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Effect of Garlic and Turmeric Powders on *In Vitro* Digestibility of the Cooked Rice

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ABSTRACT Consumers nowadays are interested in foods with low glycemic index (GI) and high indigestible carbohydrate content. In Asia, white rice is the staple food and is generally considered as a readily digestible and high GI food. Occasionally, white rice is cooked by mixing with other ingredients such as herbs and spices. This study is carried out to determine the total phenolic content and the effect of *in vitro* digestibility on cooked white rice with added garlic and turmeric powders. Rice cooked with addition of turmeric powder (3 % w/w) showed the highest total phenolic content (92.02 mg GAE/100 g) among all the cooked rice samples. The effect of incorporating garlic powder (3 % w/w) and turmeric powder (3 % w/w) into the rice preparation was determined using an *in vitro* digestion protocol. Results show that by incorporating either garlic or turmeric powder into the rice, starch digestibility was significantly reduced. Rice with added turmeric powder showed a greater reduction in digestibility with significantly lower fraction of rapidly digestible starch (41.5 %; white rice 57.6 %) beside higher fraction of slowly digestible starch (36.1 %; white rice 28.4 %) and resistant starch fraction (22.6 %; white rice 14%). Overall, both spices were able to inhibit starch digestion which can be considered as a potential ingredient in lowering starch digestibility in the cooked rice.

KEYWORDS: *In vitro* digestibility, polyphenol, rice, spices, starch
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Original Article

INTRODUCTION

In most regions of the world, foods derived from cereal wheat and rice grains are the essential staples and rich energy source. They are rank third (771 719 thousand tonnes) and fourth (769 658 thousand tonnes), respectively after the sugarcane and maize in term of world crops production (FAO, 2019). Over 90 % of the world's rice is produced and consumed in the Asia-Pacific region and the type of rice that is predominantly consumed globally is white rice, also known as polished rice (Rathna Priya *et al*., 2019). The process of milling increases the digestibility of white rice grain after being cooked due to better access of digestive enzymes to the starch granules (Li *et al*., 2014). Thus, white rice contributes significantly to dietary glycaemic load for those who consume rice as major source of energy and white rice consumption has been positively associated with the risk of type II diabetes or hyperglycaemia (Aune *et al*., 2013; Hu *et al*., 2014; Oba *et al*., 2013). From a nutritional point of view, a simple marker for the impact of carbohydrate-rich foods on post-prandial blood glucose is the glycaemic index (GI) (Jenkins *et al*., 1987). Starchy foods are known to differ in the rates at which they are digested and absorbed. Depending on the rate of starch digestion, starch is classified as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). The *in vitro* methodology for determination of RDS refer to the glucose release after incubation for 20 min adjusted for any sugar glucose present, SDS as the additional glucose release by 120 min incubation, and RS as the remaining starch (Englyst *et al*., 2018).

Despite of an increasing popularity of low carbohydrate diets, carbohydrate is important for the normal body function, thus implementation of low-carbohydrate diet or complete removal of carbohydrate from diet is not recommended as it may lead to impaired health functions especially when the dietary pattern is replaced with high animal-derived protein and fat diet (Seidelmann *et al*., 2018). Besides, for the population that accustomed to rice consumption, advocacy to reduce consumption may not be sustainable (Kaur *et al*., 2016). Therefore, efforts to reduce the rate of digestion of carbohydrate-based food have gained attention. Wee and Henry (2020) identifies some strategies that can be considered and done by the consumers in reducing the glycaemic impact in foods either by using alternative ingredients with low GI or by adding functional ingredients into the food preparation that limit its digestibility.

Rice is usually cooked (boiling or steaming) in plain form and eaten alongside with dishes of protein and vegetables. The type and quantity of condiments and accompaniments eaten with rice can have impacts on the GI (Kaur *et al.*, 2016). Several studies showed that by adding ingredients containing functional polysaccharides (Fuwa *et al*. 2016; Hu *et al*., 2014), lipids (Kaur *et al*., 2015) and polyphenol (Chusak *et al*., 2019; Quek & Henry, 2015) can lower the GI and/or reduce the rice starch digestibility. Polyphenols have a diverse range of chemical classes which include flavonoid, phenolic acid, tannin, and other substances. They are known to have hypoglycaemic, hypolipidemic, anti-inflammatory and/or anti-oxidative properties (Scalbert *et al*., 2005). However, cooking process is also expected to change the phenolic content in food and subsequently on the starch digestibility (da Silva Lindemann, 2021). In certain food culture and practices, ingredients such as herbs, spices and plant extracts and are added into the rice preparation such as *nasi biryani, nasi kerabu,* and *nasi kuning* (Chusak *et al*., 2019; Abdul Raji *et al*., 2017). Herbs and spices are widely used ingredients in many other Asian culinary dishes to provide aromatic flavour or colour. Asian population is said to typically consume 2-4 g/day of spices that are rich in polyphenols in culinary use (Madkor *et al*., 2011).

The present study is intended to identify the potential health benefit of turmeric and garlic powders beyond the customary use as natural food colourant and flavour enhancer, particularly in the reduction of starch digestibility in cooked white rice. Therefore, the objectives of this study were to determine the total phenolic content and the *in vitro* digestibility of cooked rice with the added spices.

METHODOLOGY

Sample Preparation

Garlic powder (McCormick), turmeric powder (McCormick) and white rice (Sazarice Cap Anggur) were used for the preparation of the rice samples. Garlic powder and turmeric powder of 1%, 2% and 3% of the total weight of white rice, respectively were added in the cooking of white rice. The ratio of rice grain to the water used for cooking was 1: 1.5 w/v. The rice is cooked by steaming using an electric cooker. When the electric cooker is automatically turned off, the rice is left to simmer for 5 min to obtain completely cooked rice.

Measurement of Total Phenolic Content

Folin Ciocalteu method (Gao *et al*., 2019) is used to determine total phenolic content of the cooked rice sample extracted with 80 % methanol. All samples were then sonicated (Branson B8510 DTH) for 1 hour at 40°C, followed by centrifugation for 45 min at 3421 g. Sample extract of 1 ml was added with 2.5 mL of 10% Folin Ciocalteu solution (v/v). Sodium carbonate (7.5% w/v) of 2 mL was then added and the mixture was shaken vigorously followed by heating in water bath at 45°C for 15 min. The samples were cooled at ambient temperature before the absorbance is measured by using UV/Vis Spectrophotometer (Perkin Elmer Lambda 25) at 765 nm. The amount of total phenolic content is expressed as mg of gallic acid equivalents (GAE) per 100 g.

In Vitro Digestibility Study

The digestion procedure employs a simulated oral digestion phase, gastric digestion phase followed by an intestinal digestion phase. The method by Brodkorb *et al*. (2019) is adapted for the *in vitro* digestibility study. Briefly, simulated digestion fluids were prepared which consist of (a) simulated salivary fluid (SSF) (b) simulated gastric fluid (SGF) and (c) simulated intestinal fluid (SIF) were prepared. The cooked rice sample was homogenized by using food grinder (Panasonic MX-AC 210 S) for 30 seconds to obtain a slurry state sample. Sample of 5 g was weighed into a 50 mL tubes. For the oral phase, rice sample to SSF ratio of 1:1 (w/v) was used. The mixture was incubated at 37 °C for 2 minutes. This was then followed by simulation of gastric digestion phase, whereby the pH of the mixture was adjusted to pH 3 using pH meter (Eutech) with 1M HCl before adding the porcine pepsin (Sigma) solution. The mixture was then incubated at 37 °C for 2 hr from the point when pepsin solution was added. Finally, SIF was added to the gastric chyme to simulate the intestinal phase. The pH of the mixture was adjusted to pH 7.0 by adding 1M NaOH. Pancreatin and bile (Sigma) solution were then added. The mixture was incubated at 37 °C for 2 hr. Aliquot (1 mL) at 0, 20, 60, 90 and 120 min from the beginning of intestinal digestion are transferred into centrifuge tubes containing 1 mL ethanol to stop the enzyme activity. To measure the total starch, 7M potassium hydroxide solution of 10 mL was added into the tube containing 1 mL aliquot sample taken after incubation of intestinal phase at 180 min. The tube was shaken for 30 min in an ice water bath. Then, 1 mL of mixture from the tube was added with 1 mL of 1 M acetic acid, followed by the addition of 200 μL solution of amyloglucosidase (Sigma). The tube was mix vigorously and kept at a water bath of 70 °C for 30 min before being immersed in boiling water bath for 10 min. The tube was left to cool until they reach room temperature before adding 12 mL of absolute ethanol (Systerm) to the sample. The sample was centrifuged at 2,000 g for 10 minutes to clarify the supernatant prior to subsequent quantification.

Measurement of Reducing Sugars

The dinitrosalicylic acid (DNS) colorimetric method is used to determine the sugars released during *in vitro* digestion (Englyst & Hudson, 1987). Hydrolysate (0.5 mL) was pipetted into a test tube followed by 1 mL of DNS reagent. The test tube was then heated in boiling water bath for 5 min. The tube was cooled, and the sugars released are measured as glucose. Absorbance of the samples were measured at 540 nm against the blank using UV-Visible spectrophotometer (Perkin Elmer Lambda 25).

Calculation of RDS, SDS and RS

Using the absorbance value of samples and standard, the amount of sugar released at 0, 20, 60, 90, 120 min from the beginning of intestinal phase was calculated in mg of glucose/g of starch. The amount of sample digested at the times interval is used to express the sample digestion rate. RDS is referred to the amount of sample digested at 20 minutes (G_{20}). SDS is obtained by calculating the difference between the quantity of reducing sugars present at 120 minutes (G_{120}) from that of 20 minutes (G_{20}). The fraction of starch that is left undigested is known as RS. Total starch (TS) refers to the sum of the three starch fractions. The concentration of glucose released was converted to starch by multiplying by 0.9. Each fraction of starch is calculated by using the following Equations (1)-(3) (Mishra & Monro, 2009).

$$RDS\ (mg/g\ total\ starch) = \frac{G_{20}}{TS}\ x\, 0.9 \quad (1)$$

$$SDS\ (mg/g\ total\ starch\ ) = \frac{(G_{120}-G_{20})}{TS}\ x\, 0.9 \quad (2)$$

$$RS\ (mg/g\ total\ starch\ ) = \frac{(TS-RDS-SDS)}{TS} \quad (3)$$

Statistical Analysis

All the experiments were carried out in triplicate and the results were reported as mean value ± standard deviation. One-way analysis of variance using SPSS version 25 (IBM Corporation) were used for the statistical analyses.

RESULTS AND DISCUSSION

As shown in Table 1, the total phenolic content (TPC) of cooked white rice (WR) is 2.14 ± 0.16 mg GAE/ 100 g dry weight (DW), which is significantly lower than all the other rice samples cooked with addition of powdered spices. Between the spices, rice added with turmeric powder in this study had significantly higher ($p<0.05$) TPC than the rice samples added with garlic powder. Turmeric has been reported as a raw material with high total phenolic substances (Levent *et al.*, 2021). Similarly with other food product like bread, addition of turmeric powder at 0–8% (w/w) showed that the phenolic compounds increased with the increase amount of turmeric powder (Lim *et al.*, 2011).

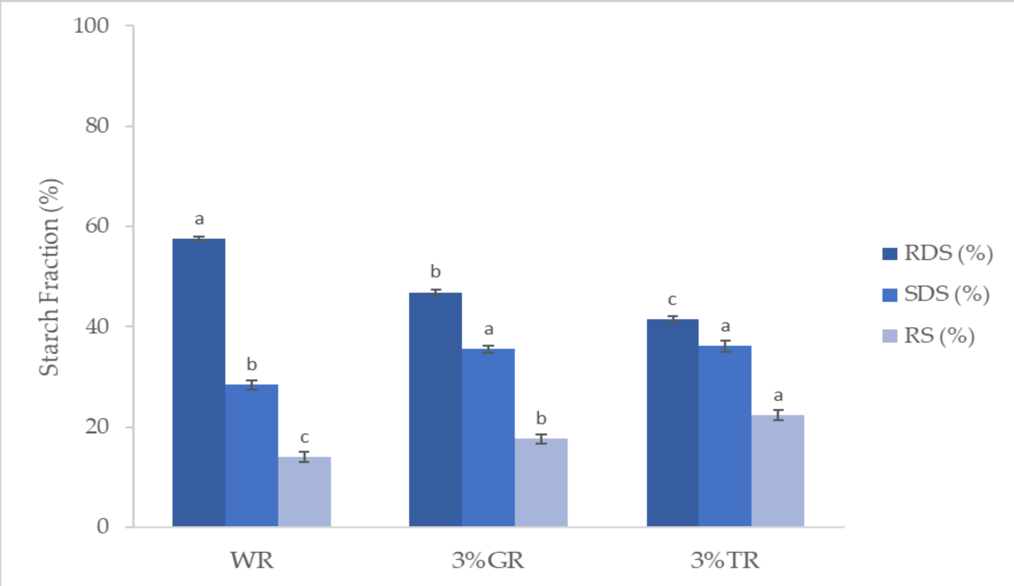
Table 1. Total phenolic content of cooked white rice and rice cooked with addition of garlic and turmeric powder at 1%, 2% and 3%

Sample	Total Phenolic Content (mg GAE/ 100 g DW)
WR	2.14 ± 0.16^{e}
1%GR	3.98 ± 0.64^{d}
2%GR	7.90 ± 0.31^{d}
3%GR	9.31 ± 0.26^{d}
1%TR	34.35 ± 3.48^{c}
2%TR	64.93 ± 3.64^{b}
3%TR	92.02 ± 1.92^{a}

The mean values and standard deviation (±) of total phenolic content. Significant difference ($p<0.05$) between sample is indicated with different letters. WR - white rice, GR – garlic rice; TR – turmeric rice

The rice cooked with 3% garlic powder (3% GR) and 3% turmeric powder (3% TR) were subsequently subjected to *in vitro* digestion and the glucose released from the starch due to the digestive enzyme breakdown was measured. In the oral and gastric phase, there were insignificant amount of glucose released due to the low transit time and low presence of carbohydrase enzyme. The intestinal phase has a longer transit time and whereby the digestion mainly takes place. Samples were collected during intestinal phase at 0, 20, 60, 90 and 120 min. Overall at all time points, the highest release of glucose was found in the WR while both 3% GR and 3% TR shows significantly lower ($p < 0.05$) released of glucose compared to WR (data not shown). These findings in agreement with previous research reported that white rice incorporated with red grape extract with high polyphenols shows a reducing effect on the release of glucose (Quek & Henry, 2015). Świeca *et al.* (2017) found that by replacing common wheat flour with functional wheat flour that contains high amount of phenolic contents, the digestibility of starch of the studied breads was reduced significantly. It is reported that phenolic compounds have the capability to bind to starch and affect digestive enzymes pancreatic α-amylase and intestinal α-glucosidase, lowering the digestibility of starch.

Starch in foods can be classified into RDS, SDS and RS depending on the rate of glucose release and absorption by the gastrointestinal tract. From the Figure 1, with addition of spices into the white rice, both 3% GR and 3% TR exhibited significantly lower RDS content as well as higher SDS and RS content than that of WR ($p<0.05$). Study by Giri *et al.* (2017) found a positive correlation between GI and RDS, while GI observed to be negatively correlated with SDS and RS. RDS is a form of starch that is digested quickly and completely in the small intestine and is linked to a rapid increase in post-prandial blood glucose level. It was determined during the first 20 minutes of enzyme digestion and describes the hydrolysis of starch chains at or near the starch granule surfaces (Ambigaipalan *et al.*, 2014).



Significant difference ($p < 0.05$) between samples is indicated with different letters.

Figure 1. Starch fraction contents of white rice (WR), and rice cooked with addition of garlic (GR) and turmeric powders (TR) at 3%

SDS is the fraction of starch that is slowly digested in the small intestine, resulting in low initial glycaemia with a steady and extended glucose release (Zhang *et al.*, 2016). SDS is widely regarded as the most favourable form of dietary starch and is beneficial for those with chronic metabolic diseases (Vinoy *et al.*, 2017). Most studies have found that heating and cooking of starch promotes the RS formation. The increase in RS content of starchy foods during processing is shown to be associated to their amylose content (Dhital *et al.*, 2010). RS is the fraction of starch that remains undigested in the small intestine after the first two hour of ingestion before undergoing fermentation in the large intestine. Phenolic compounds have previously been related to modify starch digestion, with SDS and RS fractions showing positive correlations (Camelo-Méndez *et al*., 2016). It was found that green tea polyphenol could inhibit amylolytic enzymes that may lead to inhibition of starch hydrolysis, resulting in an increase in the RS content (Yilmazer-Musa *et al.*, 2012). Therefore, the ability of garlic and turmeric to reduce starch digestibility could be due to the presence of various phytochemicals, including flavonoids and phenolics that have been found to inhibit both α-amylase and α-glucosidase (Butala *et al*., 2018; Wongsa *et al*., 2012;).

CONCLUSION

Ingredient containing polyphenol may be a useful ingredient which could be added in rice and other rice-based products to have benefits in reduction of starch digestibility to lower the food GI. The findings of this study have provided an insight on capability of some commonly used spices such as garlic and turmeric in the households in limiting the digestion of cooked rice in reducing the glycemic impact of refined starchy carbohydrates and a simple culinary method to reduce the glycemic response of rice can be recommended in communities consuming rice. However, this will involve *in vivo* study in the future to establish the postprandial glycemic response. Any positive outcome derived from the study, nevertheless, should not be considered as a replacement for a balanced and healthy diet and lifestyles.

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