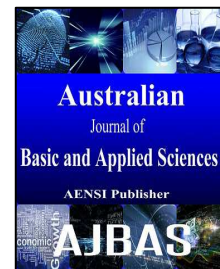




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### Investigation of the effect tea polyphenol extract to digestion properties

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#### ABSTRACT

Tea (*Camellia sinensis* L.) is a popular consumed beverage contain high amount of polyphenol, while spent teas originating as the main by product of beverage tea manufactured and still contain some level of antioxidant elements. The purpose of the present study is to investigate the hypoglycemic effect of antioxidant activity present in black tea (BT), oolong tea (OT), green tea (GT), spent black tea (SBT), spent oolong tea (SOT) and spent green tea (SGT) using suitable *in vitro* methods. In this study, *in vitro* glucose adsorption, glucose diffusion and amylolysis kinetics analysis were conducted. Tea and spent tea showed significant ( $p < 0.05$ ) glucose adsorption capacities which were directly proportional to the glucose concentration. Besides, both tea and spent tea significantly retarded the diffusion of glucose across the dialysis membrane in both glucose diffusion and amylolysis kinetics assay. From the results, all types of tea (black, oolong and green tea) shows hypoglycemic activity as indicated by significant glucose adsorption capacity, glucose diffusion retardation ability in glucose diffusion and amylolysis kinetics model systems in trend: BT > OT > GT. Furthermore, all tea showed significantly higher glucose adsorption capacity, reduction in glucose diffusion and retard amylolysis kinetics studies than their respective spent tea as in trend: BT > SBT, OT > SOT, GT > SGT

#### INTRODUCTION

Tea is derived from the leaves of the plant, *Camellia sinensis* and popular beverages worldwide. There are three major types of tea depending on the degree of fermentation. Green tea is unfermented product containing relatively large amount of polyphenolic compound compare to oolong tea and black tea, where oolong tea is partially fermented and black tea is fully fermented. Catechin and its derivatives are major group of flavonoid found in green tea. During fermentation, the bioactive polyphenol in tea leaves undergo enzymatic oxidation resulting in the formation of theaflavins and thearubigins (Lin, Y., *et al.*, 2003). Several *in vitro* and *in vivo* studies have shown that tea polyphenols are found to interact with digestive enzymes to affect metabolic system (Cabrera, C., *et al.*, 2003; Prior, R.L. and G. Cao, 1999; Zhang, J. and S. Kashket, 1998). Furthermore, there are reports on the beneficial health effects of tea consumption in diabetes; it is not known whether the spent tea, the main bio-waste from tea extracts, has potential anti-diabetic properties.

Spent tea is the main bio-waste byproduct generated in the beverage making industry that can accumulate rapidly to large amounts, leading to waste management issues. Spent tea contains many useful bioactive compounds such as polyphenolic compounds, organic acid and edible fibers, which can be recycled and reused. Several studies showed that waste obtained from natural sources has many beneficial health effects such as grape pomace on anti-hyperglycemic effects in diabetic mice (Hogan, S., *et al.*, 2010), blueberry pomace on

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improved metabolic parameter associated with metabolic syndrome (Khanal, R.C., *et al.*, 2012) and pear pomace on anti-adipogenic effects (Rhyu, J., *et al.*, 2014).

Now days, natural antioxidant can show inhibition activity towards carbohydrate hydrolyzing enzyme such as  $\alpha$ -amylase and  $\alpha$ -glucosidase. Introduction of  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitor into the diet has been proposed to be effective in retarding carbohydrate digestion. This linked to postprandial hyperglycemia and a lot of interest as a potential approach for curing type 2 diabetes mellitus as analyzed by several researcher (Kwon, Y.I., *et al.*, 2008; Ranilla, L.G., *et al.*, 2010). In some country, enzymes inhibitors such as acarbose are used as diabetic drugs. However, these drugs often reported to be associated with gastrointestinal side effects including abdominal pain, flatulence and diarrhea due to the gut microflora fermentation on the undigested carbohydrate (Derosa, G. and P. Maffioli, 2012). Besides, higher dosage of diabetic drugs may promote hypoglycemia effect. Many traditional medicinal plants such as cumin seeds, mulberry leaves and mangosteen pericarp contain natural  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitor and bioactive compound (Loo, A.E.K. and D. Huand, 2007; Ani, V. and K.A. Naidu, 2008).

Tea polyphenols can inhibit the carbohydrate hydrolysis enzymes, thus may reduce the postprandial blood glucose level (Ramesh, E.T., *et al.*, 2009). According to Honda and Hara (Hara, Y. and M. Honda, 1993), tea polyphenols have been determined to inhibit intestinal  $\alpha$ -amylase or sucrose. Several research have been proposed for the hypoglycemic effect of phytochemicals such as inhibition of carbohydrate hydrolyzing enzyme, manipulation of glucose transporters,  $\beta$ -cell reperation and enhancing insulin-releasing activity (Tiwari, A.K. and J.M. Rao, 2002). However, there are limited research on comparing the glucose adsorption, glucose diffusion and amylolysis kinetics activity of different types of tea (black tea, oolong tea and green tea) and their respective spent tea (spent black tea, spent oolong tea and spent green tea). Thus, the present study was conducted to study the effect of different types of tea and their respective spent tea on glucose adsorption, glucose diffusion and amylolysis kinetics.

## MATERIALS AND METHODS

### **Materials:**

Tea (*Camellia sinensis*) was obtained from local market in Penang, Malaysia. Three types of tea were used such as green tea (Chinese Tea Montea, Malaysia), black tea (Boh, Malaysia) and oolong tea (Chinese Tea Montea, Malaysia). For normal tea, the tea leaves were grinded into 1 mm size by using a grinder (IKA-WERKE). While for spent tea, the tea leaves were immersed in the hot boil water and stirred for 5 minutes. After that, the tea leaves were filtered by using muslin cloth and dried in the oven at 40 °C for 48 h. After that, the spent tea was grinded same as the normal tea.

Dialysis tubing cellulose membrane flat,  $\alpha$ -amylase from human saliva and glucose anhydrous were purchased from Sigma Aldrich (Subang, Malaysia). Glucose oxidase-peroxidase assay kit was purchased from Randox Laboratories Limited (Antrim, UK). Acarbose was purchased from local pharmacy store.

### **Solvent extraction of sample:**

About 1 g of different types of tea (green, black, oolong and spent tea respectively) was infused in 100 ml of 50% ethanol solvent. Extraction was carried out by stirred the tea solution using magnetic stirrer on a hot plat (magnet 4.5 x 0.5 cm; hotplate stable temperature, Cole Palmer Instrumental Company, Bunker Court, USA) at 100 x g for 3 h at (25±1 °C). After that, the tea extracts were filtered using muslin cloth and centrifuged (KUBOTA 5100 Centrifuge, Japan) for 30 minutes at 700 x g. Next, the tea extract was concentrated using a rotary evaporator (IKA-WERKE-RV06ML, Stanfer, Germany). After 30 min, the tea extract were collected and stored at low temperature and were covered with aluminium foil throughout the extraction process.

### **Quantification of glucose content by glucose oxidase peroxidase assay kit:**

In this study, glucose oxidase peroxidase assay kit was used to determine the glucose content in all samples and control. Glucose content was quantified according to the standard procedure provided by Randox Laboratory Limited. About 10  $\mu$ L of test sample was added with 1 mL of glucose oxidase peroxidase reagent and incubated at room temperature for 25 min. A standard solution was set up contained 10  $\mu$ L of 5.43 mM glucose standard and 1 mL glucose oxidase-peroxidase reagent. Then, the absorbance of sample and glucose standard was measured at 500 nm using UV-Visible Spectrophotometer (Spectro UV-160A, UV-Visible Recording Spectrophotometer, Shimadzu, Japan). The glucose content in sample was calculating using the standard formula provided in the assay kit:

Glucose concentration of sample (mM) = [(Absorbance of sample)/ (absorbance of standard)] x standard concentration (mM)

***In vitro glucose adsorption capacity:***

*In vitro* glucose adsorption capacities of different types of tea were determined according to method of Ou *et al* (2001). Briefly, 1% of tea sample was added to 25 mL of glucose solution with increasing concentration (0, 5, 10, 15, and 20 mM). Then, the mixture was stirred well and incubated in a shaker water bath at 37 °C for 6 h before it was centrifuged at 1800 x g for 20 min. After that, the glucose content in the supernatant was determined by using glucose peroxidase assay kit. Glucose bound was calculated using the following formula:

$$\text{Glucose bound} = \left[ \frac{(\text{glucose concentration of original solution}) - (\text{glucose concentration after 6 h})}{\text{weight of the sample}} \right] \times \text{volume of solution}$$

***In vitro glucose diffusion:***

*In vitro* glucose diffusion of different types of tea was determined according to method of Ou *et al* (2011). The glucose dietary fiber system comprising 25 mL of glucose solution (20 mM) and tea samples (1%) were dialyzed in a dialysis tubing membrane against 200 mL of distilled water at 37 °C in a shaker water bath. Control sample was carried out without addition of tea sample, whereas acarbose (0.2%) was carried out by substituting the test sample. Glucose content in the dialysate was determined at 60, 120, 180 and 240 min using glucose peroxidase assay kit. The glucose dialysis retardation index (GDRI) was calculated using the following formula:  $\text{GDRI} = 100 - [(\text{glucose content in sample}) / (\text{glucose content of control})] \times 100$

***In vitro amylolysis kinetics:***

*In vitro* amylolysis kinetics of different types of tea sample was determined according method of Ahmed and Urooj (2010). About 40 g of potato starch was added to 900 mL of 0.05 M phosphate buffer (pH 6.5). The solution was stirred at 65 °C for 30 min, after that the solution was made up to a final volume of 1000 mL to obtain a 4 % (w/v) starch solution. The starch- $\alpha$ -amylase-dietary fiber system was comprised of 4 % starch solution (25 mL),  $\alpha$ -amylase (0.4 %) and test sample (1 %) were dialyzed in a dialysis tubing membrane against 200 mL of distilled water at 37 °C in a shaker water bath. A control test was carried out without addition of tea sample, whereby acarbose (0.2 %) was carried out by substitute the test sample. The glucose content in the dialysate was determined at 60, 120, 180 and 240 min. An amount of 1 mL dialysate was taken for glucose determination spectrophotometrically using glucose oxidase-peroxidase assay kit. The glucose dialysis retardation index (GDRI) was calculated using the the same GDRI equation from section *in vitro* glucose adsorption capacity.

***Statistical Analysis:***

All data are presented as mean  $\pm$  SD for each assay. Statistical analysis was carried out using the statistical package SPSS 18 (Statistical Package for Social Science, SPSS Inc.) program and significance each group was verified with One-way analysis of variance (ANOVA) followed by the Duncan's multiple range test were completed compare of means.

**RESULTS AND DISCUSSIONS*****In vitro glucose adsorption capacity:***

The glucose adsorption capacities of samples at different glucose concentration are shown in Figure 1. All samples could bind glucose effectively and their glucose-binding capacities were directly proportional to the glucose concentration. The amount of glucose adsorbed by BT was significantly higher than others tea samples.

At the initial glucose concentration of 5 mM, BT adsorbed a total of 1.85 mM of glucose, which in turn amounts for 37% of the initial content compare to OT (1.78 mM, 35.6%) and GT (1.28 mM, 25.6%). The results implied that tea powder could absorb small amount of glucose even at low glucose concentration. Similar result was reported by Urooj and Ahmed (2010), indicated that *Ficus racemosa* bark powder can help to retain the glucose in the intestinal lumen even at low glucose concentration.

At glucose concentration of 20 mM concentration, BT adsorbed a total of 15.36 mM of glucose, which in turn amounts for 76.8% of the initial content compare to OT (14.8 mM, 74%) and GT (14.37 mM, 71.8%). Thus, it shows that glucose-adsorption capacities of tea powder were concentration-dependent and increased with the higher concentration of glucose solution.

In case of spent tea (SBT, SOT and SGT), the adsorption was significantly lower compared to normal tea (BT, OT and GT) respectively. This might due to the lower phenolic content in spent tea compared to normal tea. This result is in agreement with the result of Nadiah and Uthumporn (2015), who stated that spent tea exhibit lower total phenolic contents compared to normal tea.

Furthermore, the increase ability of tea powder to adsorb glucose might be attributed to the dietary fiber present in the samples are reported to adsorbed glucose (Ou, S., *ET AL.*, 2001). The mechanism of fibers such as

blocking diffusion of glucose and glucose adsorbing, reduce the concentration of the glucose in the small intestine, could help in lowering the postprandial serum glucose level (Schneeman, B.O., 1998).

#### ***In vitro glucose diffusion:***

The samples effectively inhibited the diffusion of glucose as presented in Table 1; Effect of different types of tea on glucose diffusion.

The glucose diffusion rate was significantly low among the samples especially BT which allowed the diffusion of 0.70 mmol of glucose in 60 min compared to control which was 2.86 mmol. All tea powder effectively inhibited the diffusion of glucose in the dialysis model. This is due to the lowest of glucose content in dialysate of tea compared to control and acarbose in order of green > oolong > black tea. Glucose content in dialysate from normal tea was lower than spent tea respectively at each time interval. The trend of glucose content in dialysate also correlated with normal tea in order SGT > SOT > SBT. Dialysis tubing technique is a simple model to evaluate the potential of dietary fibers to retard the diffusion and movement of glucose in the intestinal tract (Adiotomre, J., *et al.*, 1990). Thus, in this system of glucose movement is a model of the true diffusion that assisted by convective activity of gastrointestinal contractions. This dialysis model is similar to the glucose movement in human body, thus this model can be used to determine the glucose diffusion *in vitro* activities.

Glucose dialysis retardation index (GDRI) is a useful *in vitro* model to predict the effect of fiber on delaying glucose absorption in the gastrointestinal tract. It is to determine on the basis of the retardation of glucose diffusion. The GDRI value of acarbose was significantly lower compared to all types of tea samples at each time interval. Black tea exhibited highest value of GDRI at each time interval compared to oolong and green tea. The relative order of different type of teas on retardation of glucose diffusion were in order of black > oolong > green tea. The lowest glucose content in dialysate for black tea was evident of effectively inhibited of glucose diffusion as the highest value of GDRI. However, the GDRI for spent tea is lower than normal tea but in a same trend as in order of SBT > SOT > SGT.

The results suggesting the effectiveness of all tea powder samples in retarding the glucose diffusion. For all samples, the GDRI was found to decrease as the intervals of time increased. According to Ou *et al.* (2001), at beginning of dialysis, diffusion of glucose was retarded by adsorption and viscosity of fibers. This results in slower diffusion rate of glucose (higher GDRI) at the beginning, as time progress, the adsorption becomes saturated with glucose and diffusion rate increases resulting in lower GDRI values.

Even though, GT has higher total phenolics content compared to OT and BT (Nadiah, N.I. and U. Uthumporn, 2015), but GDRI of GT was significant lower compared to OT and BT. Results suggested that tea polyphenols might not be the main factor contributed to glucose diffusion retardation. Retardation of glucose by all tea powder samples could be attributed to the presence of fibers in tea powder. According to (Chau, C.F., *et al.*, 2004), they found that *in vitro* studies on insoluble fiber-rich fraction derived from *Averrhoa carambola* (starfruit) could effectively adsorb glucose and delay the glucose diffusion. Thus, this study suggested insoluble fiber particles could provide physical obstacle glucose molecules and cause entrapment of glucose within the network formed by fibers.

#### ***In vitro amylolysis kinetics:***

Amylolysis is the reaction that converts starch into simple sugars by enzyme reaction. Table 2; *In Vitro* amylolysis kinetics of different types of tea, shows the result on diffusion and GDRI in the starch- $\alpha$ -amylase-dietary fiber system of *in vitro* amylolysis kinetics. The glucose content in dialysate increased as time prolong for all tea samples, except in acarbose system where no diffusion had took place at all time intervals. This is probably due to the complete inhibition of  $\alpha$ -amylase in the system by acarbose, thus no breakdown of starch took place.

Breakdown of starch and diffusion of glucose in the system contain different types of tea was significantly lower at all time intervals compared to control, furthermore no diffusion of glucose occurred for BT, OT and GT within the first 60 min interval. The retardation of glucose diffusion is due to the inhibition of  $\alpha$ -amylase. However, after 60 min time interval, no longer 100% retardation of glucose diffusion of all types of tea samples except acarbose. This is might due to increasing of glucose concentration in dialysate as byproduct of starch hydrolysis. At 240 min, the ranking value of GDRI were in order of BT > OT > GT > SBT > SOT > SGT. Thus, it shows that black tea had highest potential in retarding of glucose diffusion compared to others types of tea samples. This is probably due to different composition of phenolic compounds such as theaflavin and thearubigins in black tea that contributing to higher degree of inhibition, thus lowering the  $\alpha$ -amylase activity.

The rate of starch hydrolysis could be delayed by tea polyphenols in different types of tea samples. Polyphenol were found to be effective inhibitors of intestinal  $\alpha$ -amylase activity, which may suggest polyphenols have potential therapeutic effect by limits the breakdown of glucose from starch, thus slow down the glucose diffusion rate (Broadhurst, C.L., *et al.*, 2000). According to Honda and Hara (1990) suggested that

black tea have highest  $\alpha$ -amylase inhibitor properties due to higher molecular weight polyphenols, theaflavin and thearubigins which more abundant in black tea compared to green and oolong tea.

Glucose content in dialysate from BT system was significant lower than SBT at all time intervals. Furthermore, BT showed significant higher GDRI compared to SBT. This is probably due to higher phenolic compounds in BT contributing to higher degree of inhibition, thus lowering the  $\alpha$ -amylase activity. The results are in good agreement with previous studies, (Gurza, I., *et al.*, 2011) had found that polyphenols extract from black tea exhibited, 100%, 99% and 91% reduction of starch hydrolysis in corn, potato and wheat starches, respectively.

Besides, the inhibition of  $\alpha$ -amylase activity by medicinal plants might be attributed to several factors such as fiber concentration, the presence of inhibitors on fibers, encapsulation of starch and enzyme by the fibers present in the sample (Ahmed, F. and A. Urooj, 2010). Thereby, decreased amylase activity is due to reducing accessibility of starch to the enzyme and direct adsorption of the enzyme on fibers. Inhibitors of carbohydrate-hydrolyzing enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and lower the postprandial plasma glucose levels (Ahmed, F. and A. Urooj, 2010).

### Conclusion:

All types of tea such as black, oolong and green tea shows significant hypoglycemic activity. In the present study, strongly suggest that black tea has high ability in glucose adsorption compared to other types of tea. The retardation of glucose diffusion were in order of black > oolong > green tea. All type of teas was potent inhibitors. However, black tea gave the highest % of retardation of glucose diffusion in the inhibition of  $\alpha$ -amylase. The inhibitory effect of  $\alpha$ -amylase were also in order of black > oolong > green tea. Besides that, spent tea was potent inhibitors too. However, the potent inhibitors of spent tea are lower than normal tea. This is due to low phenolic compounds in spent tea as spent tea is a waste byproduct of tea processing. Thus, spent tea is less effective inhibitor of glucose absorption and glucose diffusion as well as  $\alpha$ -amylase inhibitors. However, spent tea can be a significant source of bioactive phenolic compound that exert antioxidant and postprandial blood glucose-lowering effect. These results indicate that spent tea could be utilized as renewable bioresource to develop functional food, health-promoting and potential antidiabetic agents.

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