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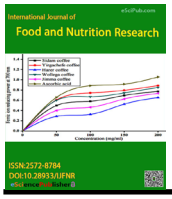

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In-Vitro Starch Hydrolysis and Prediction of Glycaemic Indices of Biscuits Produced from Wheat, African Walnut and Moringa Seed Flour Blends

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ABSTRACT

African walnut and moringa seed were procured and processed into flours. Biscuits were thus produced from different blends of wheat flour (WHF), African walnut flour (AWF) and moringa seed flour (MSF) in the ratios of (WHT:AWF:MSF) 100:0:0, 77.5:20:2.5, 75:20:5, 72.5:20:7.5, 70:20:10, 90:0:10, 80:20:0 and labelled from A to G, respectively. The produced biscuits were evaluated for dietary fibre content, in-vitro starch hydrolysis and predicted glycaemic indices. The results of dietary fibre content of the biscuits revealed that sample E was significantly higher with a value of 0.72g compare to other samples. Dietary fibre content of the biscuits increased as the level of substitution with moringa seed flour increased. Results of the in-vitro starch hydrolysis of the biscuits showed that the percentage starch hydrolysed reached its peak at 120 min of digestion and after which, a reduction steps in as digestion time increases. Equilibrium concentration, hydrolysis index and predicted glycaemic indices of the biscuits reduced as the level of substitution of moringa seed flour increased. It revealed sample E with Equilibrium concentration value of 48.06, hydrolysis index of 51.66% and predicted glycaemic index of 68.07. Thus, the blends of 70:20:10 (WHT:AWF:MSF) which represented sample E could be used as medium glycaemic index food.

Keywords: Dietary fibre; Starch hydrolysis; Glycaemic index; Biscuits; Moringa seed; African Walnut

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INTRODUCTION

African walnut botanically known as Tetracarpidium conophorum is found mainly in Nigeria, Southern Cameroons and the Congo region. Tetracarpidium conophorum is a dry nut that is enclosed in a hard shell and the plant is cultivated primarily for its nuts which are cooked and consumed. The nuts could be white or creamy on cracking the shell after it is cooked [1]. Investigations have revealed a high presence of phytochemicals and polyphenols containing ellagic and gallic acids. The presence of phenolic acids have contributed to the astringent taste associated with the nuts [2]. Other health related compounds found in the African walnut are high biological amino acids such as Arginine, Methionine and Leucine, as well as fibre and vitamins.

Osuaoha and Nwaichi [3] have reported the potential of African walnut in lowering the risk of heart disease and promoting weight loss. They further stated that the presence of seven essential amino acids in the African walnut makes the walnut a nutritionally important component in food preparations. Study conducted by Oriakin et al. [4] revealed that extracts of African walnut are rich sources of secondary metabolites and have strong antioxidant properties which exhibited good scavenging activity. Application of walnut flour in cookie production have been reported by Barber and Obinna-Echem [5], with composite formulations with wheat flour at 5 – 15% substitution levels having acceptable product quality. Their study revealed improvements in protein, fat, crude fibre and ash content of the cookies at the blend of African walnut flour to wheat flour.

Moringa oleifera is a widely grown genera Moringacea and is known in some countries as drumstick tree or horse radish tree [6]. Oparinde et al. [7] investigated the effect of moringa seed extract on diabetic induced rats and reported a significant reduction in fasting blood glucose after the rats were fed with moringa seed extracts. Al-Malki and El-Rabey [8] also reported the antidiabetic and anti-oxidant activity of moringa seed powder and affirmed its scavenging effect on nitric oxide radicals. It was further reported that moringa seed powder would be a potential source of natural antioxidant. Apart from its neutraceutical uses, moringa seed have been used in the treatment of hypercholesterolemia and hyperglycaemia as well as in nutritional supplementation. Emelike et al. [9] studied the physico-chemical and sensory properties of cookies fortified with moringa leaf paste and reported that 10% moringa leaf paste addiction to the formulation of the cookies increased the protein content of the cookies. Kiin-Kabari et al. [10] also reported on the enrichment of moringa leaf flour (MLF) in cookie production and observed the increment in the protein values of the cookies with 5% level of MLF. Moring leaf and the seed can then be prescribed in food for coronary artery disease and malnutrition control.

Adejoh et al. [11] reported the inhibitory activity of moringa seeds on alpha amylase and glucosidase digestive enzymes. The glucosidase enzymes are located at the intestinal brush boarder of the intestine which is responsible for digestion of carbohydrates and absorption of glucose in the digestive tract. The management of diabetes can be achieved by reducing post prandial hyperglycemia by delaying the activities of alpha amylase and alpha glucosidase enzymes, respectively.

Wang et al. [12] reported that dietary fibres can be classified into two major predominant types and they are composed of those fibres that are dispersed in water, which are referred to as soluble dietary fibre (SDF) and the structural non-viscous fibres which are insoluble in water. Vegetables and cereals are especially rich in water insoluble fibres, with the highest amounts found in wheat and corn. Water insoluble fibre is respon-

sible for increased stool bulk and helps to regulate bowl movement. The type, source and amount of dietary fibre influence the intestinal function in a number of ways. Also, fibres bind a large amount of water thereby enlarging the aqueous phase of the food pellets and slows down the absorption of nutrients [13]. Other benefits are prebiotic effects and cell protection.

Dietary carbohydrates are digested and absorbed at different rates and to different extents in the human small intestine, depending on their botanical source and the physical form of the food. Diets that contain large amounts of rapidly digested carbohydrates, which elevate blood glucose and insulin responses may be detrimental to health. It has been suggested that diets rich in slowly digested carbohydrates may protect against chronic diseases [14].

Starch hydrolysis is affected by the degree of gelatinization, as gelatinised starch is readily susceptible to the activity of alpha amylase. Shin et al. [15] reported that the structure of the food matrix, as well as the presence of insoluble fibres affect the rate of starch hydrolysis. Insoluble fibres in food tissues retards the rate of starch hydrolysis and the destruction of matrix through grinding or milling increases the rate of starch digestion. Tormo et al. [16] have reported that the presence of other food components such as proteins, lipids and phytic acids in legumes can reduce starch surface accessibility by blocking absorption sites needed for enzymes to bind or substrates, thus leading to low digestibility levels.

The glycaemic index is defined as the incremental area under the blood glucose response curve (the change in blood glucose level two hours after a meal) of 50g available portion of a test food expressed as a percentage of the response to the same amount of carbohydrate from a standard food (either white bread or glucose) ingested by the same subject [17]. Goni et al. [18] reported the kinetics of starch hydrolysis using the first order equation model and studied the percentage of starch hydrolysed over time period of 180 min as well as the Kinetic constant of the hydrolysed starch.

Thus, this study is intended to formulate biscuit from the blends of African walnut flour, Moringa seed flour and wheat flour and evaluate the effects of these blends on the dietary fibre content, starch hydrolysis and prediction of glycaemic index.

MATERIALS AND METHODS

Collection of Samples

Mature African walnut (Tetracarpidium conophorum) were procured from a market in Aba, Abia State, Nigeria. Moringa oleifera seeds were procured from Gaiya farms Ltd, Zaria, Kaduna State, Nigeria, Wheat flour and other ingredients used in biscuit making were purchased from Nextime Supermarket, Port Harcourt, Rivers State, Nigeria while Enzymes such as Protease (porcine), α- amylase, Amyloglucosidase (A 7420 E.C.3. 2.1.3 Sigma) and Pepsin (Cat No P6887 Sigma chemical Ltd.) used for the analysis were purchased from Sigma Aldreich Chemical Ltd, Germany. All these were transferred to the Department of Food Science and Technology Laboratory, Rivers State University, Port Harcourt for processing and analysis.

Processing of Samples

African walnut flour

African walnut flour was processed as described by Barber and Obinna- Echem [5]. The nuts were sorted according to uniform sizes, washed and boiled with distilled water (3:1) volume by weight for 90 min and allowed to cool. Unit operations of cracking and de-hulling of the shells were done manually. Particle size of nuts were reduced to 1cm in diameter and dried in an electric oven at 54^{o}C for 6 hr to allow for complete drying and ease of milling. After which, the dried African walnuts were milled and sieved using 250µm particle size sieve, package and stored for usage

as required.

Moringa seed flour

Moringa seed flour was processed as described by Ogunsina et al. [19] with some modifications. The seeds of irregular colour and shape were discarded in order to have raw materials of uniform physical characteristics. The sorted seeds were dehulled, boiled in water for 10 min and allowed to cool and water was drained. The boiled seeds were then dried in an electric oven at 54°C for 4 hr and milled to pass through an aperture size of 250µm.

Table 1. Recipe Formulation from the Blends of WHF, AWF and MSF for the Biscuit Production

	Composite Biscuit Samples						
Ingredients	**A**	**B**	**C**	**D**	**E**	**F**	**G**
Wheat flour (g)	400	310	300	290	280	360	320
Walnut flour (g)	0	80	80	80	80	0	80
Moringa seed flour (g)	0	10	20	30	40	40	0
Sugar (g)	120	120	120	120	120	120	120
Margarine (g)	180	180	180	180	180	180	180
Salt (g)	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Baking powder (g)	2	2	2	2	2	2	2
Egg (whole)	1	1	1	1	1	1	1
Vanilla (ml)	5	5	5	5	5	5	5

Key:

WHT = Wheat Flour, AWF = African walnut flour, MSF = Moringa seed flour

Sample A = 100:0:0, B = WHF 77.5:20:2.5, C = 75:20:5, D = 72.5:20:7.5, E = 70:20:10, F = 90:0:10, G = 80:20:0 (WHT: AWF: MSF)

Biscuit Production

Biscuit were produced from the formulated blends of wheat flour, African walnut flour and moringa seed flour according to the procedure illustrated by Agu and Okoli [20] with some modifications. Dry ingredients were manually mixed in a mixing bowl to achieve uniformity and margarine was rub in. One whipped whole egg and 5ml of vanilla flavour were added and mixed thoroughly again for about 10 min to form a smooth dough. The dough was moulded out of the mixing bowl and rolled on a baking table with the aid of a rolling pin. The adequately rolled out dough were cut to a biscuit size using pastry cutter and transferred to aluminium baking trays, whose surfaces have been previously lubricated with margarine to prevent burning. The surfaces of the biscuits were glazed with whipped egg. This was then transferred to a pre-heated oven at 180°C and baked for 25 min. The baked biscuits were then removed from the oven, allowed to cool before packaging for analysis.

Determination of Dietary Fibre of the Biscuit Samples

Total dietary fibre determination was carried out using AOAC 985.29 method [21]. One gram of sample was weighed into a conical flask and 50ml of borate buffer solution added to it. Then, 0.1ml of fungal α – amylase enzyme was added and transferred to a shaker water bath at a temperature of 95 – 100°C for 30 min. Thereafter, this was cooled to room temperature. The pH of the solution was adjusted to 7.5 by the addition of 0.1N sodium hydroxide (NaOH). Then, 0.1ml protease enzyme was added to the sample and heated for 30 min at 60°C, allowed to cool to room temperature and pH adjusted to 4.0 using 0.1N hydrochloric acid (HCl). An addition of

0.2ml amyloglucosidase was made and the sample heated for 30 min. The final solution was made up to 70ml and 100ml of alcohol held at 60°C was added and allowed to stand for 1 hr to precipitate the residue. Pre-weighed filter paper was used to filter the sample and dried in an oven at 105°C for 1 hr and re-weighed.

One of the weighed samples (filter paper + residue) was used for protein analysis and the other for fat. Thus, dietary fibre content was calculated using the following formula:

$$Dietary\ fibre\ (5) = \frac{\text{Wt of residue} - \text{protein Ash} - \text{Blank}}{Wt\ of\ sample} \times 100$$

In-Vitro Starch Hydrolysis/Predicted Glycaemic index

The Starch hydrolysis and predicted glycaemic index was determined using the modified Goni et al. [18] as described by Frei et al. [22] Fifty milligrams of sample was weighed and 10ml of HCl-KCl buffer was added to adjust pH to 1.5. Then 0.2ml of a solution containing 1mg of pepsin (Cat. No P6887, Sigma) from porceine gastric mucosa were added to the sample and incubated for 1 hr at 40°C in a water bath shakings at intervals. The volume was made up to 25ml by adding phosphate buffer (pH 6.9) containing 2.5ml of α – amylase from porceine pancreas. The flasks were then incubated in a water bath at 37°C with moderate agitation. Aliquots of 1ml were taken from each flask at intervals of 30 min from 0 hr to 3 hr. Alpha amylase was inactivated by placing the tubes containing the aliquots in a boiling water bath for 5 min and allowed to cool. Then 3ml of 0.2m sodium acetate buffer (pH 4.5) and 0.06ml of amyloglucosidase enzyme were added. This was then incubated for 45 min at 60°C. The rate of starch digestion was expressed as a percentage of total starch hydrolysed at 30, 60, 90, 120, 150 and 180 min. Final glucose concentration was determined using the DNS method.

Preparation of 3.5 Dinitrosalicylic Acid (DNS)

Seventy five grams (75g) of sodium potassium tatarate was dissolved in 125ml of distilled water and 2.5g of 3,5, dinitrosalicylic acid was dissolved in 50ml of 2N sodium hydroxide solution. Both solutions were mixed and 250ml of distilled water was added. A known quantity of digested sample (0.5ml) was added to 1ml of 3.5 Dinitrosalicylic acid with slight agitation, heated for 5 min and allowed to cool. Thereafter, 3.5ml of distilled water was added and absorbance read at 540nm against a glucose standard. A plot of absorbance was done against glucose concentration of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/l. A standard graph was obtained and values read.

The Area under hydrolysis curve for each sample, as well as the reference sample was calculated using the trapezoid method [17] expressed in Microsoft excel as; = B1 + B2/2 * A2 – A1.

Where;

A represents Time in min on the X axis

B represents % of Starch hydrolysed on the Y axis

Hydrolysis index (HI) was obtained by dividing the area under curve of each sample by the area under curve of the reference sample (white bread).

$$Hydrolysis\ index\ (H.I) = \frac{Area\ under\ curve\ of\ sample}{Area\ under\ curve\ of\ Reference\ sample}$$

Glycaemic index (GI) was estimated using the model: $(\mathrm{GI}) = 39.71 + (0.549xH.I)$.

Statistical Analysis

Data obtained were expressed as the mean ± SEM of triplicate samples using the Gensat software. Statistical significance was investigated using one way Analysis of Variance (ANOVA) and means separated using Duncan Multiple

Range test at 5% level of probabaility.

RESULTS AND DISCUSSION

Dietary Fibre Content of the Biscuit Samples

Results of the dietary fibre content of biscuit samples produced from wheat flour (WHF), African walnut flour and Moringa seed flour (MSF) blends is presented in Table 2. The biscuits showed increased amounts of dietary fibre as the substitution levels of Moringa seed flour (MSF) increased. The biscuit samples were observed to contain the values of 0.13, 0.25, 0.30, 0.40, 0.72, 0.43 and 0.21% of dietary fibre for samples ranging from A to G, respectively. Anudeep et al. [23] reported that Moringa oleifera seeds is a potential source of soluble dietary fibre. This report is in agreement with the result obtained in this study as sample E with the highest moringa blend correlated with the highest dietary fibre content of the biscuit sample. Carclona et al. [24] stated that dietary fibre rich diet has been shown to reduce blood glucose by increasing early insulin secretion and improving insulin sensitivity. They went further to report that digestion and absorption of dietary fibre can be affected by dosage, food matrix gender and differences in gut microbial population. The majority of the dietary fibre ingested reaches the colon thereby undergoing intensive metabolism prior to absorption.

Table 2. Dietary fibre content of biscuit samples produced from WHF, AWF and MSF blends

Samples	Dietary Fibre Content (%)
A	0.13 ± 0.01^{e}
B	0.25 ± 0.00^{d}
C	0.30 ± 0.00^{c}
D	0.40 ± 0.06^{b}
E	0.72 ± 0.01^{a}
F	0.43 ± 0.10^{b}
G	0.21 ± 0.00^{d}

Mean values with different superscripts within a column are significantly different ($p \leq 0.05$), ± SEM of triplicate samples.

Key:

WHT = Wheat Flour, AWF = African walnut flour, MSF = Moringa seed flour

Sample A = 100:0:0, B = WHF 77.5:20:2.5, C = 75:20:5, D = 72.5:20:7.5, E = 70:20:10, F = 90:0:10, G = 80:20:0 (WHT: AWF: MSF)

In-Vitro Starch Hydrolysis of the Biscuit Samples

The in-vitro starch hydrolysis curve of biscuit samples produced from wheat flour (WHF), African walnut flour (AWF) and Moringa seed flour (MSF) blends is shown in Figure 1. The result revealed that sample A (control) had the maximum hydrolysis percentage after 120 min of enzyme hydrolysis with a value of 78.44%, followed by sample G, B, C, D, E and F with the values of 65.98%, 64.37, 63.07, 55.97, 52.65 and 52.43%, respectively. White bread used as a reference sample had a value of 85.68% which was higher than all the biscuit sample. The digestion curve of the African walnut biscuit reached a plateaued at 120 min of hydrolysis, after which the values reduced. The values obtained showed that the amount of starch hydrolysed was highest at 120 min for all the samples formulated as well as the reference sample white bread. This result was in agreement with that reported by Englyst and Englyst [25]. According these authors, slowly digestible starch is the portion of starch that is digested after rapidly digestible starch but not longer than 120 min; that is, between 30 and 120

min of starch digestion in-vitro. It is completely but slowly digested in the human small intestine. Slowly digestible starch (SDS) offers a stabilizing and sustaining effect on blood glucoses levels and subsequently lowers glycaemic index. This observation was in agreement with Williamson [26] that reported that the presence of enzyme inhibitors such as procyanidin, narigenin would slow the rate of starch digestion. Also, the influence of polyphenols contained in the moringa seed flour and African walnut flour many have an effect on alpha and beta amylase activity and consequently affect the rate of starch digestion. Thus, as the level of substitution of the flour blend with moringa seed flour increased, the result showed a decrease in the level of starch hydrolysed over a period of 120 min. This implies that the increase in the moringa seeds flour led a slow release of starch by hindering the activity of alpha enzyme. The amount of starch hydrolysed over a period of time have an effect on the glyceamic index. This revealed that moringa have an effect of lowering starch hydrolysis percentage.

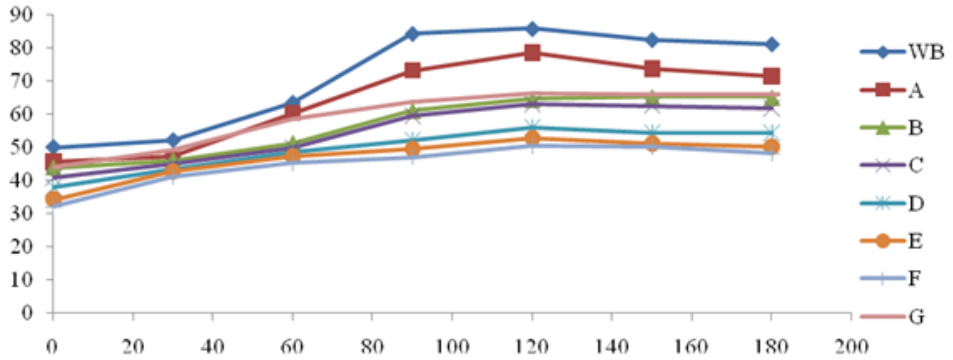

Figure 1. Starch hydrolysis of biscuit samples produced from wheat flour (WHF), African walnut flour (AWF) and Moringa seed flour (MSF) blends.

Key:

WHT = Wheat Flour, AWF = African walnut flour, MSF = Moringa seed flour

Sample A = 100:0:0, B = WHF 77.5:20:2.5, C = 75:20:5, D = 72.5:20:7.5, E = 70:20:10, F = 90:0:10, G = 80:20:0 (WHT: AWF: MSF)

The Equilibrium concentration, Hydrolysis Index and Predicted Glycaemic Indices of the Biscuit Samples

The Equilibrium concentration, Hydrolysis index and predicted Glycaemic indices of biscuit samples produced from wheat flour (WHF), African walnut flour (AWF), and Moringa seed flour (MSF) blends is presented in Table 3. Equilibrium concentration represents the percentage of starch hydrolysed after 180 min of starch digestion. Low equilibrium values generally suggest higher resistance to enzymatic hydrolysis [27]. Results of the hydrolysis index of the samples ranged from 51.66% for sample F to 100% for the reference sample white bread. The hydrolysis index represents the ratio of the area under the curve of the sample to the area under the curve of the reference sample. The results also revealed reducing values of hydrolysis index with increased moringa substitution levels with sample F containing 90% wheat flour and 10% MSF being significantly lower ($p \leq 0.05$) than other samples.

The values obtained for predicted glycaemic index of the biscuit samples showed that sample F was significantly lower with a value of 68.07. Oboh and Erema [28] reported similar values of 64.23% estimated glycaemic index for plantain.

The value of the predicted glycaemic index of the reference sample was 94.61% and the reduction in values could be attributed to the contributory effect of moringa seed flour. Gupta et al. [29] reported the blood glucose reduction effect of moringa seed on Streptozocin (STZ) induced rats.

Investigations have revealed that low glycaemic index diets improve metabolic consequence of insulin resistance as well as improvement in glucose and lipid metabolism [30].

Table 3. Hydrolysis and Estimated Glycaemic Indices of Biscuit Samples Produced from WHF, AWF and MSF Blends

Sample	AUC	C∞	H.I %	E.G.I
WB	29,058.40	81.04	100	94.61
A	22,380.68^{a}	71.32^{a}	77.01^{a}	81.98^{a}
B	19,458.55^{c}	65.03^{c}	66.96^{c}	76.47^{c}
C	18,760.02^{d}	61.78^{d}	64.55^{d}	75.14^{d}
D	16,601.85^{e}	54.21^{e}	57.13^{e}	71.04^{e}
E	15,592.74^{f}	50.06^{f}	53.66^{f}	69.16^{f}
F	15,011.59^{g}	48.06^{g}	51.66^{g}	68.07^{g}
G	20,125.73^{b}	66.04^{b}	69.25^{b}	77.72^{b}

Mean values with different superscripts within a column are significantly different ($p \leq 0.05$), ± SEM of triplicate samples.

Key:

AUC = Area under curve, H.I. = Hydrolysis index, E.G.I. = Estimated Glycaemic index, WB = White Bread

WHT = Wheat Flour, AWF = African walnut flour, MSF = Moringa seed flour

Sample A = 100:0:0, B = WHF 77.5:20:2.5, C = 75:20:5, D = 72.5:20:7.5, E = 70:20:10, F = 90:0:10, G = 80:20:0 (WHT: AWF: MSF)

CONCLUSION

Addition of moringa seed flour to wheat flour and African walnut flour in biscuit formulation and production improved the dietary fibre content of the biscuits and reduced the equilibrium concentration, hydrolysis index and predicted glycaemic indices of the samples. Therefore, making sample E and F a medium glycaemic index food with the potential of reducing post prandial blood glucose level.

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