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Starch digestibility of cooked rice as influenced by the addition of different tea types (*Camellia sinensis*): An *in vitro* study

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ABSTRACT

The stability of tea catechin is influenced by various factors such as tea types, pH and digestive processes. The study aimed to investigate the effect of different tea types on the stability of tea catechin and their impact on starch digestibility and glycemic response to different types of cooked rice. Cooked rices were co-digested with green tea, oolong tea and black tea, and the catechin profiles were correlated with the inhibition of enzymatic digestion.

The findings revealed that the green tea exhibited to be the most potent inhibitory effect on starch digestion. In addition, due to its highly porous structure, glutinous rice showed a higher starch hydrolysis rate and glycemic index than jasmine rice. The estimated glycemic index (eGI) of cooked jasmine rice co-digested with green, oolong, and black teas were 61.95 ± 1.07, 64.62 ± 1.12, and 67.14 ± 0.87, respectively, while eGI values of cooked glutinous rice were 77.55 ± 1.15, 79.98 ± 0.98, 81.45 ± 0.75, respectively. The findings indicates that epigallocatechin (EGC) achieves the highest stability. Overall, the results provided compelling evidence that tea types and rice structure affect the regulation of starch digestion and eGI of cooked rice.

1. Introduction

Rice (*Oryza sativa*) is a staple food that sustains two-thirds of the world's population with a global annual production of 505 million tons in 2021 alone and rising (Susmitha et al., 2022). Along with corn and wheat, it has a global annual production of 505 million tons in 2020–2021 (Susmitha et al., 2022). With the population expansion in Asia, Latin America, and Africa, the per capital consumption of rice is only rising. Brown rice is rich in fiber, energy, minerals, proteins, vitamins, and other biomolecules that are beneficial to health (Sen et al., 2020). However, during the polishing process, fat, protein, vitamins, and polyphenols are removed along the bran which results in retention of only carbohydrates in the white rice (Reddy et al., 2017). Consequently, white rice is generally considered to have a high glycaemic index (GI) as starch makes up 80% of the dry weight of the rice. Consumption of high amounts of food with high GI value can lead to several health diseases such as type II diabetes, cardiovascular diseases, and cancer. Previous studies have revealed the effect of tea polyphenols on the ability to inhibit starch digestive enzymes in other starchy foods yet on Thai Jasmine rice and glutinous rice (Goh et al., 2015).

Tea (*Camelia sinensis*) is one of the most popular beverages consumed worldwide. Generally, tea is classified into three types based on its degree of fermentation: green tea (non-fermented tea), oolong tea (partially fermented tea), and black tea (fully fermented tea) (Chen et al., 2021). 78% of the tea consumed worldwide is black tea, consumed primarily in Western countries. Green tea on the other hand represents the 20% is green tea world consumption which is predominantly consumer in countries and the rest 2% is oolong tea, which is mainly consumed in Southern China (Khan & Mukhtar, 2007).

The possible beneficial health effects of tea due to its high

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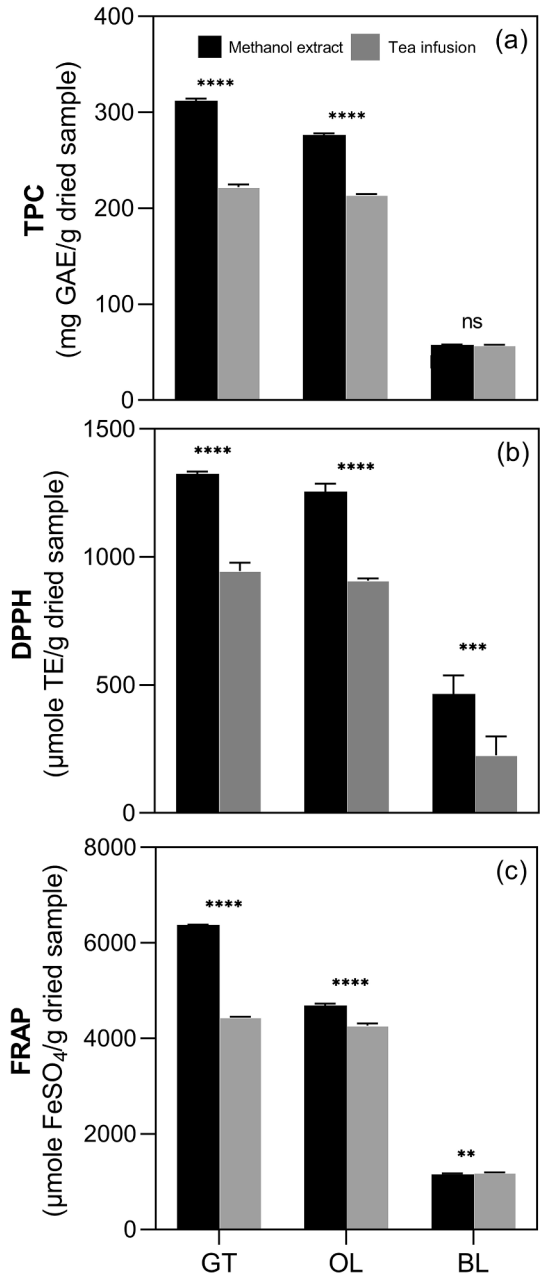

Fig. 1. Total polyphenol content (TPC), 2,2-diphenylpicrylhydrazyl free-radical scavenging (DPPH) and ferric reducing antioxidant power (FRAP) values of methanol extracts and infusions from dried green tea (GT), oolong tea (OL), and black tea (BL) leaves.

polyphenol compounds content have become more interesting (Shang et al., 2021). Previous studies have shown that polyphenolic compounds present in tea extracts have the potential to reduce the risk of a variety of diseases, such as diabetes, by inhibiting digestive enzymes, thereby lowering blood glucose levels (de Paulo Farias et al., 2021; Gutierrez et al., 2020; Ishmael et al., 2019). Catechins (flavan-3-ols) are the main phenolic compounds in tea.

Following the consumption of tea, catechins appear to be rapidly absorbed at their maximum concentration. After entering the animal body, tea polyphenols exert antioxidant effects by increasing the activity of the antioxidant enzyme (Lima Tribst et al., 2020). (−)-epigallocatechin-3-gallate (EGCG) is found primarily in green tea, and theaflavin-3,3-digallate (flavan-3-ols) is a major component of black tea (Musial et al., 2020). Theaflavins and thearubigins are catechin-derived compounds. These compounds are formed during fermentation (oxidative processing) and are involved in the manufacture of oolong and black tea. Even though few studies have already investigated the effects of different tea types on the ability to inhibit starch digestion in cooked rice, their inhibitory effect on Thai jasmine and glutinous rice have not been investigated.

Therefore, this study aimed to investigate the effects of different types of tea on the stability of tea catechins and their inhibition of digestion of cooked rice starch during *in vitro* gastrointestinal digestion and to evaluate the relationship between catechin profiles and their ability to inhibit starch digestion. However, the effect on sensory evaluation needs to be further investigated.

2. Materials and methods

2.1. Chemicals

Standards for (+)-catechin (C), (−)-epicatechin (EC), (−)-gallocatechin (GC), (−)- epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-catechin gallate (CG), (−)- epigallocatechin gallate (EGCG), and (−)-gallocatechin gallate (GCG) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol was obtained from RCI Labscan Chemical (Bangkok, Thailand). Trifluoroacetic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Deionized water was obtained using an in-house Milli-Q water purification system (Millipore, Billerica, MA, USA). Pepsin (P7000, porcine gastric mucosa, ≥250 units/mg solid), pancreatin (hog pancreas, 4 × USP), and invertase (invertase, Baker's yeast, grade VII, 301 U/mg solid) were purchased from Sigma-Aldrich, Ltd. (St. Louis, MO, USA). Amyloglucosidase (3260 U/ml) was purchased from Megazyme International Ireland, Ltd. (Wicklow, Ireland). Folin-Ciocalteu's phenol reagent ferric chloride was purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). (St. Louis, MO, USA). Gallic acid monohydrate, 2,2-diphenyl-1-picrylhydrazyl, 2,4,6-tris(2-pyridyl)-s-triazine 5 (TPTZ), and trolox-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid were purchased from Sigma–Aldrich. Sodium carbonate, sodium acetate, potassium chloride, and ferrous sulfate were purchased from Ajax Finechem Pty Ltd. (Wellington, New Zealand). Hydrochloric acid was purchased from Merck (Kenilworth, NJ).

2.2. Samples

Tea leaves of Chinese tea plant No.12 (*C. sinensis* var. *sinensis*) were harvested and processed for each different tea variation at Suwirun farm tea plantation in Chiang Rai Province, Thailand. Processed tea leaves of all the team samples i.e., green tea (GT), oolong tea (OL), and black tea (BL) were packed in an aluminium-sealed bag and transferred for analysis at Mae Fah Luang University, Chiang Rai Province, Thailand. White Thai Jasmine rice 105 (*Oryza sativa L.*) or HM and white glutinous rice RD6 (*Oryza sativa* var. *glutinosa*) or ST were obtained from the Pitchaphak Rice Shop, Chiang Rai Province, Thailand.

2.3. Sample preparation

2.3.1. Tea infusion

5 g of tea leaves sample were mixed with 500 mL of hot water (95 °C) for 5 min with continuous shaking. The tea samples were then filtered through Whatman No. 1 filter paper (GE Healthcare UK, Buckinghamshire, UK). The filtered portion was immediately cooled to 37 °C in a water bath (Donlao & Ogawa, 2019).

2.3.2. Methanol extraction

To extract the compounds from the dried tea leaves, 1 g of ground dried tea leaves was mixed with 20 mL of 70% (v/v) methanol and kept at 70 °C for 10 min in a water bath (Memmert, Schwabach, Germany).

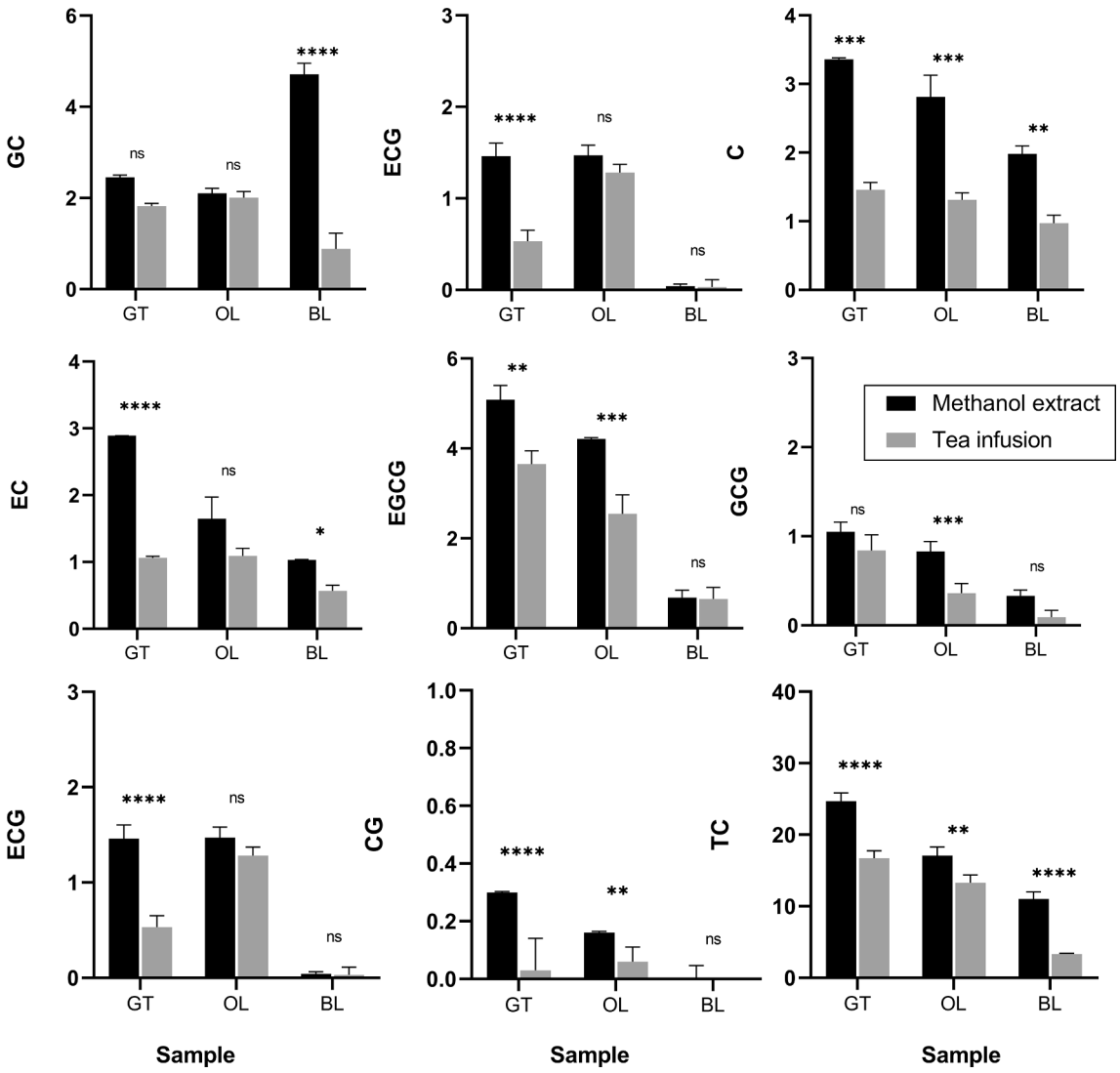

Fig. 2. Individual catechins and total catechins content in tea methanol extracts and tea infusion.

During the incubation, the samples were vigorously remixed for 5 and 10 min. The samples were centrifuged using a centrifuge machine (Hettich, Universal 32R, Germany) at 1800xg for 10 min, and the supernatant was collected. The procedure was then repeated. The supernatants were combined, and the volume was adjusted to 50 mL with cold 70% (v/v) methanol (Donlao & Ogawa, 2019).

2.3.3. Cooked rice preparation

Thai jasmine rice (Thai Hommali rice 105) was prepared following Tamura's experimental rice cooking method (Tamura et al., 2016) with some modifications. Briefly, 40 g white rice was mixed with 200 mL water and placed in an electric rice cooker (Sharp Co., Osaka, Japan). Rice samples were cooked for 45 min. The cooked rice sample was subsequently removed, wrapped with plastic film, and kept in a water bath at 30 °C for 30 min before further processing. For glutinous rice (RD6), the grain sample was soaked in water for 19 h. The soaked grain was steamed for 45 min using a steamer according to the traditional Thai cooking method. After cooking, the cooked samples were wrapped in plastic film and incubated at 30 °C for 30 min to stabilize the moisture in the grains. The total starch content of cooked grain samples was determined using a total starch assay kit (K-TSTA 07/11; Megazyme International, Wicklow, Ireland).

2.4. Moisture content determination

Aluminium cans and lids were dried in a hot-air dryer at 105 °C for 3 h and transferred to a desiccator to cool them down. The dried cans and lids were weighed, filled with all the tea samples then dried at 105 °C for 24 h in a hot-air dryer (Memmert, Schwabach, Germany). After drying, the can filled with the dried samples was reweighed. The moisture content of dried tea leaves was expressed on a wet basis (w.b.) (Donlao & Ogawa, 2019).

2.5. Total polyphenol content (TPC) determination

TPC was determined using the Folin-Ciocalteu assay (Wang et al., 2000). Five hundred microliters of extracted samples, 2.5 mL Folin-Ciocalteu's reagent (10 % v/v), and 2 mL sodium carbonate (7.5 g/100 mL water) were added to the test tubes and mixed well. The mixture was kept at room temperature for 1 h, and the absorbance was measured at 765 nm using a UV–VIS spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States). TPC was reported as milligram gallic acid equivalent (GAE) per gram of dried tea samples.

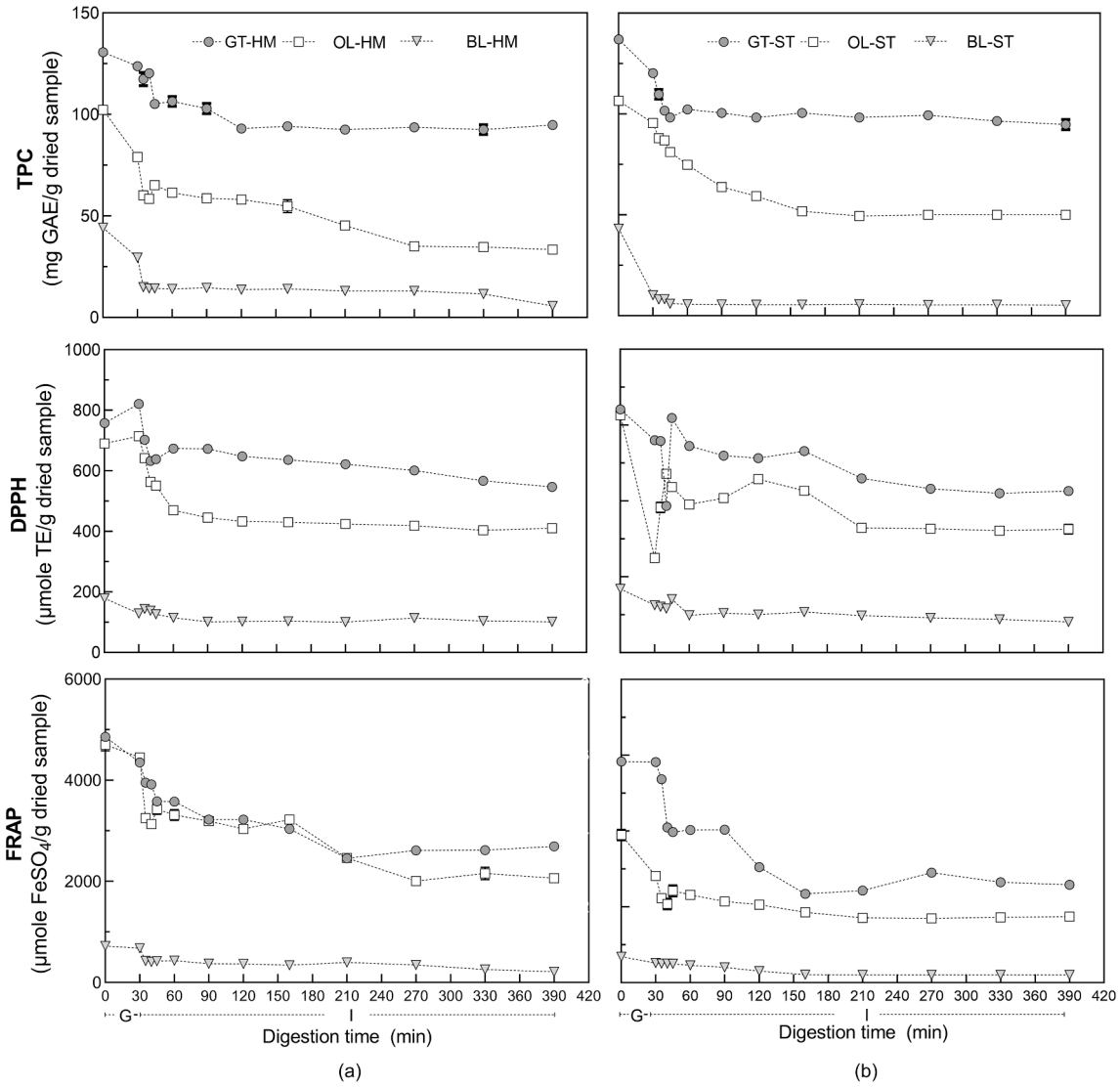

Fig. 3. Total polyphenol content (TPC), 2,2-diphenylpicrylhydrazyl free-radical scavenging (DPPH) and ferric reducing antioxidant power (FRAP) of green tea (GT), oolong tea (OL) and black tea (BL) treated with jasmine rice or HM (a) and glutinous rice or ST (b) during *in vitro* digestion.

2.6. Antioxidant activities

2.6.1. 1,1 -diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging assay

The DPPH free radical scavenging assay was conducted following the method described by Molyneux (2004), with slight modifications. Extracted samples were diluted (1–20-fold) using distilled water, and then 100 μL of the diluted extracted sample was mixed with 3.9 mL 60 μM DPPH solution. The mixture was kept in the dark at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. The radical scavenging activity was expressed as μmol Trolox equivalent (TE) per gram of dried tea samples.

2.6.2. Ferric reducing antioxidant power

The ferric-reducing antioxidant power (FRAP) was determined as described (Benzie & Szeto, 1999) with slight modifications. FRAP reagent was prepared from a mixture of 300 mM acetate buffer (adjusted to pH 3.60), 10 mM 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3$ at a ratio of 10:1:1 (v/v). Extracted samples (400 μL) were mixed with 2.6 mL FRAP reagent and then incubated at 37 °C for 30 min in a temperature-controlled water bath. Absorbance was measured at 595 nm using a spectrophotometer. FRAP was expressed as μmol $FeSO_4$ equivalents per gram of the dried sample.

2.7. Analysis of tea catechin

The analysis of tea catechins using HPLC was performed according to the International Organization for Standardization (ISO1 4502–2, 2005). The tea infusion and methanol extracts were filtered through a 0.45 μM nylon syringe filter (Whatman International Ltd.) and then transferred to glass autosampler vials (Aijiren Technology Co., Ltd., Zhejiang, China) before being injected into an HPLC system (Waters Alliance e2695 Separations Module, Waters Corporation, MA, USA). The injection volume for all the samples was 10 μL. The gradient mobile phase was comprised of a solution of 0.05% (v/v) trifluoroacetic acid in water and 99.9% acetonitrile. Both solutions were filtered through a 0.45 μM cellulose acetate filter (Whatman International Ltd.) The gradient was as follows: 87% of 0.05% (v/v) trifluoroacetic acid and 13% 99.9% acetonitrile solution. Separation and quantification of tea catechins were achieved using a commercially available column, platinum C18-EPS 3μ (53 mm × 7 mm) supplied by GRACE (W. R. Grace & Co.–Conn, Maryland, USA). The temperature of the column was maintained at 30 °C. Elution was performed at a solvent flow rate of 2.0 mL/min for 10 min. A photodiode array (PDA) detector (Waters 2996,

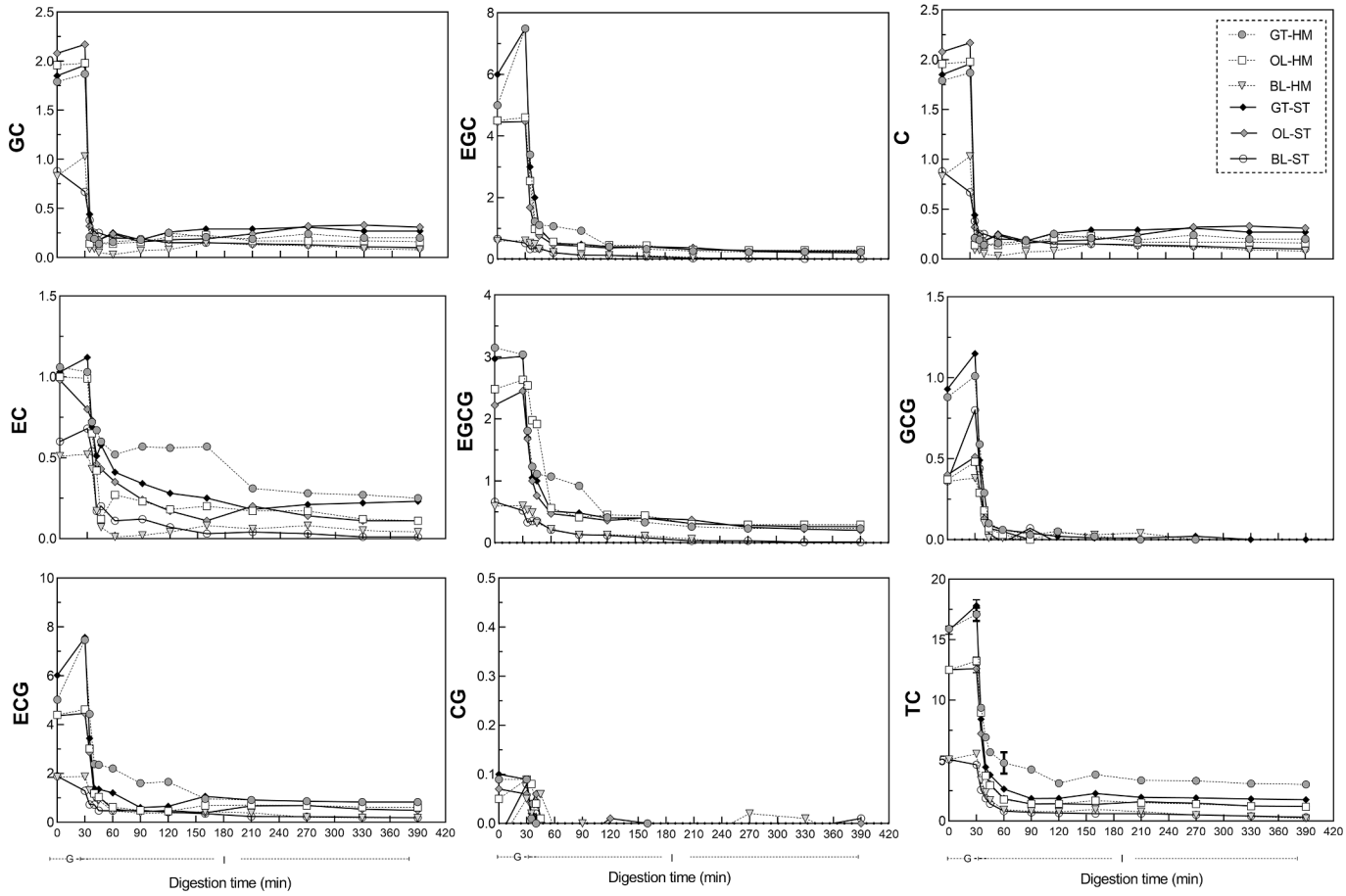

Fig. 4. Individual and total catechins content of green tea (GT), oolong tea (OL) and black tea (BL) treated with jasmine rice (HM) and glutinous rice (ST) during *in vitro* digestion.

Waters Corporation) was used to detect the eluent at 210 nm. Standard curves were constructed at various concentrations by using a mixture of catechin standards. Catechins were prepared at nine different concentrations (0.20–100.00 μg/mL) and analyzed in duplicate. Catechins were identified by comparing the retention times and spectral data with those of authentic standards, and quantification was performed using the external standard method. The amount of catechins in the tea extracts was expressed as g/100 g of the dried sample.

2.8. Simulated in vitro gastrointestinal digestion

A two-stage model of *in vitro* gastrointestinal digestion (Tamura et al., 2016) was used to characterize the stability and antioxidant activity of polyphenolic compounds, along with the influence of tea infusions on starch digestion during gastric and intestinal digestion. The TPC and antioxidant activity were assessed at various stages of digestion by performing chemical assays to measure the digestive recovery and residual activity of tea polyphenols. Pepsin solution was prepared by, dispersing 0.24 g pepsin from porcine gastric mucosa (activity of 800–2500 U/mg protein; Sigma Aldrich, St. Louis, MO, USA) in 50 mL gastric fluid buffer (adjusted to pH 1.20) using a magnetic stirrer for 10 min. For the preparation of intestinal enzyme solution, 0.2 g pancreatin from porcine pancreas (Sigma Aldrich) and 4 mL amyloglucosidase (Megazyme, Co. Wicklow, Ireland) were added to 25 mL intestinal fluid buffer mixture and mixed using a magnetic stirrer (Color Squid White, IKA Works, Wilmington, NC, USA) for 10 min. To achieve 4% starch, 8.82 g of cooked rice samples and 161.18 g of tea infusion were added to a glass reactor before starting the co-digestion while the water was replaced for the control sample. The reactor was connected to a temperature-controlled water bath (Memmert, Schwabach, Germany) and the liquid sample in the reactor was continuously agitated at 200 rpm with a magnetic stirrer. The temperature of the reactor was maintained at 37 °C throughout the experiment, and the pH of the sample was adjusted to 2.00 with 3 M HCl. The gastric phase was started by the addition of pepsin solution (19 mL), and the pH was immediately adjusted to 1.20 ± 0.01 with 0.5 M HCl. A liquid sample (0.5 mL) was taken from the reactor at intervals (0 and 30 min) and then placed into a centrifuge tube containing 4.5 mL 70% (v/v) methanol to terminate enzyme reactions. After 30 min of the gastric phase, the pH of the sample was adjusted to 6.00 using 3 N NaOH to inactivate the pepsin. The small intestinal phase was initiated by the addition of an intestinal enzyme solution (23 mL) and the pH was adjusted to 6.80 ± 0.01 0.5 M NaOH. The samples were maintained under intestinal conditions for 3 h. At intervals (5, 10, 15, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 min), a liquid sample (0.5 mL) was taken from the reactor and placed into a centrifuge tube containing 4.5 mL 70% (v/v) methanol. After completion of the small intestinal phase, the tubes containing the liquid samples were centrifuged at 1800 × g for 10 min and used for the analysis of TPC, catechin content, and antioxidant activity.

2.9. Glucose content and kinetics of starch hydrolysis

The released glucose was measured using a D-glucose assay kit (GOPOD Format K-GLUK 07/11; Megazyme International). 100 μL of supernatant from the previous step was digested with 0.5 mL of invertase/amyloglucosidase in acetate buffer at pH 5.20 (Monro et al., 2009) and incubated in a water bath at 37 °C for 10 min. Then, 0.1 mL of the mixture was combined with 3 mL of GOPOD solution and subsequently

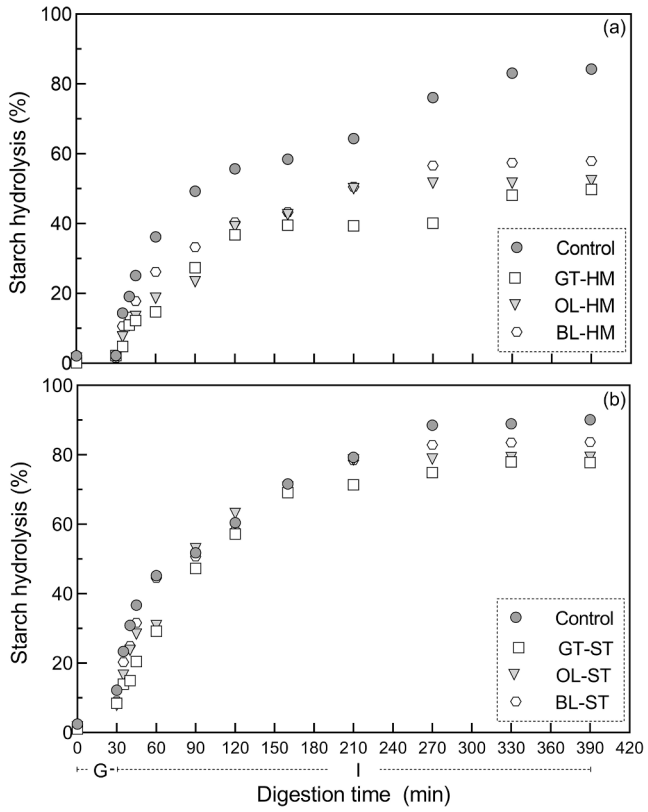

Fig. 5. Changes in starch hydrolysis (%) of cooked jasmine rice or HM (a) and glutinous rice or ST (b) treated with tea infusion from green tea (GT), oolong tea (OL), black tea (BL) during simulated *in vitro* starch digestion.

Table 1
Kinetics of starch hydrolysis, the equilibrium concentration of hydrolyzed starch (C_∞), kinetic constant (k), hydrolysis index (HI) and estimated glycemic index (eGI) for cooked rice samples including jasmine rice (HM) and glutinous rice (ST) treated with green tea (GT), oolong tea (OL) and black tea (BL) during simulated *in vitro* digestion.

Sample (s)	C_∞ (%)	k (s^{-1})	R^2	HI	eGI
Control-HM	90.88 ± 6.72a	$6.8 \times 10^{-3} \pm 1 \times 10^{-3}$a	0.96	69.28 ± 2.49a	77.75 ± 1.37a
GT-HM	53.83 ± 5.23d	$6.6 \times 10^{-3} \pm 2 \times 10^{-3}$a	0.95	40.51 ± 1.94d	61.95 ± 1.07d
OL-HM	61.13 ± 5.51c	$6.4 \times 10^{-3} \pm 1 \times 10^{-3}$a	0.96	45.38 ± 2.04c	64.62 ± 1.12c
BL-HM	63.97 ± 4.25b	$7.2 \times 10^{-3} \pm 9 \times 10^{-4}$a	0.97	49.96 ± 1.58b	67.14 ± 0.87b
Control-ST	93.80 ± 3.33a	$9.1 \times 10^{-3} \pm 8 \times 10^{-4}$a	0.98	79.91 ± 1.23a	83.61 ± 0.68a
GT-ST	86.81 ± 5.63a	$7.5 \times 10^{-3} \pm 1 \times 10^{-3}$a	0.97	68.93 ± 2.09c	77.55 ± 1.15c
OL-ST	86.69 ± 4.81a	$8.9 \times 10^{-3} \pm 2 \times 10^{-3}$a	0.96	73.35 ± 1.78b	79.98 ± 0.98b
BL-ST	89.17 ± 3.84a	$9.1 \times 10^{-3} \pm 1 \times 10^{-3}$a	0.98	76.02 ± 1.42b	81.45 ± 0.78b

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 ($P < 0.05$).

incubated in a water bath at 50 °C for 20 min. The absorbance of the solution was measured within 1 h at 510 nm using a spectrophotometer. The results are presented as the percentage of starch hydrolysis using 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 amount of hydrolyzed starch, S_i is the initial amount of starch, and G_p is the amount of glucose produced. A conversion factor of 0.9, which is generally calculated as the ratio of the molecular weight of the starch monomer to the molecular weight of glucose (162 / 180 = 0.9), was used (Goñi et al., 1997) The first-order equation model following Goñi et al. (1997), as shown below, was applied to describe the kinetics of starch hydrolysis.

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

where k is the kinetic constant, t is the time, C corresponds to the percentage of hydrolyzed starch at time t, and $C\infty$ is the equilibrium concentration of starch in the simulated small-intestinal phase.

The hydrolysis index (HI), which indicates starch hydrolysis in the samples, was calculated by dividing the area under the starch hydrolysis curve during the simulated small intestinal phase by the starch hydrolysis area of a reference sample. The estimated glycemic index (eGI), which indicates the carbohydrates consumed in different types of food based on the postprandial level of blood glucose, was calculated according to the equation described by the equation as shown below. (Goñi et al., 1997)

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

2.10. Statistical analysis

The data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test using the SPSS software (version 16.0). Statistical significance was set at $P < 0.05$.

3. Results and discussion

3.1. Changes in phytochemical and antioxidant activities in tea extracts

Phenolic compounds due to its antioxidant properties are the main quality parameters for health benefit studies (Yao et al., 2006; Zaiter et al., 2016). Fig. 1. shows the TPC, DPPH, and FRAP values of the methanol extracts and infusions made from dried green tea, oolong tea, and black tea leaves. Methanol is an effective solvent for extracting polyphenolic compounds (Siddhuraju & Becker, 2003). In this study, methanol extraction was performed to determine the actual content of chemical compounds in tea extracts for comparison with the actual amount that the human body receives. The TPC exhibited the highest level in green tea, followed by oolong tea, and black as polyphenol compounds could be oxidized during the fermentation process (Anesini et al., 2008). These data were in accordance to data reported by Yang and Kong (2015).

The antioxidant activity correlated well with TPC values. Green tea had the highest antioxidant activity in both the methanol extract and the infusion. For the methanol extract, the DPPH and FRAP values of green tea were 1324 μmol TE/g dried sample and 6373 μmol $FeSO_4$/g dried sample, respectively, followed by oolong tea, which was 1255 μmol TE/g dried sample, 4693 μmol $FeSO_4$/g dried sample, and black tea, 466 μmol TE/g dried sample and 1484 μmol $FeSO_4$/g dried sample, respectively. The DPPH and FRAP values of green tea infusions were 945 μmol TE/g dried sample and 4446 μmol $FeSO_4$/g dried sample, followed by oolong tea 908 μmol TE/g dried sample, 4021 μmol $FeSO_4$/g dried sample, and black tea 225 μmol TE/g dried sample and 904 μmol $FeSO_4$/g dried sample, respectively. The thermal degradation that occurs during tea processing and the oxidation of polyphenol content in tea extracts may be responsible for the reduction in antioxidant activity (Antony & Farid, 2022). The individual and total catechins are shown in Fig. 2. From the 8 types of catechins were investigated in all three tea samples, EGC showed the highest content (1.91 to 6.23 g/100 g dried sample) mirroring the previous findings of study done by (Donlao & Ogawa, 2019). EGC, C, EC, EGCG, GCG, CG, and total catechins were higher in green tea than in oolong tea and black tea (Ishmael et al.,

2019) whereas GC was higher in black tea owing to the epimerization of EGC (Timmel et al., 2013). However, ECG and CG were not found in black tea, which decreased considerably because of the conversion and oxidation of theaflavins, especially during the drying step (Lee et al., 2019). During the fermentation process, the catechin derivatives are oxidized to form polymeric compounds, such as thearubegins and theaflavins (Abudureheman et al., 2022; Anesini et al., 2008).

3.2. Changes of tea constituents during in vitro gastrointestinal digestion (TPC, DPPH, FRAP)

To exert their physiological functions, total polyphenols and antioxidants must be absorbed into the gastrointestinal tract. The effects of gastrointestinal digestion on tea polyphenols have also been reported (Annunziata et al., 2018). The changes in TPC, DPPH, and FRAP during *in vitro* digestion are shown in Fig. 3. The co-digestion of tea extracts with jasmine rice and glutinous rice is shown in Fig. 3a and Fig. 3b, respectively. In this experiment, *in vitro* digestion was performed within 360 min. Based on these results, TPC exhibited a decrease after gastrointestinal digestion. GT infusion in both jasmine rice and glutinous rice demonstrated the highest stability (94.82 ± 0.82 and 94.82 ± 2.46 mg/g dried sample, respectively), followed by OL (of 33.49 ± 1.14 and 50.02 ± 0.00 mg/g dried sample), and black tea (5.49 ± 0.35 and 5.22 ± 0.09 mg/g dried sample). TPC levels might have declined due to unstable pH during digestion as well as digestive enzymes (Friedman & Jürgens, 2000). Additionally, the distribution of –OH radicals from the phenolic rings led to a decrease in TPC after they reached the intestinal phase because of the changing pH conditions (Qin et al., 2022). Therefore, pH changes that result in the transformation of phenolic compounds and digestive enzymes could be the reason for TPC loss after digestion (Diep et al., 2022).

Green tea displayed the highest antioxidant activity in both rice samples, followed by oolong tea and black tea. This observation can be attributed to the influence of pH and digestive enzymes, which can impact the molecular structure of phenolic substances and ultimately result in the degradation of these compounds. (Annunziata et al., 2018). Fig. 4 presents the individual and total catechin contents of GT, OL, and BL extracts treated with HM and ST rice during *in vitro* digestion. Among both rice samples, green tea exhibited the highest catechin content, followed by oolong tea and black tea. Specifically, EGC displayed the highest catechin content. Notably, *in vitro* gastrointestinal digestion was found to significantly impact the stability of catechins. (Governa et al., 2022). Catechin was observed to be more stable at low pH levels; Consequently, upon entering the intestinal phase, which has a higher pH than the gastric phase, the catechin content tends to decrease (Food et al., 2018; Jang et al., 2014). Additionally, black tea with catechin levels due to the fermentation showed the lowest concentration of catechins. It is worth noting that some catechins may not be detected at the end of the digestion process.

3.3. Effect of tea extracts on starch digestibility

Fig. 5 shows the effect of tea extracts from the three different types of tea on the *in vitro* starch digestibility of jasmine and glutinous rice. The samples were co-digested with tea extracts and observed for 360 min and compared with the control samples. According to these findings, owing to the lack of amylases in the pepsin solution, minimal starch digestion occured during the gastric phase (Larner & McNickle, 1955). The hydrolysis rate of cooked rice was the lowest when treated with GT, followed by OL and BL. This is partially in line with the previous study (Ismail et al., 2018) which found that GT was more effective than OL and BL in inhibiting starch digestion. Furthermore, the starch hydrolysis rate in ST is higher than that of HM because of the amylose and amylopectin concentrations; rice with low amylose content, such as HM, has a higher starch hydrolysis rate and glycemic index than rice with intermediate and high amylose content like an ST (Hu et al., 2004; Thuengtung et al., 2018).

Similarly, (Thuengtung et al., 2018) found a high starch hydrolysis rate and glycemic index in Kum Luempua, waxy rice with low amylose content. In addition to amylose and amylopectin concentrations, rice structure also affects starch hydrolysis. Glutinous rice is cooked by steaming, which leads to more disruption in the internal grain tissue, resulting in a porous structure compared to jasmine rice cooked using a rice cooker (Thuengtung et al., 2018). A result on the kinetics of starch hydrolysis of ST as shown in Table 1 has a significantly higher kinetic constant than that of HM. This indicated a greater enzymatic reaction rate between α-amylase and starch; thus, glucose was released rapidly because ST is a highly porous material, whereas the competitive binding reaction of catechins against α-amylase was lower than that of HM.

The hydrolysis profile was fitted using a first-order nonlinear kinetic model, where C is the hydrolysis rate (%), C_∞ is the equilibrium hydrolysis rate, and k is the kinetic constant indicating the speed of hydrolysis (Goñi et al., 1997). The kinetic models for HM and ST are shown in Table 1. The highest values of C_∞, HI, and eGI were found in the control in both rice samples, followed by BL, OL, and GT, which could be influenced by the degree of fermentation of the tea samples. Green tea showed the highest inhibitory effect, similar to a previous study that found the highest inhibitory effect in green tea treated with plantain and yam (Ishmael et al., 2019). Green tea contains catechin, which inhibits the action of α-amylase, one of the most important enzymes involved in starch digestion (Hara & Honda, 1990). The table shows that the eGI of HM was 77.75; however, after treatment with GT, it was 61.95, accounting for 20%, OL for 17%, and BL for 14%. Consequently, the eGI of ST was 83.61% and that of GT was reduced by nearly 7%. 4% of the total tea is made up of OL, whereas 2 is BL. This may be due to the relationship between tea extracts, starch substrates, and enzymes. As a result, when glucose release is low, the process switches from substrate to product. Over time, the reaction reached equilibrium and the release of glucose peaked. The starch hydrolysates competed with the tea extract for binding sites, resulting in diminished inhibition. The components in tea extracts may react differently to the enzyme system, resulting in an observed trend that is either inhibiting or encouraging (Kan et al., 2020). This result may be due to the direct interaction between catechin and starch. According to a previous study, catechin interacts directly with starch via hydrophobic forces and hydrogen bonding, limiting the accessible surface of starch granules for enzymatic reactions (Amoako & Awika, 2016). Also, polyphenols adsorbed on granular starch may act as enzyme inhibitors against α-amylase activity (Sun et al., 2018). Consequently, the binding of polyphenols to starch is expected to reduce starch digestion via both pathways. Moreover, the potential of GT to lower the glycemic index of HM and ST may be confirmed. However, as previously stated, amylose content, cooking method, and cooking time can all alter the hydrolysis rate and, thus, the eGI value. Moreover, throughout the last century, the relationships between the gut microbiota and several diseases, including cancer, diabetes, inflammatory bowel disease, obesity, and disorders of the liver, have drawn attention since there are phenol-microbial interactions (Guo et al., 2019). Inhibiting a variety of pathogens, enhancing intestinal barrier function, improving lactose digesting, and regulating colonic microbiota have all been seen to occur in the gut with Lactobacillus and Bifidobacterium. Some research studies found that polyphenols can inhibit those microbes (Liao et al., 2016). Yet, it is still unknown how tea catechins affect intestinal micro-ecology, as well as the possible processes and useful applications of these biologically active substances. This research revealed the changes in catechin profiles during *in vitro* digestion and the high stability of some individual catechins i.e., EGC, EGCG, ECG etc. which can be interactive towards the action of digestive enzymes and result in lower glucose released during the digestion. However, the interaction of individual catechins toward digestive enzymes i.e., α-amylase and α-glucosidase was unclear in this study. Thus, this can be studied in further study through the molecular docking model and catechin analysis using the HPLC technique during *in vitro*

digestion.

4. Conclusion

In this study, the effects of different types of tea on the stability of tea catechins and their inhibition of starch digestion of cooked rice during simulated *in vitro* gastrointestinal digestion were investigated. The results revealed that the tea types had different effects on inhibiting starch digestion. Green tea was the most effective, followed by oolong and black teas. The inhibition of starch digestion was attributed to interactions between catechin and digestive enzymes. Overall, glutinous rice showed a significantly higher hydrolysis rate and glycemic index than jasmine rice which is attributed to the highly porous structure of glutinous rice hence difficult for catechins against α-amylase. Moreover, the rice variety and cooking methods play an important role in starch hydrolysis since glutinous rice has a significantly higher kinetic constant than jasmine rice. As it was discovered that EGC is the most essential component of starch digestibility. The presence of tea catechins in the digestive tract is a possible way to lower the glycaemic index of cooked Thai jasmine and glutinous rice. The effect on sensory evaluation, particle sizes, solvent-to-material ratio and molecular docking model and catechin analysis using the HPLC technique during *in vitro* digestion are suggested to be further investigated.

CRediT authorship contribution statement

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

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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