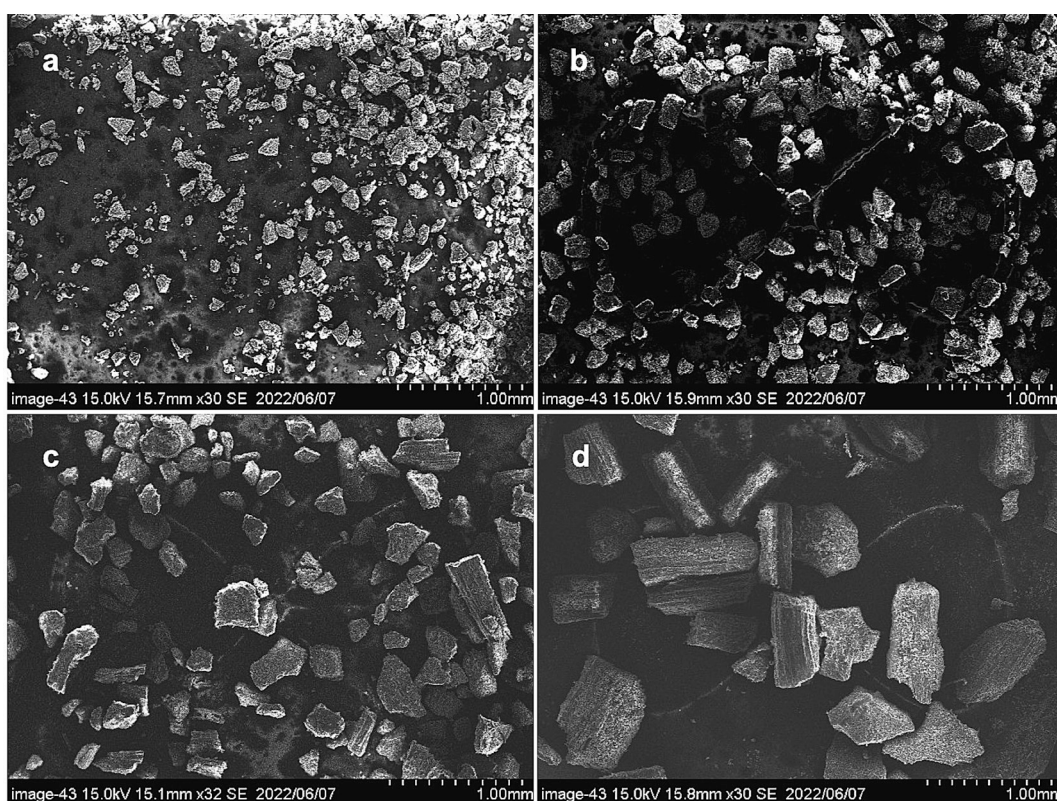


Fig. 1. MLP at different sieve opening sizes (µm); 150 (a), 250 (b), 355 (c), 500 (d) under scanning electron microscope at 1000× magnification.

considered practical for the industry, as it mitigates production complexities and reduces production costs. These fractions still retain valuable bioactive compounds. Memete et al. revealed that predominant phytochemicals in mulberry leaves (*Morus sp.*) were rutin, isoquercitrin, astragaln, skimmin, and chlorogenic acid. While the main composition of twig, petiole, and midrib contained reducing sugars, *i.e.*, arabinose, glucose, fructose, maltose, and stachyose [29]. Another study revealed marker compounds (*i.e.*, flavonoids, stilbenoids, phenolic acid, and coumarin) in different morphological parts of white mulberry (*Morus Alba L.*). Leaves showed higher contents of skimmin, chlorogenic acid, rutin, isoquercitrin, astragaln and quercitrin, while twig, petiole and midrib showed higher concentrations of 4-hydroxycinnamic acid and oxyresveratrol [30]. In the present study, the bioaccessibility (%) of TPC in RG samples showed over 100 % ranging from 131 to 191 % (Table 1.) because of higher TPC released after digestion compared to before

digestion. This might be due to the breaking of the bonds among these compounds resulting in the fragmentation of polyphenolic compounds under acidic conditions since phenolic compounds could be fragmented during digestion under the action of pepsin, trypsin and the acidic condition [31]. The bioaccessibility (%) of total phenolic content (TPC) in MLP774-RG and MLP774-RS was notably the highest among the samples, showing 191 % and 123 %, respectively. This phenomenon may be attributed to the liberation of larger, bound phenolic compounds during the infusion process, which subsequently undergo fragmentation into free forms during digestion. Notably, larger particle sizes contain greater proportion of bound phenolic compounds associated with carbohydrates, such as pectin, starch, and hemicelluloses, in comparison to free phenolic compounds [32]. This is consistent with previous studies, which reported approximately 62 % to 90 % increases in total phenolic content in both non-fermented and fermented *Aloe vera* juice following

the gastric phase of *in vitro* digestion. This increase reflects excessive bioaccessibility (>100 %), as it exceeds the initial content prior to digestion. Besides, some individual phenolic compounds demonstrated excessive bioaccessibility after *in vitro* digestion, including ellagic acid, resveratrol, taxifolin, quercetin, and kaempferol. The low pH and enzymatic action during gastric digestion promote the release of phenolic compounds bound to carbohydrates, enhancing their bioaccessibility [33]. Smaller particle sizes ($\leq 404 \mu\text{m}$) tend to exhibit lower bioaccessibility, particularly after the gastric phase. This phenomenon may be attributed to a decrease in phenolic compounds that bind to carbohydrates in these finer particles due to the super fine grinding. Furthermore, total flavonoid content (TFC) exhibited lower bioaccessibility compared to total phenolic content (TPC), ranging from 35.5 to 66.9 % in RG samples and from 35.4 to 69.1 % in RS samples. The decreased bioaccessibility was attributed to the lower stability of various flavonoids during the intestinal phase, which is sensitive to alkaline conditions, leading to chemical degradation. Besides, the concentration of TFC was significantly increased, particularly in MLP160, compared to the control (ML). TFC in the RG sample was increased from 12.7 ± 4.5 to 21.7 ± 0.8 mgQUE/g (42.3 % increased), and in the RS sample was increased from 16.6 ± 1.1 to 32.9 ± 1.1 mgQUE/g (49.9 % increased). This finding aligns with the previous study conducted by Shu et al., [18] which demonstrated that reducing particle size through superfine grinding resulted in an increase in polyphenols and flavonoids. This increase was attributed to the breakdown of bound polyphenols and flavonoids into free compounds, which enhanced the extraction and dissolution rate of these compounds from the matrix during infusion. Hence, superfine grinding of MLP into $160 \mu\text{m}$ of particle size was most effective in increasing flavonoid content in the infusion.

3.3. Change in starch hydrolysis during *in vitro* digestion as affected by infusions prepared from different particle size of MLP

Percentage of starch hydrolysis (%SH) associated with enzymatic reaction rate in which starch was hydrolyzed through the action of digestive enzymes, *i.e.*, α -amylase, α -glucosidase into glucose. Glucose release was further calculated into %SH against digestion time (min). Fig. 3. shows the changes in starch hydrolysis during the *in vitro* digestion of RG and RS samples. The simulated gastric phase began from 0 to 30 min, while after 30 min of digestion represents the simulated small intestinal phase. The results showed that starch hydrolysis was initiated at the beginning of the small intestinal phase in both RG and RS due to the presence of pancreatic α -amylase in small intestinal fluid, which could hydrolyze starch to maltose and glucose. The results also revealed that RG and RS samples reached the equilibrium state at about 300 min of digestion. Remarkably, MLP infusion significantly decreased SH in RG

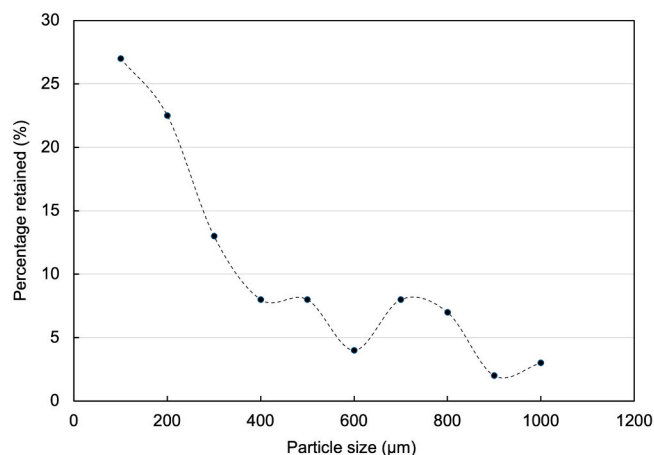


Fig. 2. Particle size distribution curve.

samples in relation to the smaller particle size of MLPs. However, RS samples did not exhibit a similar trend in SH as observed in RG samples. Particle size reduction showed no correlation with SH. This can be attributed to the tendency of homogenized samples to release glucose from starch granules rapidly, which alters the enzymatic reaction rate in the random manner. Hence, the curve-fitting model based on the first-order equation was applied to the simulated small intestinal phase to calculate the area under the curve, the kinetics of starch hydrolysis, and the estimated glycemic index, providing a better understanding of the differences in kinetic starch hydrolysis between RG and RS.

3.4. Change in kinetic starch hydrolysis during *in vitro* digestion, as affected by infusions prepared from different particle size of MLP

Remarkably, MLP infusion apparently decreased SH in RG, while SH decreased slightly in RS. The kinetics of starch hydrolysis was evaluated from the starch hydrolysis curve using a non-linear regression model. The kinetic constant (k) indicates the enzymatic reaction rate between digestive enzymes, such as α -amylase and α -glucosidase, and starch. This constant is associated with the conversion of the enzyme-substrate complex into products, such as glucose [34]. The current study demonstrated that MLP infusion in RG samples significantly decreased the k value in all samples except for the MLP774 infusion (Table 2) compared to the control (CT), which exhibited 0.02 min. This might be due to the presence of a lower concentration of TFC in MLP774 compared to smaller particle size of MLPs. Remarkably, k values were not significantly different in RS samples. Nevertheless, k values in RS samples were higher than in RG samples. This finding revealed the differences in the morphological structure of cooked rice affected the enzymatic reaction rate. This was well agreed with studies of Tamura et al. [35] and Thuengtung et al. [36]. As cooked rice slurry was initially homogenized prior to digestion, it resulted in a highly porous structure, which made it easier for enzymes to access starch granules [34]. Also, the presence of α -amylases in the intestinal juice induced rapid hydrolyzation of starch into maltose and glucose, respectively [36]. The current study exhibited different morphological structure of cooked rice as well as pulverization significantly affected to the k and C_{∞} values. Since pulverization increased the extraction and dissolution rate of phytochemicals from its matrix led to a higher concentration of TFC in MLP infusions compared to ML infusion. Several studies revealed that 1-deoxyojirimycin and chlorogenic acid were the main active constituent's retardation against starch digestion. 1-Deoxyojirimycin demonstrated inhibitory effect against α -glucosidase [15]. Chlorogenic acid inhibited the action of α -amylase, which acted as competitive and non-competitive enzyme inhibitors [37]. Previous studies reported one of the mechanisms associated with the hypoglycemic effect of *Morus alba* L. leaves. It possessed inhibition against porcine pancreatic α -amylase by 15 %, leading to retardation in starch hydrolysis rate due to the presence of flavonoids, naphthelene and hexadecanoic acid [38]. Additionally, quercetin acted as competitive enzyme inhibitor decreased the binding affinity and the dissociation constant of α -amylase toward starch [39]. Interestingly, the current study also found that MLP250-RG and MLP160-RG infusions exhibited significantly lower estimated glycemic index (eGI) values, specifically 74.5 ± 0.2 and 73.4 ± 0.6 , respectively. The eGI value was decreased by approximately 15 % compared to CT-RG. However, eGI was decreased approximately 3 % in MLP404-RS and MLP160-RS, showing 82.6 ± 1.4 and 83.9 ± 1.2 , respectively. Our results demonstrated that MLP160 was the most effective particle size to reduce the *in vitro* starch digestibility, in which highly associated with the presence of highest concentration of TFC in both rice grain and slurry.

4. Conclusion

Pulverizing dried mulberry leaves into different particle sizes enhanced their physical and functional properties. The particle size

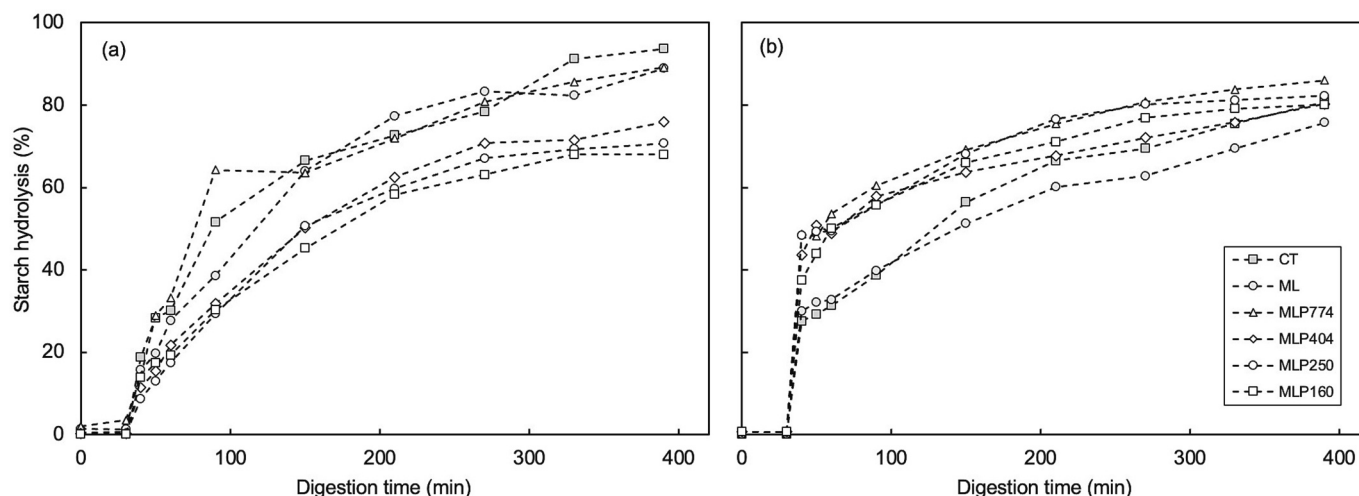


Fig. 3. Changes in starch hydrolysis (%) for cooked rice grain (a) and slurry (b) co-digested with different particle sizes of MLP infusion during simulated *in vitro* digestion. CT, cooked rice without infusion; ML, dried mulberry leaf; MLP774, particle size of 774 μm ; MLP404, particle size of 404 μm ; MLP250, particle size of 250 μm ; MLP160, particle size of 160 μm .

Table 2

Kinetics of starch hydrolysis, equilibrium concentration of hydrolyzed starch (C^{∞}), kinetic constant (k), hydrolysis index (HI) and estimated glycemic index (eGI) for intact grain (RG) and slurry (RS) cooked rice co-digested with different particle size of MLP infusion during simulated *in vitro* digestion.*

Sample	C^{∞} (%)	k (min)	HI	eGI
CT-RG	85.9 ± 5.1^a	0.02 ± 0.01^a	85.6 ± 1.9^a	86.7 ± 1.1^a
ML-RG	88.4 ± 3.5^a	0.01 ± 0.01^b	80.1 ± 1.3^c	83.7 ± 0.7^c
MLP774-RG	82.1 ± 3.9^{ab}	0.02 ± 0.01^a	83.3 ± 1.1^b	85.5 ± 0.6^b
MLP404-RG	77.3 ± 2.5^{bc}	0.01 ± 0.01^b	66.8 ± 0.7^d	76.4 ± 0.4^d
MLP250-RG	74.1 ± 1.5^{cd}	0.01 ± 0.01^b	63.4 ± 0.4^e	74.5 ± 0.2^e
MLP160-RG	69.6 ± 3.6^d	0.01 ± 0.01^b	61.3 ± 1.1^f	73.4 ± 0.6^e
CT-RS	75.2 ± 6.5^a	0.05 ± 0.02^a	83.4 ± 1.2^{ab}	85.5 ± 0.6^{ab}
ML-RS	72.9 ± 6.2^a	0.04 ± 0.02^a	80.2 ± 1.1^{bc}	83.8 ± 0.6^{bc}
MLP774-RS	76.3 ± 7.1^a	0.06 ± 0.02^a	85.4 ± 2.6^a	86.6 ± 1.4^a
MLP404-RS	69.2 ± 6.8^a	0.07 ± 0.03^a	78.1 ± 2.5^c	82.6 ± 1.4^c
MLP250-RS	74.4 ± 6.6^a	0.06 ± 0.02^a	83.2 ± 2.4^{ab}	85.4 ± 1.3^{ab}
MLP160-RS	72.9 ± 5.7^a	0.04 ± 0.02^a	80.7 ± 2.1^{bc}	83.9 ± 1.2^c

Different letters in the same column indicate significant differences ($P < 0.05$). CT, cooked rice without infusion; ML, dried mulberry leaf; MLP774, particle size of 774 μm ; MLP404, particle size of 404 μm ; MLP250, particle size of 250 μm ; MLP160, particle size of 160 μm .

* Values of C^{∞} , k , HI, and eGI are presented as the mean \pm SD ($n = 3$).

distribution mainly ranged from 100 to 200 μm . *In vitro* starch digestion showed that infusion from mulberry leaf powder for 160 μm reduced eGI, with a 15 % and 3 % in cooked rice grain and slurry, respectively. Rice structures influence the distinct kinetic of starch hydrolysis. Smaller particle sizes correlated with higher phytochemical concentrations and reduced starch digestibility, particularly flavonoids. The bioaccessibility was influenced by the particle size of the powder. Our investigation demonstrates association among flavonoid concentration and estimated glycemic index, with implications for designing proper particle size of powder in the food industry. Further investigation should explore individual phytochemicals' roles using molecular docking model.

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Ethical statement

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

CRediT authorship contribution statement

Thiraphong Aumasa: Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Gunthawan Apinanthanuwong:** Writing – review & editing, Investigation. **Jaspreet Singh:** Writing – review & editing. **Lovedeep Kaur:** Writing – review & editing. **Jinhu Tian:** Writing – review & editing. **Suphat Phongthai:** Writing – review & editing. **Yardfon Tanongkankit:** Writing – review & editing. **Utthapon Issara:** Writing – review & editing. **Yukiharu Ogawa:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Natthawuddhi Donlao:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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.

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References

- [1] M. Shahwan, et al., Role of polyphenols in combating type 2 diabetes and insulin resistance, *Int. J. Biol. Macromol.* 206 (2022) 567–579.
- [2] Organization, W.H., *Global Status Report on Noncommunicable Diseases 2010*, World Health Organization, 2011.
- [3] H. Sun, et al., IDF diabetes atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045, *Diabetes Res. Clin. Pract.* 183 (2022) 109119.
- [4] D.J. Jenkins, et al., Glycemic index of foods: a physiological basis for carbohydrate exchange, *Am. J. Clin. Nutr.* 34 (3) (1981) 362–366.

- [5] S.N. Bhupathiraju, et al., Glycemic index, glycemic load, and risk of type 2 diabetes: results from 3 large US cohorts and an updated meta-analysis, *Am. J. Clin. Nutr.* 100 (1) (2014) 218–232.
- [6] R.M. Welch, R.D. Graham, Breeding for micronutrients in staple food crops from a human nutrition perspective, *J. Exp. Bot.* 55 (396) (2004) 353–364.
- [7] R.M. van Dam, A global perspective on white Rice consumption and risk of type 2 diabetes, *Diabetes Care* 43 (11) (2020) 2625–2627.
- [8] V.M. Butardo Jr., N. Sreenivasulu, B.O. Juliano, Improving Rice grain quality: state-of-the-art and future prospects, *Methods Mol. Biol.* 1892 (2019) 19–55.
- [9] A. Barrett, et al., Inhibition of α -amylase and glucoamylase by tannins extracted from cocoa, pomegranates, cranberries, and grapes, *J. Agric. Food Chem.* 61 (7) (2013) 1477–1486.
- [10] C. Hansawasdi, J. Kawabata, α -Glucosidase inhibitory effect of mulberry (*Morus alba*) leaves on Caco-2, *Fitoterapia* 77 (7–8) (2006) 568–573.
- [11] S.C. Forester, Y. Gu, J.D. Lambert, Inhibition of starch digestion by the green tea polyphenol, (–)-epigallocatechin-3-gallate, *Mol. Nutr. Food Res.* 56 (11) (2012) 1647–1654.
- [12] K. Noppharat, N. Jaipakdee, E. Limpongsa, Development of herbal capsules containing mulberry leaf and black tea extracts using the modified liquid-solid techniques, *Int. J. Appl. Pharm.* 4 (11) (2019) 25–31.
- [13] G. Giuberti, G. Rocchetti, L. Lucini, Interactions between phenolic compounds, amylolytic enzymes and starch: an updated overview, *Curr. Opin. Food Sci.* 31 (2020) 102–113.
- [14] T. Thaipitakwong, S. Numhom, P. Aramwit, Mulberry leaves and their potential effects against cardiometabolic risks: a review of chemical compositions, biological properties and clinical efficacy, *Pharm. Biol.* 56 (1) (2018) 109–118.
- [15] J. Józefczuk, et al., Mulberry leaf extract decreases digestion and absorption of starch in healthy subjects—a randomized, placebo-controlled, crossover study, *Adv. Med. Sci.* 62 (2) (2017) 302–306.
- [16] P. Barnwal, et al., Influence of pin and hammer mill on grinding characteristics, thermal and antioxidant properties of coriander powder, *J. Food Sci. Technol.* 52 (12) (2015) 7783–7794.
- [17] W. Xiao, et al., A method for producing superfine black tea powder with enhanced infusion and dispersion property, *Food Chem.* 214 (2017) 242–247.
- [18] Y. Shu, et al., Effect of particle size on the bioaccessibility of polyphenols and polysaccharides in green tea powder and its antioxidant activity after simulated human digestion, *J. Food Sci. Technol.* 56 (3) (2019) 1127–1133.
- [19] A. Zaiter, et al., Effect of particle size on antioxidant activity and catechin content of green tea powders, *J. Food Sci. Technol.* 53 (4) (2016) 2025–2032.
- [20] N. Donlao, Y. Ogawa, Impacts of processing conditions on digestive recovery of polyphenolic compounds and stability of the antioxidant activity of green tea infusion during in vitro gastrointestinal digestion, *LWT* 89 (2018) 648–656.
- [21] M. Tamura, et al., Changes in histological tissue structure and textural characteristics of rice grain during cooking process, *Food Struct.* 1 (2) (2014) 164–170.
- [22] T. Aumasa, et al., The role of herbal teas in reducing the starch digestibility of cooked rice (*Oryza sativa* L.): an in vitro co-digestion study, *NFS J.* 33 (2023) 100154.
- [23] N. Donlao, Y. Ogawa, Impact of postharvest drying conditions on in vitro starch digestibility and estimated glycemic index of cooked non-waxy long-grain rice (*Oryza sativa* L.), *J. Sci. Food Agric.* 97 (3) (2017) 896–901.
- [24] I. Goñi, A. Garcia-Alonso, F. Saura-Calixto, A starch hydrolysis procedure to estimate glycemic index, *Nutr. Res.* 17 (3) (1997) 427–437.
- [25] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, [14] analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, in: *Methods in Enzymology*, Academic Press, 1999, pp. 152–178.
- [26] V.D. Tambe, R.S. Bhambar, Estimation of total phenol, tannin, alkaloid and flavonoid in *Hibiscus tiliaceus* Linn. wood extracts, *J. Pharmacogn. Phytochem.* 2 (4) (2014) 41–47.
- [27] X. Cai, et al., Estimation of the bioaccessibility and bioavailability of Fe, Mn, Cu, and Zn in Chinese vegetables using the in vitro digestion/Caco-2 cell model: the influence of gut microbiota, *Food Funct.* 8 (12) (2017) 4592–4600.
- [28] G. Kulić, V. Radojičić, Analysis of cellulose content in stalks and leaves of large leaf tobacco, *J. Agric. Sci. (Belgrade)* 56 (3) (2011) 207–215.
- [29] A.R. Memete, et al., Phytochemical composition of different botanical parts of *Morus* species, health benefits and application in food industry, *Plants (Basel)* 11 (2) (2022).
- [30] J.-H. Kim, E.-J. Doh, G. Lee, Quantitative comparison of the marker compounds in different medicinal parts of *Morus alba* L. using high-performance liquid chromatography-diode Array detector with Chemometric analysis, *Molecules* 25 (23) (2020) 5592.
- [31] Q. Zhang, et al., Changes in bio-accessibility, polyphenol profile and antioxidants of quinoa and djlulis sprouts during in vitro simulated gastrointestinal digestion, *Food Sci. Nutr.* 8 (8) (2020) 4232–4241.
- [32] S.X. Liu, et al., Impact of particle size fractions on composition, antioxidant activities, and functional properties of soybean hulls, *J. Food Meas. Charact.* 15 (2021) 1547–1562.
- [33] R.B. Cuvás-Limon, et al., Effect of gastrointestinal digestion on the bioaccessibility of phenolic compounds and antioxidant activity of fermented *Aloe vera* juices, *Antioxidants (Basel)* 11 (12) (2022).
- [34] R.L.J. Almeida, et al., Effect of enzymatic hydrolysis on digestibility and morpho-structural properties of hydrothermally pre-treated red rice starch, *Int. J. Biol. Macromol.* 222 (2022) 65–76.
- [35] M. Tamura, et al., Impact of structural characteristics on starch digestibility of cooked rice, *Food Chem.* 191 (2016) 91–97.
- [36] S. Thuengtung, et al., In vitro examination of starch digestibility and changes in antioxidant activities of selected cooked pigmented rice, *Food Biosci.* 23 (2018) 129–136.
- [37] Z. Karim, M. Holmes, C. Orfila, Inhibitory effect of chlorogenic acid on digestion of potato starch, *Food Chem.* 217 (2017) 498–504.
- [38] P. S, et al., Potent α -amylase inhibitory activity of Indian Ayurvedic medicinal plants, *BMC Complement. Altern. Med.* 11 (1) (2011) 5.
- [39] L. Sun, F.J. Warren, M.J. Gidley, Natural products for glycaemic control: polyphenols as inhibitors of alpha-amylase, *Trends Food Sci. Technol.* 91 (2019) 262–273.