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Effect of Tea, Coffee and Cocoa Drinks on *in-vitro* Starch Hydrolysis from Rice Composite Diet

Israel Olusegun Otemuyiwa*, Oluwaseun Francis Oguntola,
Steve Adeniyi Adewusi

Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria

Email address

otemuyisegun@oauife.edu.ng (I. O. Otemuyiwa),

muyiwasegun100@yahoo.com (I. O. Otemuyiwa)

*Corresponding author

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Abstract

This study evaluated the influence of polyphenol – carbohydrate interaction on *in-vitro* starch hydrolysis from rice diet using tea, coffee and cocoa drinks as the source of polyphenols. The rate of starch hydrolysis in rice and composite diets were studied using controlled enzymatic digestion with pepsin, amylase and amyloglucosidase. The glucose released was quantified using dinitrosalicylic acid, and the result obtained was used to calculate the glycemic index (GI). Fourier-Transform Infrared (FT-IR) spectroscopic method was used to identify starch-polyphenol interaction in the composite diets. The results indicated that the equilibrium concentration of the composites ranged from 32 to 48.5%, the kinetic constant from 0.024 to 0.04, the hydrolysis index from 55.10 to 88.2% and GI from 69.9 to 88.1%. Comparing the result with rice alone, black and green tea caused 25.7% reduction in GI, coffee (17%) cocoa (15.5%) and chocolate (6.68%). The FT-IR spectra of the composites indicated spectra shifts in wavelength recorded at 3394cm^{-1} , 1026 and 1367 corresponding to O-H bonds, O-C bonds and C-O-H bonds, respectively, supporting the possibility of starch-polyphenol complex formation. The study revealed that tea, coffee and cocoa drinks can help control the release of glucose from carbohydrate foods, hence, intake of the drinks could help regulate glucose homeostasis in patients suffering from type 2-diabetes. The study provides an insight that consumption of beverages is not only for pleasure but as nutraceutical.

1. Introduction

Polyphenols are multifunctional dietary components; evidence of the role of polyphenols in the prevention of chronic degenerative diseases is emerging, and the effect of their action on carbohydrate metabolism is one of the proposed activities. Many of the potential health benefits of polyphenol have been associated with their specific chemical and biological properties including their ability to interact and bind non-covalently to macronutrients in foods [1].

Current dietary guidelines for prevention of obesity and other non communicable diseases focus on lowering dietary fat and increasing complex carbohydrate intake such as starch and dietary fiber [2]. Food products such as potatoes, white rice, and bread serve as a major source of carbohydrate and are known to have high GI - an index that provides a measure of the increase in blood glucose levels after consumption of a food.

Diabetes mellitus is one of the most common metabolic disorders, according to the

International Diabetes Federation; thus, there are approximately 366 million individuals around the world suffering from diabetes and it is assumed that this figure could increase to 552 million by the year 2030. *Diabetes mellitus* is characterized by hyperglycemia (that is, high blood glucose level) due to partial or absolute lack of insulin activity (Type I) and/or insulin resistance (Type II). Many factors have been implicated to play crucial roles in the development of hyperglycemia and progression of diabetes in which obesity and dietary attributes are considered major contributors [3]. Impaired carbohydrate metabolism and developing insulin resistance is the main metabolic disorder in non-insulin dependent *diabetes mellitus* leading to hyperglycemia, altered digestion and absorption of dietary carbohydrate, depletion of glycogen storage, increased gluconeogenesis and excess output of hepatic glucose; β -cell dysfunction, insulin resistance of peripheral tissue and defect in insulin signaling pathways [1].

Tea, traditionally, has been considered to have health-promoting potential in most part of the world. This is due to the various polyphenolic compounds present in them [4]. Consumption of tea and beverages are common especially among youths and elites as part of breakfast and when consuming foods considered as 'light' foods like bread, biscuits, snacks and even rice. Polyphenols have been reported to interact with carbohydrates and such interactions have been observed to reduce starch digestibility and glycemic index of food [2].

It may be needful to examine the magnitude of the effect of polyphenol interaction on carbohydrate catabolism in foods in order to evaluate its effectiveness in controlling glucose release from carbohydrate sources and hence, the glycemic index. Therefore, the objectives of this study were to carry out *in-vitro* starch digestibility study of composite diets consisting of rice and either tea, cocoa or coffee drinks and to predict the effect of these drinks on its glycemic index; and also to measure the infrared vibrational bands of the composite diets in order to confirm the possibility of polyphenol-starch interactions.

2. Materials and Methods

2.1. Sample Preparation

The samples consist of green tea, black tea, coffee, and two brands of cocoa drinks labeled chocolate and coco obtained from Obafemi Awolowo University Supermarket, Ile-Ife, Nigeria while Aroso rice was purchased from Odo-Ogbe Market, Ile-Ife, Nigeria. The rice sample was washed with distilled water, the water was drained off and the rice was air-dried, milled and kept in refrigerator until used.

2.2. Preparation of the Tea and Beverages

Ten tea bags of each brand were taken; the contents were pooled together and properly mixed before samples were taken for analysis. Tea infusion was prepared by introducing 2.00 g of tea into boiling water (200 ml) for 10 min. The

solution was filtered using cheese cloth. Coffee, chocolate and coco-drink were prepared by dissolving 2.00 g of each in boiling water (200 ml) and stirred. The beverages were analysed immediately after preparation.

2.3. Determination of Total Starch on Rice Sample

The rice sample (1.00 g) was weighed into a thimble and placed in the soxhlet extractor containing 85% (v/v) ethanol to extract sugar for 2 hours. The residue was dried at 60°C to constant weight. The residue (200mg) was weighed into a flat-bottom flask, 110 ml of 0.7M HCl and about 3 glass beads were added. The content of the flask was placed in a boiling water bath inside a fume cupboard and refluxed for 2.5h with occasional shaking; the volume of the liquid was maintained by adding water periodically. After refluxing, the hydrolysate obtained was cooled and neutralized to pH 7.0 using 5.0M NaOH. The hydrolysate was poured into a 500ml standard flask and made up to volume with distilled water. 50ml aliquot was filtered and kept for the determination of the reducing sugar.

2.4. In-Vitro Starch Hydrolysis of Rice and Tea, Cocoa, Coffee Composite Meals

The rice sample (250g) was weighed into 100ml beaker; 20ml of distilled water was added, placed on hot plate and heated at 100°C for 20 min (to gelatinize the starch).

The rice composite diets were prepared by adding (20ml) of tea infusion, cocoa and coffee drinks separately to the gelatinized rice while 20ml of distilled water was added to the gelatinized rice (rice alone) as the reference diet. The diets were prepared in triplicate.

To the rice composite diets were added 20 mL of HCl - KCl buffer (pH 1.5) and homogenized using a basic homogenizer (Kika Labortechnik 725, Janke and Kukul GmbH & Co., Stanfen Germany) at 9500 rpm for 1 min. The homogenate was then digested with 20 mg of pepsin prepared in HCl - KCl buffer pH 1.5 (Sigma; CAS 2001/75-6, code 10132561, 666 IU /mg, porcine gastric mucosa) for 2 h; after which, the mixture was adjusted to pH 6.9 by 10 mL Tris - maleate buffer. Further digestion was carried out with 2.6mL α -amylase in 5 mL of Tris - maleate buffer and incubated at 37°C in a shaking water bath. Aliquots (1.0 mL) were withdrawn at 30 min intervals, the enzyme inactivated at 100°C for 5 min and finally hydrolyzed with amyloglycosidase (Sigma, Number 10105-5GF, 70 IU /mg, *Aspegilius niger*) (5ml). Glucose was determined using dinitrosalicylic acid method [5]. Starch hydrolysis was carried out for 180 min. The experiment was performed in triplicate for each sample.

The rate of starch digestion was expressed as the percentage of total starch hydrolyzed at different times. The digestibility curve for each food sample was fitted into the first-order kinetic equation [6].

$$C_t = C_\infty (1 - e^{-kt})$$

Where C_t is the percentage of starch hydrolyzed at time t (min).

C_∞ is the equilibrium starch hydrolysis after 180 min. and k is a pseudo-first order rate constant.

The rate constant (k) and equilibrium constant (C_∞) were calculated from the equation based on the data obtained from starch hydrolysis.

Hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of the sample by the corresponding area of a reference food (Aroso rice) expressed as a percentage [6]

Glycemic index (GI) was estimated using the equation of Goni *et al.*[7]:

$$GI = 39.71 + (0.549 \times HI)$$

Standard stock solution of glucose (5.0mg glucose/ml), 0.0 ml, 1.0ml, 2.5ml, 4.0ml and 5.0ml equivalent to 0, 200,500, 800 and 1000 μ g of glucose/2ml were measured into 25ml volumetric flask each and made up to volume with distilled water.

To each of the amyloglucosidase digestate, 0.1ml of 0.1M sodium hydroxide and 0.1ml of the dinitrosalicylic acid reagent were added and placed in a water bath for 15 minutes for development of orange-red colour in a test-tube. The content was then transferred into a 25ml standard flask and made up to the mark with distilled water. The absorbance was taken with a UV spectrometer at 590nm and the glucose released was extrapolated from the glucose standard using a linear curve equation $y = 0.001x + 1.621$ with a correlation coefficient $R^2 = 0.997$.

4. Results and Discussion

2.5. Fourier Transform Infrared (FT-IR) Spectroscopy of Starch - Polyphenol Interaction

The rice sample (1.0 g) was placed in 100 mL beaker; distilled water (10 mL) was added and heated at 100°C for 5 min to gelatinize the starch. The gelatinised starch was centrifuged and the supernatant was discarded, the residue was air-dried, powdered. The rice sample was prepared with the beverages as described. The powdered samples were taken for FT-IR analysis.

The methanolic extract of tea, cocoa and coffee prepared by extracting 5.00 g each of the samples with 25ml methanol-water (70:30), the solution was filtered and solvent was evaporated. The residue was taken FT-IR analysis.

The spectra were obtained using an FT-IR spectrophotometer (Shimadzu, Japan). A KBr pellet was made by accurately weighed 100mg of potassium bromide (KBr) and 2mg of the sample, (KBr) and pressed into a disc of *ca.* 1 mm thick. The FT-IR spectra was analysed within the range of 4000-500 cm^{-1} and the spectral was analysed using chemstation computer software.

3. Statistical Analysis

Results were expressed as mean and standard deviation of triplicate analysis and the data were treated with one-way analysis of variance (ANOVA), and Duncan Multiple test was used to test the level of significant difference ($P < 0.05$). Microsoft Excel package and GraphPad InStat version 3.06 for Windows (2003) were used for the analysis of data.

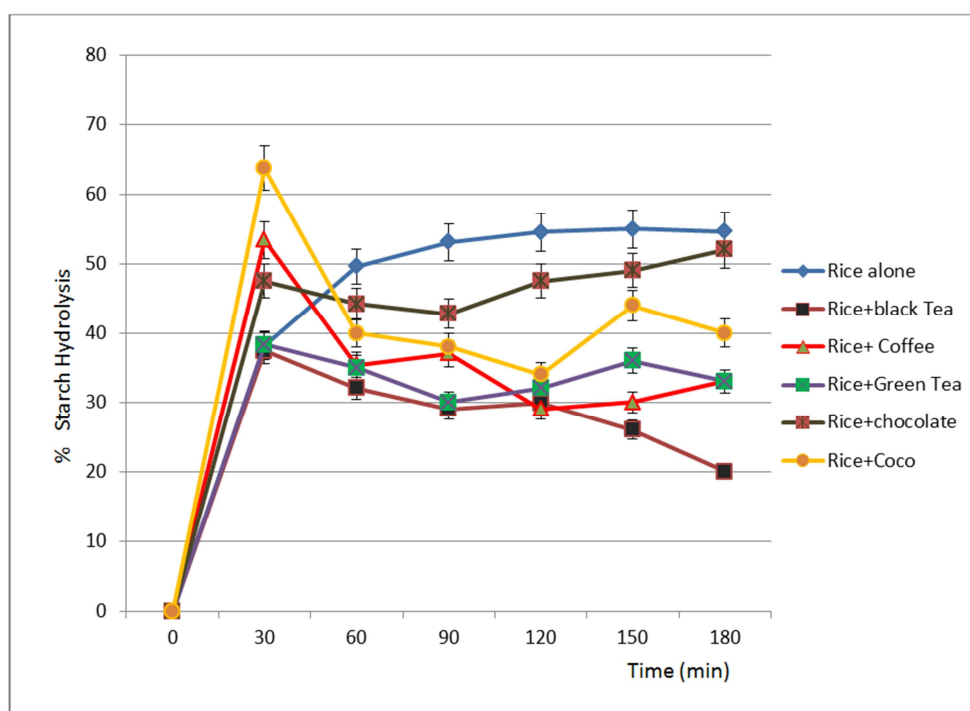


Figure 1. Starch hydrolysis pattern of Rice-tea, coffee and cocoa drink composite diets compared to rice alone (reference diet).

The results of the interaction in food model on the rate of starch hydrolysis are presented in Figure 1 while the kinetic parameters are shown in Table 1. As shown on Table 1, the percentage equilibrium concentration (Ca) showing the amount (%) of starch hydrolysed within the period of 180min ranged from 32.0 to 55.0 %, the values obtained for black and green tea were significantly lower than the control (rice alone). Also the kinetic constant (K) which represents the rate of starch hydrolysis, ranged between 0.024 and 0.04, coco and chocolate composites recorded K values that were not significantly different ($P \leq 0.05$) from control diet (rice alone). The hydrolysis index ranged from 55.1 to 73.7 % while the

rice alone (reference diet) is assigned 100% hydrolysis. The glycemic index of the composited diets ranged from 69.9 to 88.1%, the hydrolysis index and glycemic index in decreasing order (Table 1) indicated chocolate > coco > coffee > tea (green and black). The result also indicated that the addition of tea (green and black) resulted in decreased glycemic index by 26%, coffee (17.5%), coco (16.5%) and chocolate reduced glycemic index by 6.9%. It was observed from Figure 1 that glucose release within 30 min of digestion was higher in coco composite diet (64%), chocolate (47.5%) and coffee (53.4%) as against 38% released from tea composite diet and reference diet (rice alone).

Table 1. Equilibrium concentration (Ca), Kinetic constant (K), Hydrolysis Index (HI) and Glycemic Index of Rice – tea, cocoa and coffee composite meals^{*1, 2}.

Sample	Ca	K	AUC	HI	GI
Rice – alone ^{*3}	55.0 ± 2.0 ^a	0.04 ± 0.006 ^a	8526 ± 15 ^a	100 ± 0.0 ^a	95 ± 3.0 ^a
Rice-green tea	36.2 ± 0.9 ^d	0.024 ± 0.002 ^b	4729 ± 14 ^c	55.5 ± 0.9 ^d	70 ± 2.5 ^c
Rice-coffee	40.8 ± 0.4 ^c	0.032 ± 0.001 ^b	5953 ± 9 ^d	69.8 ± 1.2 ^c	78 ± 3.8 ^{bc}
Rice-chocolate	48.5 ± 0.7 ^b	0.04 ± 0.003 ^a	7516 ± 22 ^b	88.2 ± 6.0 ^b	88 ± 4.0 ^{ab}
Rice –coco	40.4 ± 1.2 ^c	0.04 ± 0.001 ^a	6200 ± 10 ^c	72.7 ± 2.0 ^c	80 ± 2.2 ^{bc}
Rice - black tea	32.0 ± 1.0 ^e	0.03 ± 0.001 ^b	4698 ± 20 ^c	55.1 ± 6.5 ^d	69.9 ± 4.0 ^c

^{*1} Means and standard deviations of triplicate analysis

^{*2} Values with the same superscript in a column were not significantly different ($P \leq 0.05$).

^{*3} rice alone- reference diet

The high reducing effect of tea might be due to the presence of complex polyphenols with several hydroxyl groups such as theaflavin and thearubigin [8]. The mechanism of action of polyphenols in reducing the rate of glucose release from carbohydrate sources have been attributed to interactions that could lead to inhibition of α -amylase and amyloglucosidase and also prevent active glucose transport [4, 9].

Coco and chocolate drink are rich in the flavanols including catechin and epicatechin, most of which were destroyed during heat and other processing to which cocoa was subjected to, this could have reduced the compounds that could have interacted with the starch components [9]. Heating or hot water could result in polymerisation of the flavanols, thus reducing the available functional groups for reactivity [10]. Whichever mechanism, the result was a reduced percentage inhibition in glucose released. The high rate of sugar hydrolysis observed within 30 min of digestion could be traced to addition of sugar to the refined coco and chocolate during processing in order to mask bitterness and thus improve the taste. That added sugar will, of course, be first hydrolysed before starch.

The findings of this study confirmed the report of *in-vivo* experiments that polyphenol and fibre rich dried fruit attenuate starch-derived post-prandial glucose and insulin response [11].

The spectra of FT-IR analysis and diagnostic bands observed were reported in Figure 2 and Table 2 respectively. Information from FT-IR spectra showed that the intense peaks at 3500-3200 cm^{-1} indicated the presence of O-H groups in rice starch. Likewise, the strong peaks at 3000-2850 cm^{-1} assigned to the C-H stretching and peaks at 1030-990 cm^{-1} would be characteristic of the

anhydroglucose ring O-C stretch. The peaks at around 1450 and 1200 cm^{-1} were characteristic of C-O-H. while peaks at 1600-1500 cm^{-1} attributed to the bending of water in starch. The spectral of the composite diets (rice and beverages) showed that the O-H bond of rice starch alone changes from 3394 cm^{-1} to 3392 cm^{-1} in rice-green tea composite for instance, which could be due to the strengthening of the hydrogen bond, such that a shift to a lower wave-number was observed. This band exhibited the red-shift phenomenon. A broad band due to hydrogen-bonded hydroxyl groups (O-H) in rice starch appears at its most intense at 3394 cm^{-1} , which is attributed to the complex vibrational stretches associated with free, inter- and intra-molecular bound hydroxyl groups (O-H stretching [12]. The measurement of the O-H stretching band has been proven to be a sensitive indicator of the strength of the hydrogen bond. The O-H stretching band also shows a slight change in its wave number and intensity depending on the formation of hydrogen bonds. The broadening of O-H stretching band has often been observed in strong hydrogen bonds [13, 4, 14]. Overall, non-covalent binding of polyphenols with macronutrients is primarily driven by Van der Waals' interactions; these interactions are modulated by characteristics such as degree of polymerization, molecular flexibility, number of external hydroxyl groups, or number of terminal galloyl groups. From the macronutrient standpoint, electrostatic and ionic interactions are generally predominant with carbohydrates, while hydrophobic interactions are generally predominant with lipids. The FT-IR technique detects the absorption of different bond vibrations in starch molecules and it is sensitive to changes in molecular structure such as starch chain conformation, helicity and crystallinity [1].

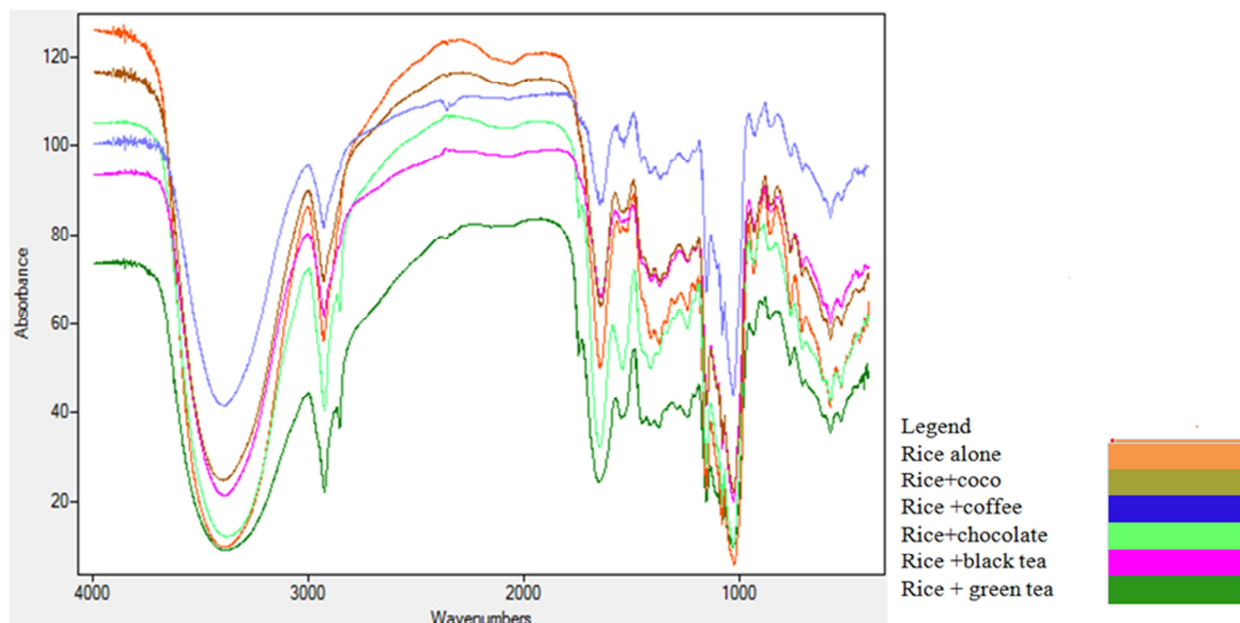


Figure 2. FT-IR spectra of rice starch and rice - beverages composite diets.

Table 2. Characteristics band of starch in rice and tea, coco and coffee drinks composite diets.

Spectra bands	Assignment	Rice alone	Rice-green tea	Rice-black tea	Rice-coco	Rice-chocolate	Rice-coffee
Intense peak wavelength (cm ⁻¹)							
3500 – 3200 cm ⁻¹	O – Hof starch	3394	3392	3385	3381	3381	3381
3000 – 2850 cm ⁻¹	C – H	2928	2928	2928	2926	2929	2926
1030 - 990 cm ⁻¹	O – C	1062	1024	1028	1026	1024	1026
1450 - 1200 cm ⁻¹	C – O – H	1367	1367	1367	1373	1371	1411
1600 – 1500 cm ⁻¹	O – Hof water	1539	1539	1546	1548	1556	1541

5. Conclusion

The study revealed that tea, cocoa and coffee drinks caused marked reduction in the rate of hydrolysis of rice starch as well as its glycemic index under *in-vitro* condition. Both green and black tea recorded high reduction impact. This shows that polyphenol containing compounds have potential application to ameliorate health problems related to Type II diabetes. The study also provides an insight that consumption of beverages is not only for pleasure but could also provide nutraceutical compounds.

Conflict of Interest

None declared by the authors

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